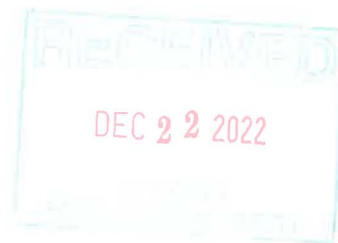




December 20, 2022

Susan J. Carlson, Ph.D., Director
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835



via UPS

Re: Replacement GRAS Notification for Pea Protein Fermented by Shiitake Mycelia

Dear Dr. Carlson,

In accordance with 21 CFR §170, Subpart E - Generally Recognized as Safe (GRAS) Notice, I am submitting, as the agent of the Notifier, MycoTechnology, Inc., a **replacement** GRAS Notification regarding the conclusion of GRAS status for the use of pea protein fermented by shiitake mycelia, as a food ingredient, formulation aid and texturizer in foods where protein is used for nutritional purposes and in foods needing protein-source properties. MycoTechnology does not intend to add FPP to infant formula or to meat and poultry applications that come under USDA jurisdiction.

Enclosed is one electronic copy of the Notification on CD. This file is intended to **replace** the Notification for pea protein fermented by shiitake mycelia that was previously submitted on behalf of MycoTechnology, Inc. via WebTrader on May 27, 2022.

Please contact me with any questions.

Best regards,



G. Craig Llewellyn, Ph.D.
Principal Toxicologist and Scientific Director

GCL/mor

Enclosure

GRAS Notice for Pea Protein Fermented by Shiitake Mycelia

Prepared for:
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive College Park, MD
20740 USA

Date:
December 16, 2022

GRAS Notice for Pea Protein Fermented by Shiitake Mycelia

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GRAS Notice for Pea Protein Fermented by Shiitake Mycelia

Part 1: §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, MycoTechnology, Inc. hereby informs the United States (U.S.) Food and Drug Administration (FDA) that pea protein fermented by shiitake mycelia, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on MycoTechnology's view that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Section 1.4 below. This conclusion is largely based on the fact that this product as produced by the notifier is substantially similar to pea and rice protein fermented by shiitake mycelia, which is manufactured by the notifier (GRAS No. 848), using the same strain and through an equivalent manufacturing process, but with pea as the primary protein raw material.

In addition, as a responsible official of MycoTechnology, the undersigned hereby certifies that all data and information presented in this Notice represents a complete, representative, and balanced submission, and considered all unfavorable as well as favorable information known to MycoTechnology and pertinent to the evaluation of the safety and GRAS status of the pea protein ingredient for use as a food ingredient, as described herein.

Signed,



12/16/2022

Dan Zhao, MS
Manager, Global Regulatory Strategy
MycoTechnology, Inc.
dzhao@mycoiq.com

1.1 GRAS Notice Submission

MycoTechnology, Inc. submits this GRAS Notification in accordance with the requirements of Title 21 of the Code of Federal Regulations (CFR) Part 170, Subpart E.

1.2 Name and Address of Notifier

MycoTechnology, Inc.
18250 E 40th Ave Suite 50
Aurora, CO
80011 USA

1.3 Common or Usual Name of Notified Substance

The common name is **pea protein fermented by shiitake mycelia**
The trade name of this product is FermentIQ™

1.4 Conditions of Use

Pea protein fermented by shiitake mycelia, which is FermentIQ™ pea protein (FPP), containing approximately 77% protein, is intended for use as a food ingredient, formulation aid and texturizer in foods where protein is used for nutritional purposes and in foods needing protein-source properties such as promotion of ease of dry flow, masking of off-flavors, texturing of meat analogues, increased water holding capacity and gelation, and increased water-solubility. In addition, it can be texturized by high pressure/temperature extrusion to add mouth feel to food products. Intended food categories include baked goods and bakery mixes, beverages and beverage bases, breakfast cereals, coffee and tea, dairy product analogues, grain products and pastas, milk products, nut and nut products, plant protein products, snack foods, soups and soup mixes. The proposed ingredient will be used as a substitute for and/or in conjunction with other sources of protein in conventional food products. MycoTechnology does not intend to add FPP to infant formula or to meat and poultry applications that come under USDA jurisdiction.

The U.S. FDA has raised no questions on the use of pea and rice protein fermented by shiitake mycelia (GRN 848) under intended conditions of use. FPP is comparable to the pea/rice blend fermented by shiitake mycelia described in GRN 848 and currently recognized as a GRAS food ingredient, but with pea protein as the primary protein raw material.

1.5 Statutory Basis for GRAS Status

Pursuant to 21 CFR Part, the proposed use of pea protein fermented by shiitake mycelia manufactured by MycoTechnology, Inc. has been concluded to have GRAS status, on the basis of scientific procedures.

The GRAS determination is based on information generally available in the public domain pertaining to the safety of commonly consumed plant proteins and the method of production using shiitake mycelia, and on the consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of pea protein fermentation by shiitake mycelia as a food ingredient [see Appendix A, titled “GRAS Panel Statement on the Generally Recognized as Safe (GRAS) Conclusion for the Proposed Uses of Pea Protein Fermented by Shiitake Mycelia”].

1.6 Premarket Exempt Status

Pea protein fermented by shiitake mycelia (FPP) is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act (FFDCA) based on the conclusion that the

notified substance is GRAS under the conditions of intended use.

1.7 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the U.S. FDA for review and copying upon request during business hours at the offices of:

MycoTechnology, Inc.
18250 E 40th Ave Suite 50
Aurora, CO
80011 USA

MycoTechnology will supply additional data and information should the FDA have any questions regarding this notification during or after the Agency's review of the Notice.

1.8 Freedom of Information Act, 5 U.S.C. 552

It is MycoTechnology's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore none of the data and information presented herein are exempt from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

2.1.1 Common or Usual Name

Pea protein fermented by shiitake mycelia

2.1.2 Synonyms

FermentIQ™; FermentIQ™ Pea Protein; FPP

2.1.3 Chemical and Physical Characteristics

The subject of this GRAS Notice is pea protein fermented by shiitake mycelia (FPP). Fermentation with shiitake mycelia is performed to improve organoleptic quality and functionality properties of the input pea protein raw material; however, the input pea protein is not substantially modified by the fermentation process. FPP contains ≥77% protein on a dry weight basis (DWB). The shiitake mycelia biomass in the final product is <0.1%.

2.2 Method of Manufacturing

The manufacturing of pea protein fermented by shiitake mycelia (FPP) involves successive fermentations of a primary culture of shiitake mycelia to build up an amount of a pure shiitake mycelial biomass, followed by a main fermentation step where the built-up shiitake mycelia biomass is combined with sterile input pea protein material and allowed to ferment for up to 40 hours. At the end of the fermentation, the contents of the fermentation tank are concentrated and spray dried. The resultant material is called pea protein fermented by shiitake mycelia. All input materials into the manufacturing process are safe and suitable for the described use in food ingredient production. The main fermentation step allows the shiitake mycelia biomass developed from the initial fermentations to improve the organoleptic qualities (as measured by human sensory testing; described at Section 2.5.4) of the input pea protein raw material. The input pea protein raw material is not substantially modified (see Section 2.5.3). A comprehensive safety assessment for the shiitake mycelia is presented in Part 6.

2.2.1 Raw Materials and Processing Aids

All raw materials, processing aids, and equipment used to manufacture pea protein fermented by shiitake mycelia (FPP) are food-grade ingredients¹ permitted by US regulation or have GRAS status.

¹ Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA, CODEX, United States Pharmacopeia, European Pharmacopoeia).

2.2.1.1 Pea protein raw material

The pea protein raw material used to produce FPP is obtained from commercial suppliers and meets specifications and quality criteria defined by MycoTechnology (see Table 2.2.1.1-1). The pea protein is a free-flowing, cream colored powder with a protein content of not less than 80% (dry basis). The pea protein is obtained from the mechanically milled and wet fractionated portion of de-hulled yellow peas (*Pisum sativum*). Supporting documentation is on file with MycoTechnology.

Table 2.2.1.1-1. Pea Protein Raw Material Specification

| Parameter | Specification | Test Method |
|----------------------------------|-------------------|--------------------------------|
| Appearance | Light yellow | Visual against standard |
| Odor | Inherent pea odor | Sensory |
| Protein, % dry weight (DW) basis | ≥80% | AOAC 990.03 AOAC 992.15 |
| Aerobic Plate Count | ≤10,000 cfu/g | AOAC 990.12 (Petrifilm) |
| Yeast | ≤100 cfu/g | AOAC 997.02 |
| Mold | ≤100 cfu/g | AOAC 997.02 |
| Coliforms | ≤10 cfu/g | AOAC 991.14 |
| E. coli SSP | ≤10 cfu/g | AOAC 991.14 |
| Salmonella SSP | Negative/25g | AOAC 2004.03 AOAC-RI 121501 |
| Mercury | <1 ppm | ICP-MS |
| Cadmium | <0.5 ppm | ICP-MS |
| Arsenic | <1 ppm | ICP-MS |
| Lead | <1 ppm | ICP-MS |
| Mycotoxins | <5 ppb | HPLC AOAC 991.31 (Mod) |

2.2.1.2 Shiitake Mushroom Mycelia

The strain of shiitake used to produce FPP was originally obtained from Pennsylvania State University (<https://plantpath.psu.edu/about/facilities/mushroom/cultures-spawn>; ID No. WC 1008). The strain was genotyped by a third-party lab and the cultures were identified as *Lentinula edodes* (100% match) by internal transcribed spacer sequencing data (ITS; 28SDNA; MycoTechnology, unpublished data). Based on the ITS results, it was concluded that the microorganism used in the manufacturing process to produce FPP is *L. edodes*. Under conditions of use in aqueous culture, *L. edodes* grows as a vegetative form (Tsifileva et al, 2005; Aminuddin et al, 2013; Aminuddin et al, 2007). This vegetative form is identified herein as ‘shiitake mycelia’. Based on this information, MycoTechnology, Inc. concludes that the organism used in the manufacturing process of FPP is the vegetative form of *L. Edodes* or shiitake mycelia.

A safety assessment of shiitake mycelia is presented in Part 6 and is fully discussed in GRN 848.

2.2.1.3 Additional Raw Materials and Processing Aids

All remaining raw materials and processing aids used to produce FPP, including carrot powder, mango puree, rice protein, maltodextrin, and a commercial anti-foam preparation are considered safe and suitable.

Maltodextrin ((C₆H₁₀O₅)_n, CAS Reg. No. 9050-36-6) is a non-sweet nutritive saccharide polymer that consists of D-glucose units linked by [alpha]-1-4 bonds and has a dextrose equivalent (D.E.) of less than 20. It is prepared by partial hydrolysis of corn starch, potato starch, or rice starch with safe and suitable acids and enzymes, meeting specifications in Food Chemicals Codex, 3d ed., 3d supp. (1992), p. 125. Maltodextrin as used in FPP is listed in 21 CFR 184.1444 as affirmed GRAS and is consistent with current Good Manufacturing Practice (cGMP, 21 CFR 184.1444). The carrot powder and mango puree used in the production of FPP (to support the fermentation process as sources of carbohydrates and micronutrients for mycelium growth) are composed of 100% organic carrots and organic mango respectively. The antifoam agent used in the production of FPP is made with ingredients that are compliant with 21 CFR 173.340 Defoaming agents. A small amount (<0.5% in finished product dry mass) of rice protein, previously recognized as GRAS for use in food (e.g. GRN 609), is also used to support the fermentation process. With the exception of mango puree, these ingredients are also described in GRN 848.

2.2.2 Manufacturing Process

Pea protein fermented by shiitake mycelia (FPP) is manufactured consistent with current Good Manufacturing Practices (cGMP) as defined in 21 CFR §117 at a facility with an established Hazard Analysis and Critical Control Points (HACCP) plan. Supporting documentation is on file with MycoTechnology.

The manufacturing is initiated by starting the growth of pure cultures of shiitake mycelia on agar plates developed from a confirmed shiitake spawn culture stored at -80°C. The identity of shiitake spawn is discussed in Section 2.2.1.2. The grown cultures on agar plates are used to initiate liquid cultures of shiitake mycelia in shake flasks. For the shake flask cultures, the media is an approximately 2% slurry of pea protein concentrate, rice protein concentrate, supplemented with maltodextrin, mango puree, carrot powder and antifoam agent. Prior to culture inoculation, the media is sterilized by heat treatment and the inoculation with shiitake mycelia is carried out using sterile procedure. The inoculated shake flasks are incubated until the shiitake mycelia has achieved the desired level of growth in the shake flasks. The entirety of the volume of the shake flasks is then transferred into the first of three “seed development” bioreactors to continue to build shiitake mycelia biomass, as described in the following paragraphs.

The shiitake mycelia biomass-building process is continued in the “seed development” bioreactor process using three separate fermentations in three progressively larger bioreactors. The three successive fermentations (“Fermentation 1”, “Fermentation 2,” and “Fermentation 3”) are carried out in these progressively larger bioreactors. Prior to inoculation, all bioreactor media is sterilized at 121°C for 90 minutes and cooled down using air and water circulation on the vessel’s cooling jacket, and all inoculations are carried out with sterile procedure to maintain a pure shiitake mycelia culture.

After inoculation of the first bioreactor (Fermentation 1) with the shake flask cultures, the cultures are allowed to grow for 24 to 48 hours. During all fermentations, purity of the culture and growth of the shiitake mycelia are confirmed via microscopy. At the conclusion of Fermentation 1, the entirety of the volume of the bioreactor is transferred into the second bioreactor, together with fresh media to fill to volume, to initiate Fermentation 2. Fermentation 2 is carried out for 24 to 48 hours. At the conclusion of Fermentation 2, the entirety of the volume of the second bioreactor is then transferred into the third bioreactor together with fresh media to fill to volume, to begin Fermentation 3. Fermentation 3 is carried out for 24 to 48 hours. At the conclusion of Fermentation 3, the shiitake mycelia biomass has reached a biomass level of approximately 2 g/mL. Log phase of

the shiitake mycelia is maintained between shake flasks and the seed fermentations by use of similar media, temperature, and agitation. The growth of the shiitake biomass is confirmed by pH monitoring. Change in pH is a lead indicator of growth of shiitake mycelia (Aminuddin et al., 2013).

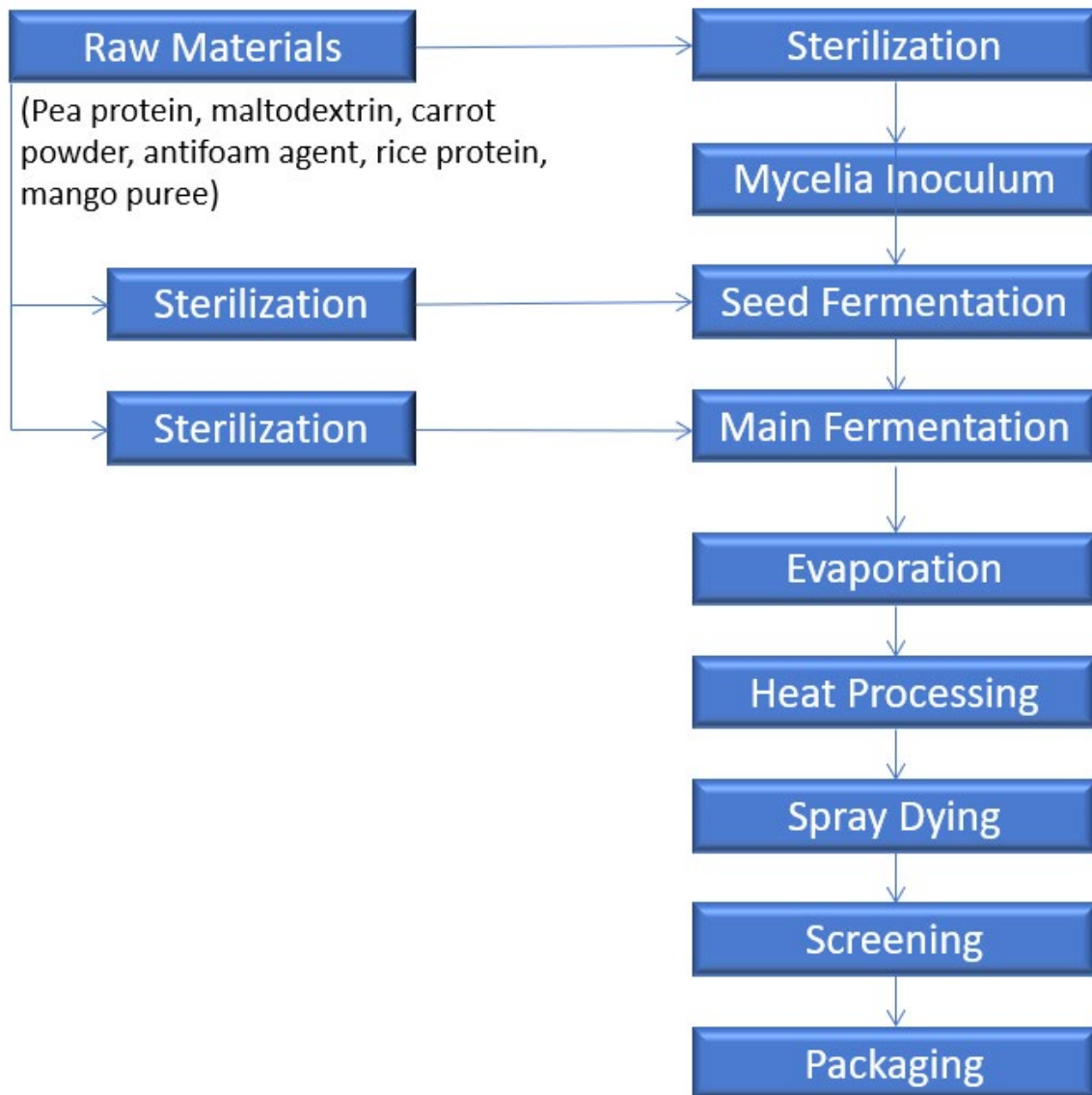
In the final fermentation stage of the manufacturing process (called herein “main fermentation”) for FPP, the entirety of the volume from Fermentation 3 is inoculated into the main fermenter, for an input volume of 4% of the volume of the main fermenter. The remainder of the input is the main fermentation media. The difference between the main fermentation media and the media used for the seed fermentation is that the main fermentation media component is pea protein at significantly higher concentrations (i.e. 95% total protein concentrates) than found in the seed fermentation media. Prior to inoculation, all media is sterilized at 121°C for 90 minutes, and all inoculations are carried out with sterile procedure. The main fermentation process is completed in up to 40 hours.

It is known from the literature that a significant change in media will induce a lag phase in an aqueous shiitake culture; specifically, the literature shows that after inoculation of shiitake mycelia into liquid media, a period of 6 to 10 days of culture time is required before appreciable increase is observed in mycelial biomass (Cavallazzi et al., 2005). The main fermentation phase is carried out for no longer than 40 hours under conditions known to induce lag phase in shiitake mycelia. The pH of the main fermentation does not change, indicating no mycelial growth is occurring. MycoTechnology, Inc. therefore concludes that the shiitake mycelia culture enters lag phase upon inoculation into the main fermentation and remains in lag phase throughout the duration of the main fermentation.

The manufacturing process is continued by heat processing and spray-drying steps at the conclusion of the main fermentation. The fermentation process is terminated by heat treatment (~ 65°C for 60 minutes), followed by an evaporator/concentration step. Then the product is heated to 80°C for 1 minute as safety control, followed by spray drying. The spray dried powder is the manufacturing process final output. As discussed below, the input pea protein raw material is not substantially modified after fermentation (see Section 2.5.3).

The flow chart for the manufacture of FPP is provided in Figure 2.2.2-1 and is consistent with the process described in GRN 848 for pea and rice protein fermented by shiitake mycelia.

Figure 2.2.2-1. Process Flow Chart



2.3 Product Specifications and Batch Analysis

2.3.1 Product Specifications

Appropriate food-grade specifications have been established for pea protein fermented by shiitake mycelia (FPP) (Table 2.3.1-1). All cited analytical methods are validated for their intended purpose.

Table 2.3.1-1. Product Specifications for FPP

| Parameter | Specification | Test Method |
|----------------------------------|---------------|--------------------------------------|
| Protein, % dry weight (DW) basis | ≥77 (DW) | AOAC 990.03 AOAC 992.15 |
| Ash | ≤10% | AOAC 942.05 |
| Moisture | ≤6% | AOAC 925.09 AOAC 985.14 |
| Total Fat | ≤10% | AOAC 996.06 mod |
| Carbohydrate (by calculation) | ≤15% | FDA CFR 21 - Calculated |
| pH | >5.5 | AOAC 943.02 AOAC 981.12 |
| Aerobic Plate Count | <10,000 cfu/g | AOAC 990.12 Petrifilm AOAC 966.23 |
| Yeast | <100 cfu/g | AOAC 997.02 Petrifilm BAM Ch 18 |
| Mold | <100 cfu/g | AOAC 997.02 Petrifilm BAM Ch 18 |
| Coliforms | <10 cfu/g | AOAC 991.14 Petrifilm |
| E Coli SSP. | <10 cfu/g | AOAC 991.14 Petrifilm |
| Salmonella SSP. | Negative/25g | AOAC 2004.03 AOAC-RI 121504 |
| Listeria monocytogenes | Negative/25g | AOAC 2004.02 AOAC-RI 061703 |
| Aflatoxin B1 | <5 ppb | AOAC 999.07 Mod |
| Aflatoxin B2 | <5 ppb | AOAC 999.07 Mod |
| Aflatoxin G1 | <5 ppb | AOAC 999.07 Mod |
| Aflatoxin G2 | <5 ppb | AOAC 999.07 Mod |
| Aflatoxin Total | <5 ppb | AOAC 999.07 Mod |
| Arsenic | <0.1 ppm | AOAC 2011.19 AOAC 993.14 Mod |
| Cadmium | <0.1 ppm | AOAC 2011.19 AOAC 993.14 Mod |
| Lead | <0.1 ppm | AOAC 2011.19 AOAC 993.14 Mod |
| Mercury | <0.1 ppm | AOAC 2011.19 AOAC 993.14 Mod |

2.3.2 Batch Analysis

Analysis of 3 non-consecutive batches of FPP produced by fermentation with shiitake mycelia demonstrates the manufacturing process produces a consistent product that meets the established product specifications. Summaries of the batch analysis for each pea protein product is presented in Table 2.3.2-1.

Table 2.3.2-1. Analytical Data from 3 Non-Consecutive Batches of FPP

| Parameter | Specification | Batch ID: Lot 101355 | Batch ID: Lot 101646B | Batch ID: Lot 101602B |
|----------------------------------|---------------|-------------------------|--------------------------|--------------------------|
| Protein, % dry weight (DW) basis | ≥77 | 78.32 | 78.45 | 78.68 |
| Ash (%) | ≤10% | 5.03 | 5.30 | 5.67 |
| Moisture (%) | ≤6% | 2.4 | 4.4 | 2.7 |
| Total Fat (%) | ≤10% | 9.11 | 9.51 | 9.18 |
| Carbohydrate by calculation (%) | ≤15% | 7.02 | 5.79 | 5.89 |
| pH | >5.5 | 6.37 | 6.61 | 6.59 |
| Aerobic Plate Count (cfu/g) | <10,000 | 1,500 | <10 | <10 |
| Yeast (cfu/g) | <100 | <10 | <10 | <10 |
| Mold (cfu/g) | <100 | <10 | <10 | 20 |
| Coliforms (cfu/g) | <10 | <10 | <10 | <10 |
| E Coli SSP (cfu/g) | <10 | <10 | <10 | <10 |
| Salmonella SSP | Negative/25g | Negative/25g | Negative/25g | Negative/25g |
| Listeria monocytogenes | Negative/25g | Negative/25g | Negative/25g | Negative/25g |
| Aflatoxin B1 (ppb) | <5 | <5 | <5 | <5 |
| Aflatoxin B2 (ppb) | <5 | <5 | <5 | <5 |
| Aflatoxin G1 (ppb) | <5 | <5 | <5 | <5 |
| Aflatoxin G2 (ppb) | <5 | <5 | <5 | <5 |
| Aflatoxin Total (ppb) | <5 | <5 | <5 | <5 |
| Arsenic (ppm) | <0.1 | <0.010 | 0.0182 | 0.0157 |
| Cadmium (ppm) | <0.1 | 0.0392 | 0.0375 | 0.0376 |
| Lead (ppm) | <0.1 | 0.0121 | 0.012 | 0.0144 |
| Mercury (ppm) | <0.1 | <0.005 | <0.005 | <0.005 |

2.3.3 Stability

The FPP product should be stored in a cool, dry location, and in the original sealed package away from odorous material. The protein content of this product is stable under accelerated conditions. The FPP product has a shelf life of 24 months from date of manufacture.

2.4 Technical Effect of Pea Protein Fermented by Shiitake Mycelia (FPP)

FPP is intended to be used as a substitute for, and/or in conjunction with, pea protein, rice protein and other protein sources in conventional food products. FPP also has beneficial functional properties in food/beverage systems: ease of dry flow, masking of off-flavors, texturing of meat analogues, increasing water holding capacity and gelation, and increase of water-solubility as compared to conventional products. Intended food categories include baked goods and baking mixes, beverages and beverage bases, breakfast cereals, coffee and tea, dairy product analogues, grain products and pastas, milk products, nut and nut products, plant protein products, snack foods, soups and soup mixes.

2.5 GRAS Material Characterization

2.5.1 Physical Characteristics of FPP

Appearance: Powder

Color: Light cream

Aroma: Low aroma

Taste: Clean taste

2.5.2 Composition of FPP

The FPP product is comprised of primarily protein ($\geq 77\%$; based on 95% total pea protein raw material input), fat, carbohydrates and up to 5% adjunct material (e.g., remainder of fermentation media components such as carrot powder and maltodextrin (refer to Section 2.2.1)). A minimal amount of protein ($< 0.05\%$) may also be contributed by the shiitake mycelium (based on $< 0.1\%$ shiitake biomass in the final FPP product). Nutritional and compositional data and amino acid profile for FPP is presented in 2.5.2-1.

Table 2.5.2-1. Nutritional and Compositional Data for FPP

| Parameter | Results | | |
|--------------------------------|----------------|----------------|----------------|
| Batch ID | 101355 | 101646B | 101602B |
| Protein (% DW) ¹ | 78.32 | 78.45 | 78.68 |
| Protein (% as is) ² | 76.44 | 75 | 76.56 |
| Moisture and Volatiles (%) | 2.4 | 4.4 | 2.7 |
| Ash (%) | 5.03 | 5.3 | 5.67 |
| Total fat as Triglycerides (%) | 9.11 | 9.51 | 9.18 |
| Carbohydrates (%) Calculated | 7.02 | 5.79 | 5.89 |
| Fiber (%) | 6.8 | 6 | 6.9 |
| Sucrose (%) | 0.62 | 0.65 | 0.68 |
| Total sugars (%) | 1.11 | 1.13 | 1.19 |
| Amino Acid (%) | 78.23 | 74.39 | 75.31 |
| Tryptophan (%) | 0.71 | 0.72 | 0.74 |
| Cystine (%) | 0.6 | 0.88 | 0.65 |
| Methionine (%) | 0.86 | 1.18 | 0.82 |
| Alanine (%) | 3.52 | 3.23 | 3.29 |
| Arginine (%) | 6.53 | 6.33 | 6.47 |
| Aspartic Acid (%) | 9.25 | 8.54 | 8.7 |
| Glutamic Acid (%) | 13.34 | 13.01 | 13.26 |
| Glycine (%) | 3.28 | 3.03 | 3.1 |
| Histidine (%) | 1.9 | 1.86 | 1.9 |
| Isoleucine (%) | 3.79 | 3.58 | 3.65 |
| Leucine (%) | 6.84 | 6.35 | 6.48 |
| Phenylalanine (%) | 4.31 | 3.98 | 4.08 |
| Proline (%) | 3.4 | 3.18 | 3.23 |
| Serine (%) | 4.1 | 3.78 | 3.88 |
| Threonine (%) | 2.98 | 2.67 | 2.74 |
| Lysine (%) | 5.77 | 5.48 | 5.61 |
| Tyrosine (%) | 2.94 | 2.7 | 2.76 |
| Valine (%) | 4.11 | 3.89 | 3.95 |

¹% Protein (DW) = $\frac{\% \text{ Protein (As Sampled)}}{(100 - \% \text{ Moisture})} \times 100$

²Protein content calculated using a nitrogen to protein conversion factor of 6.25.

2.5.3 Similarity of FPP to input pea protein starting material

A comparison of the amino acid profiles of pea protein and FPP are provided in Table 2.5.3-1. The overall typical amino acid profile of FPP generally aligns with the amino acid values of pea protein.

Table 2.5.3-1. Amino Acid Profile Comparison of FPP with Pea Protein

| Amino Acids | Pea Protein Raw Material* | FPP Finish Product* |
|-------------------|---------------------------|---------------------|
| Alanine % | 3.36 | 3.35 |
| Arginine % | 6.94 | 6.44 |
| Aspartic Acid % | 9.14 | 8.83 |
| Glutamic Acid % | 13.48 | 13.20 |
| Glycine % | 3.18 | 3.14 |
| Histidine % | 2.00 | 1.89 |
| Isoleucine % | 3.88 | 3.67 |
| Leucine % | 6.71 | 6.56 |
| Phenylalanine % | 4.29 | 4.12 |
| Proline % | 3.41 | 3.27 |
| Serine % | 3.84 | 3.92 |
| Threonine % | 2.83 | 2.80 |
| Lysine % | 6.00 | 5.62 |
| Tyrosine % | 2.94 | 2.80 |
| Valine% | 4.15 | 3.98 |
| Cystine % | 0.68 | 0.71 |
| Methionine % | 0.84 | 0.95 |
| Tryptophan % | 0.83 | 0.72 |
| Total Amino Acid% | 78.50 | 75.98 |

*Data presented in this column is average amino acid value from 3 lots of unfermented pea protein and 3 lots of FPP.

The total amino acid content (%) of FPP remained substantially unchanged ($\leq 3\%$ difference) from the raw material. Individual amino acid values are slightly lower in FPP ($< 0.5\%$ differences) probably due to the dilution of FPP product with about 5% of other materials such as, carrot powder and maltodextrin. In summary, the composition of the input pea protein material remains largely unchanged after fermentation with shiitake mycelia.

2.5.4 Proposed Mechanism for Improvement to Organoleptic Properties of Input Pea Protein

Vegetable-derived protein isolates and concentrates possess objectionable flavor compounds that can arise from oxidative deterioration of unsaturated fatty esters in protein-bound lipids (Rackis et al., 1979). Schindler identified several volatile organic compounds (VOC) in pea protein extracts which impart undesirable organoleptic qualities impacting their acceptance by consumers (Schindler et al., 2012; Table 1). In many cases, the VOCs associated with undesirable organoleptic qualities are below limits of detection (LOD) or limits of quantification (LOQ) by contemporary analytical techniques (Rackis

et al., 1979; Sessa and Rackis, 1977; Buttery et al., 1988). On the other hand, human sensory (taste and smell) testing can be reliably used to detect the presence of these VOCs at levels undetectable by contemporary analytical techniques (Yoshikawa et al., 1965).

Consistent with the literature discussed above, MycoTechnology, Inc.'s analysis of organoleptic qualities (by human sensory analysis) of the input pea protein used to produce FPP found undesirable levels of green pea, yellow pea/beany, chalky, and cardboard notes in the input pea protein (MycoTechnology; unpublished data on file). Sensory studies on FPP compared to unfermented pea protein standard show that FPP has improved organoleptic qualities (i.e. flavor, taste, and aroma attributes reported by trained descriptive panelists) as compared to the unfermented pea protein standard. These results indicate that the manufacturing process described in section 2.2 remediates the undesirable organoleptic qualities associated with the control pea protein starting material including 30% reduction in aroma intensity, green pea flavor reduced by 53% and yellow pea/beany flavor reduced by 41% (MycoTechnology; unpublished data on file).

Consistent with the discussion provided in GRN 848, the improvement in organoleptic qualities of FPP during the fermentation process described in section 2.2 may be due to the secretion of enzymes by the shiitake mycelia during the main fermentation step which act to modify certain VOCs known to impart unpleasant organoleptic qualities of pea protein concentrates. As discussed above (see Section § 2.2.2), MycoTechnology concluded that shiitake mycelia are in lag phase during the main fermentation step, but the literature shows that even during lag phase, shiitake mycelia remain metabolically active, due to adaptation of the organism to a change in media (Cavallazzi, 2005). Shiitake mycelia are known to secrete a number of fungal enzymes, such as pectinases; cellulases; amylases; laccases; laminarinases; and xylanases (Mata et al., 2016). In particular, it is known that Shiitake mycelia constitutively express laccases (Matjuskova et al., 2017), and expression of laccases in shiitake mycelia may be upregulated or stimulated by the presence of lignin-derived phenols and or polymeric lignin materials (Matjuskova et al., 2017; Agrawal et al., 2018). Copper-containing laccases have the ability to oxidize a wide range of aromatic and non-aromatic compounds which includes substituted phenols, some inorganic ions, and a variety of non-phenolic compounds (Agrawal et al., 2018). Laccase is currently used in the food industry for a variety of functional applications including improvement of food sensory parameters (Osma et al., 2010). For example, Schroeder et al. (2008) demonstrated that laccase treatment of apple juice degraded the levels of certain phenolic compounds, guaiacol and 2,6-dibromophenol, responsible for off-flavors in apple juice. Other mechanisms such as physical trapping of volatiles and thermal reactions during the sterilization and drying of the protein blends may also contribute to the changes in olfactory character (Clark et al., 2022).

From its critical evaluation of the available information summarized above, MycoTechnology concludes that confirmed improvements to the organoleptic qualities of FPP relative to the protein input are likely due to the action of secreted enzymes (e.g. laccase) from shiitake mycelia to modify molecules that confer unpleasant organoleptic qualities to pea protein concentrates.

2.5.5 Absence of Viable Shiitake Mycelia in FPP

The main fermentation phase for FPP is carried out under conditions known to induce lag phase in shiitake mycelia (Cavallazzi et al., 2005). Since the pH of the main fermentation does not change, it may be concluded that no mycelial growth is occurring. Thus, MycoTechnology concludes that the shiitake

mycelia culture enters lag phase upon inoculation into the main fermentation and remains in lag phase throughout the duration of the main fermentation. The fermentation process concludes with a thermal deactivation step at about 65 °C for 60 minutes which would kill any remaining live shiitake mycelia.

MycoTechnology confirmed this by conducting an experiment to demonstrate the presence and viability of shiitake mycelia present in FPP. A sample was obtained from the stock 15 kg bags (commercial product size). A total of 4 independent FPP batches were tested in triplicate. For each batch, one gram of FPP was analyzed by plating in two different shiitake solid growing media (plates); for each medium 3 technical replicates were used. Sterile water was used to ensure the quality and sterility of the plates. Blended shiitake mycelium was added as a positive control to ensure that mycelium would grow on the selected media. The samples were incubated at 26 °C in the dark for seven days.

The results presented below demonstrate the absence of fungal/mycelia growth in any of the sample plates at the end of the 7-day incubation period. Fungal/mycelia growth was reported in the positive control plates that contained shiitake mycelium as expected. Results for all samples are shown in Table 2.5.5-1. The report for this study is found in Appendix B.

Table 2.5.5-1. Shiitake Mycelia Growth Results

| Sample Lot | MYPGA+AKS Replicate 1 | MYPGA+AKS Replicate 2 | MYPGA+AKS Replicate 3 | PDA Replicate 1 | PDA Replicate 2 | PDA Replicate 3 |
|---|--------------------------|--------------------------|--------------------------|--------------------|--------------------|--------------------|
| 101646 | ND | ND | ND | ND | ND | ND |
| 101355 | ND | ND | ND | ND | ND | ND |
| 101602 | ND | ND | ND | ND | ND | ND |
| 101905 | ND | ND | ND | ND | ND | ND |
| Negative Control (Sterile H ₂ O) | ND | ND | ND | ND | ND | ND |
| Positive Control Blended shiitake flask | D | D | D | D | D | D |

*ND- No mycelial growth detected.

D- Mycelial growth detected.

Part 3: §170.235 Dietary Exposure

3.1 FPP Application Usage Estimates

The proposed uses of MycoTechnology, Inc.'s pea protein fermented by shiitake mycelia (FPP) as a food ingredient in multiple food categories are summarized in Table 3.1-1 below.

Table 3.1-1. Application of Usage Estimates

| Food Category | Use Level (%) |
|------------------------------|---------------|
| Baked Goods and Baking Mixes | 5 -15 |
| Beverages and Beverage Bases | 40 |
| Breakfast Cereals | 15 |
| Coffee and Tea | 10 |
| Dairy Product Analogues | 10 - 25 |
| Grain Products and Pastas | 20 |
| Milk Products | 15 |
| Nut and Nut Products | 10 |
| Plant Protein Products | 12 - 30 |
| Snack Foods | 4 |
| Soups and Soup Mixes | 5 |

3.2 FPP Daily Consumption Calculation

FPP is intended to be used as a substitute for, and/or in conjunction with, pea protein, rice protein and other protein sources in conventional food products. Target product categories include food products needing protein-source properties such as promotion of ease of dry flow, masking of off-flavors, texturing of meat analogues, increasing water holding capacity and gelation, and increase of water-solubility. MycoTechnology concludes that the intended uses of FPP will not result in an increase in the overall consumption of protein.

3.2.1 FPP Estimated Daily Intake

Estimates for the intake of FPP were based on its proposed food uses and use levels (Table 3.1-1, above) in conjunction with food consumption data included in the U.S. National Center for Health Statistics' National Health and Nutrition Examination Surveys (NHANES) 2015-2016 or 2017-2018. Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food uses of FPP and the percentage of consumers was determined. The per person (g/day) and per kilogram body weight (g/kg bw/day) intakes were reported for the total population and various population groups as presented below in Tables 3.2.1-1 and 3.2.1-2. The complete report for the intake assessment of FPP is included in Appendix C.

Among the total population (ages 2 years and older), the mean and 90th percentile consumer-only intakes of FPP were 22 and 54 g/person/day, respectively. Among the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of FPP on an absolute basis, at 25 and 60 g/person/day, respectively, while children ages 2 to 5 years had the lowest mean and 90th percentile consumer-only intakes of 12 and 27 g/person/day, respectively.

Table 3.2.1-1. Summary of the Estimated Daily Intake of FPP from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

| Population Group | Age Group (Years) | Per Capita Intake (g/day) | | | Consumer-Only Intake (g/day) | | |
|------------------|-------------------|---------------------------|-----------------------------|------|------------------------------|------|-----------------------------|
| | | Mean | 90 th Percentile | % | n | Mean | 90 th Percentile |
| Children | 2 to 5 | 12 | 27 | 99.8 | 468 | 12 | 27 |
| Children | 6 to 11 | 15 | 31 | 99.0 | 672 | 15 | 31 |
| Female Teenagers | 12 to 19 | 20 | 43 | 97.8 | 432 | 21 | 43 |
| Male Teenagers | 12 to 19 | 19 | 41 | 97.8 | 429 | 19 | 41 |
| Female Adults | ≥20 | 22 | 56 | 96.5 | 2,076 | 23 | 56 |
| Male Adults | ≥20 | 24 | 59 | 96.8 | 1,888 | 25 | 60 |
| Total Population | ≥2 | 21 | 53 | 97.1 | 5,965 | 22 | 54 |

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (ages 2 years and older) mean and 90th percentile consumer-only intakes of FPP were determined to be 0.34 and 0.79 g/kg body weight/day, respectively. Among the individual population groups, children ages 2 to 5 years were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 0.75 and 1.48 g/kg body weight/day, respectively. Male adults had the lowest mean consumer-only intake of 0.29 g/kg body weight/day, while male teenagers had the lowest 90th percentile consumer-only intake of 0.70 g/kg body weight/day, respectively.

Table 3.2.1-2. Summary of the Estimated Daily Per Kilogram Body Weight Intake of FPP from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

| Population Group | Age Group (Years) | Per Capita Intake (g/kg bw/day) | | | Consumer-Only Intake (g/kg bw/day) | | |
|------------------|-------------------|---------------------------------|-----------------------------|------|------------------------------------|------|-----------------------------|
| | | Mean | 90 th Percentile | % | n | Mean | 90 th Percentile |
| Children | 2 to 5 | 0.75 | 1.48 | 99.8 | 460 | 0.75 | 1.48 |
| Children | 6 to 11 | 0.46 | 0.89 | 99.0 | 670 | 0.46 | 0.91 |
| Female Teenagers | 12 to 19 | 0.34 | 0.73 | 97.8 | 425 | 0.35 | 0.73 |
| Male Teenagers | 12 to 19 | 0.29 | 0.70 | 97.8 | 426 | 0.30 | 0.70 |
| Female Adults | ≥20 | 0.30 | 0.76 | 96.5 | 2,058 | 0.31 | 0.77 |
| Male Adults | ≥20 | 0.28 | 0.71 | 96.8 | 1,873 | 0.29 | 0.72 |
| Total Population | ≥2 | 0.33 | 0.79 | 97.1 | 5,912 | 0.34 | 0.79 |

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

To allow comparisons to current dietary reference intakes for protein, the estimated total intake of protein from FPP consumption (g/person/day) from all proposed food uses was calculated assuming a

maximum protein content of approximately 79% in FPP (Table 3.2.1-3). Among the total population (ages 2 years and older), the mean and 90th percentile consumer-only intakes of protein from FPP were determined to be 17 and 43 g/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of protein on an absolute basis, at 20 and 48 g/person/day, respectively, while children ages 2 to 5 years had the lowest mean and 90th percentile consumer-only intakes of 10 and 22 g/person/day, respectively.

Table 3.2.1-3. Summary of the Estimated Daily Intake of FPP as Protein^a from Proposed Food Uses in the U.S. by Population Group (2017-2018 NHANES Data)

| Population Group | Age Group (Years) | Per Capita Intake (g/day) | | Consumer-Only Intake (g/day) | | | |
|------------------|-------------------|---------------------------|-----------------------------|------------------------------|-------|------|-----------------------------|
| | | Mean | 90 th Percentile | % | n | Mean | 90 th Percentile |
| Children | 2 to 5 | 10 | 22 | 99.8 | 468 | 10 | 22 |
| Children | 6 to 11 | 12 | 24 | 99.0 | 672 | 12 | 24 |
| Female Teenagers | 12 to 19 | 16 | 34 | 97.8 | 432 | 16 | 34 |
| Male Teenagers | 12 to 19 | 15 | 33 | 97.8 | 429 | 15 | 33 |
| Female Adults | ≥20 | 17 | 44 | 96.5 | 2,076 | 18 | 45 |
| Male Adults | ≥20 | 19 | 47 | 96.8 | 1,888 | 20 | 48 |
| Total Population | ≥2 | 17 | 42 | 97.1 | 5,965 | 17 | 43 |

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

^a Calculation: (Estimated Daily Intake of FermentIQ™ Pea Protein)*(Maximum protein content, 79.31%)

Based on the above information, the conservative 90th percentile estimate of the daily consumption of FPP from proposed food categories is 54 g/person/day for the total population (2 years and older). Assuming a maximum protein content of approximately 79% for the FPP ingredient, the conservative 90th percentile estimate of the daily consumption of protein from FPP in these categories is approximately 43 g/day for the total population (2 years and older).

3.2.2 Comparison of FPP Estimated Daily Intake to Background Intake Levels for Protein

The Institute of Medicine (IOM) Recommended Dietary Allowance (RDA) for protein varies from approximately 10 g/day (for infants) to 71 g/day (for pregnant and lactating women), with the RDA for adult females and males as 46 and 56 g/day, respectively (IOM, 2005). The FDA daily reference value (DRV) for protein is 50 g/day for adults and children 4 or more years of age and 13 g/day for children 1 through 3 years of age (FDA, 2016). As noted in GRNs 803 and 851 for pea protein, background dietary intake of protein by adults at the 90th percentile was estimated to range from 76 to 142 g/day using data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 (IOM, 2005). Average protein intake for the U.S. population using food consumption data from the National Health and Nutrition Examination Survey (NHANES 2011-2012) was estimated to be in the range of 80-103 g/day in men and 58.8-75.5 g/day in women (GRN 683 [FDA, 2017]). Additionally, as discussed in GRN 608 (pea protein concentrate [FDA, 2016a]), the Reference Amount Customarily Consumed (RACC) for peas is 85 g/serving (21 CFR 101.12), with the 90th percentile daily intake estimated to be approximately doubled the RACC (FDA, 2006), i.e. 170 g/day. Since peas contain ~24% protein (GRN 608; USDA NNDSE, 2018a), it can be determined that the amount of protein that would be provided by peas is 20.4 g protein per serving (mean), and 40.8 g protein per day (90th percentile). Based on the intake estimates

for FPP presented above, the estimated 90th percentile intake of protein from FPP from all proposed uses is lower than or within the range of these recommended and/or background intake levels of protein in the diet. Since FPP is intended as a source of protein that will substitute for other proteins in the diet, this ingredient will not result in an overall increase in the consumption of protein in the diet. As discussed in GRN 575 for oat protein (p. 000026-27) and the associated FDA Response Letter (FDA, 2015), it is reasonable to expect that most of the U.S. population's intake of protein is expected to remain in the form of unprocessed foods including meat, poultry, fish, and legumes. The mean protein intake from FPP consumption is similar to or below current FDA and IOM recommendations for protein in the diet further supporting a conclusion of safety.

3.3 Dietary Exposure to Shiitake Mycelia via Consumption of FPP at Estimated Consumption Levels

As discussed above in section 3.2, for the Total Population All-Users Consumption dietary exposure of FPP from all proposed food categories is estimated to be 54 g/day at the 90th percentile of consumption, and 22 g/day at the mean level of consumption.

The level of shiitake mycelia present in the FPP is determined as follows: MycoTechnology, Inc. has confirmed that the shiitake mycelia, when added as input into the main fermentation step, enters lag phase upon inoculation (see Method of Manufacture section 2.2). Lack of additional biomass accumulation of the shiitake mycelia during the main fermentation is confirmed by microscopy and pH monitoring. The input shiitake mycelia in the final fermentation step is <0.1 wt.%. Therefore, MycoTechnology concludes that the relative amount of shiitake mycelia in the FPP matches the relative amount of the input shiitake mycelia, which is <0.1 wt.%.

At <0.1% w/w shiitake mycelia in FPP, for an estimated Total Population All-Users Consumption at the worst-case 90th percentile level of estimated daily consumption of FPP of 54 g, MycoTechnology, Inc. concludes that the corresponding level of shiitake mycelia dietary exposure is 54 mg per person per day. At <0.1% w/w shiitake mycelia in FPP, for an estimated Total Population All-Users Consumption mean level of estimated daily consumption of FPP per person of 22 g, MycoTechnology, Inc. concludes that the corresponding level of shiitake mycelia dietary exposure is 22 mg per person per day. At a mean body weight of 60 kg this would result in an intake of approximately 0.9 mg/kg bodyweight/day at the 90th percentile level of estimated daily consumption of FPP or 0.4 mg/kg bodyweight/day at the mean level of estimated daily consumption for the Total Population.

The safety assessment of the consumption of shiitake mycelia at estimated dietary exposures in FPP is presented below in section 6.2.4.

3.4 Dietary Exposure Conclusions

The FPP product will be used as source of protein in a number of food products. Most of the population's protein intake is derived from, and will continue to be derived from, unprocessed foods, including meat, poultry, fish, and legumes. FPP will be added to products as a competitive protein alternative ingredient on the market in a similar manner to other products on the market such as pea and rice protein. Thus, the addition of FPP ingredients will simply serve as a replacement for these other competitive protein sources and will not increase overall consumer exposure to protein. In addition, considering the current FDA and IOM recommendations for protein in the diet, even overly exaggerated

protein intake estimates from current proposed uses of FPP would not pose any human safety concern.

As discussed below in section 6.2.4, consumption of shiitake mycelia at estimated dietary exposures in FPP is also considered to be safe.

Part 4: §170.240 Self Limiting Levels of Use

The use of the pea protein fermented by shiitake mycelia (FPP) as a food ingredient is limited by the level that can technically be added to a given food without jeopardizing its quality and consumer acceptability. The self-limiting level of use is independent of safety (toxicity, allergenic, etc.) concerns.

Part 5: §170.245 Experience Based on Common Use in Food Before 1958

The statutory basis for the GRAS conclusion for pea protein fermented by shiitake mycelia (FPP) is based on scientific procedures; therefore, information regarding experience based on common use of the notified substance in food prior to 1958 is not applicable. The historical consumption of the GRAS material raw ingredient (pea) and fermentation organism (shiitake mushroom) is discussed below in §170.250 Part 6 (GRAS Narrative) as supporting information.

Part 6: §170.250 GRAS Narrative and Safety Rationale

FPP is composed of pea protein fermented with *L. edodes* (shiitake) mycelium. Based on its method of the manufacture (section 2.2) and demonstrated confirmation that the input raw materials are not substantially modified following fermentation (section 2.5.3), the safety of FPP can be established through the safety of the ingredients used to produce it. FPP is substantially similar to pea and rice protein fermented by shiitake mycelia (GRN 848), using the same strain and through an equivalent manufacturing process, with pea protein as the primary protein raw material.

6.1 Safety of FPP Raw Materials

The safety of pea protein raw material was reviewed in GRN 848 (pea and rice protein fermented by shiitake mycelia). The GRAS status of pea protein has been reviewed by FDA (581, 608, 788, 803, 804, 851, and 948) without any questions. Other raw materials used in the process to manufacture FPP such as maltodextrin, carrot powder, rice protein and mango puree, have been commonly consumed prior to 1958 or previously recognized as GRAS and thus meet the standard for generally recognized as safe ingredients. The antifoam used in the production of FPP is made from vegetable oil, which is a safe food processing aid. Antifoam agent is considered a secondary direct food additive according to the Code of Federal Regulations, Title 21, and is compliant with 21 CFR 173.340. These raw materials are substantially degraded or removed during the production process and residues are either not expected or at the lowest possible quantities having no function in the final product.

6.2 Safety of *L. edodes* (Shiitake) Mycelia Fermentation Organism

The strain of shiitake (*L. edodes*) used to produce FPP was originally obtained from Pennsylvania State University and grown in aqueous culture as the vegetative form (shiitake mycelia). The relative amount of inactive shiitake mycelia in FPP is estimated at <0.1%. Based on the weight-of-evidence evaluation described below, including bridging to the safety and historical consumption of the fruiting bodies of shiitake mushrooms (*L. edodes*), MycoTechnology concludes that the use of *L. edodes* (shiitake) mycelia in the fermentation of FPP is safe.

6.2.1 Historical Consumption of *L. edodes* (Shiitake Mushroom)

The fruiting bodies of *L. edodes*, also known as shiitake, are a commonly consumed food. As a source of diverse secondary metabolites, fungi have a long history of use in both culinary and medicinal applications (VanderMolen et al., 2017). The shiitake mushroom is the second most widely produced mushroom in the world (Bisen et al., 2010). The world mushroom industry markets more than 2 million tons of mushrooms per year and is still expanding (Nakamura, 1992).

Mushrooms have nutritional value since they contain protein (~2.26 % protein), providing essential amino acids, and fiber (Finimundy, 2014). Edible mushrooms are a high nutritional quality food and have been used as an alternative to dietary protein in countries with high malnutrition rates (Finimundy, 2014, Canadian Nutrient File for Shiitake Mushroom, Food code 6904). The chemical and nutritional characteristics of mushrooms vary in function after harvest, and processing (Finimundy, 2014).

In a review of the nutritional compounds found in *L. edodes*, Finimundy (2014) reported that the dietary fiber present in *L. edodes* consists of soluble and insoluble fractions. Water-soluble β -glucans and

proteins are found in the soluble fraction. In the non-soluble fraction, polyuronide (acidic polysaccharide), hemicellulose, β -glucan chains with hemicellulose, lignin, and chitin are found. *L. edodes* provides a nutritionally significant content of vitamins B₁, B₂, B₁₂, C, D, and E. The aroma components include alcohols, ketones, sulfides, alkanes, and fatty acids. The main constituents which are volatile include matsutakeol (1-octen-3-ol) and ethyl, n-amyl ketone (Finimundy, 2014). The characteristic aroma of shiitake was identified as 1,2,3,5,6-Pentathiepane (Finimundy, 2014). *L. edodes* mycelium are composed of glycoproteins containing glucose, galactose, xylose, arabinose, mannose, and fructose (Coates, 2010).

6.2.2 Similarity of Shiitake Mushroom and Shiitake Mycelia Composition

The life cycle of mushrooms starts with a spore which produces a primary mycelium. When the mycelium originating from two spores mates, a secondary mycelium is produced. This mycelium continues to grow vegetatively. When vegetative mycelium has matured, its cells are capable of a phenomenal rate of reproduction which culminates in the development of the mushroom fruitbody. The fruitbody represents the last functional change in the mushroom life cycle and it is tertiary mycelium. The entire mushroom is composed of compressed mycelia (Stamets & Chilton, 1983). The shiitake mushroom is largely made up of bundles of mycelia composing the pileus (cap) and stalk, and having only a small portion of tissue, located underside of the mushroom cap that differentiates into gills (lamella) to produce spores (basidiospores) for reproduction of the shiitake organism. Thus, shiitake mushroom itself, aside from gill tissue on undersides of caps producing spores, is, physically indistinguishable from its parent mycelia (Stamets & Chilton, 1983; Liu et al., 2016). From this information, MycoTechnology, Inc. concludes that the shiitake mycelia and shiitake mushroom compositions are physically very similar, and the safety demonstrated for shiitake mushroom is directly applicable to shiitake mycelia.

Van der Molen et al. (2017) (discussed further below in § 6.3.3) reported on a comparison of 1:1 methanol-chloroform (MeOH-CHCl₃) extracts of culinary mushrooms and identified similarity of 98% in the composition of shiitake mycelia culture to grocery store shiitake mushrooms using ultrahigh-performance liquid chromatography-photodiode array-evaporative light scattering-high resolution mass spectrometry (UHPLC-PDA-ELS-HRMS) analysis, confirming that the mycelium were not substantially different from the fruiting bodies used as food. The polarity of 1:1 MeOH-CHCl₃ is such that most organic soluble molecules are extracted efficiently. Total unevaluated peak area was 2%. In another approach, shiitake fungal raw material (i.e. mycelia) extract was subjected to a targeted UHPLC-PDA-HRMS/MS protocol that screened for the presence of cytotoxins and mycotoxins from a database of over 300 fungal secondary metabolites (El-Elmat et al., 2013 as reported in Van der Molen et al., 2017). The shiitake fungal raw material extract yielded matches for fungal metabolites from this database based on retention time, UV data, HRMS data, and MS/MS data. The cytotoxic metabolite ergosterol peroxide was detected in the shiitake fungal raw material extract, and in the store-bought culinary shiitake mushroom extract. MycoTechnology, Inc. concludes that the shiitake mycelia and shiitake mushroom compositions are virtually identical and the safety demonstrated for shiitake mushroom is directly applicable to shiitake mycelia.

Song et al. (2018) reported on the differential expression of 11,675 total genes known to shiitake and identified that 9,595 of these are not differentially expressed between mycelia and fruit body. There is an approximately an 82% identity in expression activity between shiitake mycelia and shiitake fruiting body tissue. While Song et al. (2018) reported that gene expression levels differ, the authors attribute the differential expression to overexpression of genes in the mature fruiting body stage (the mushroom)

related to “DNA replication, recombination, repair, chromatin structure, and the associated dynamics” and the transcripts from the fruiting body are “significantly enriched in ‘replication and repair’ and ‘transcription’ pathways for premeiotic replication, karyogamy, or meiosis.” The differential expression reported by Song et al. (2018) appears to be primarily related to the reproductive activity related to shiitake fruiting in the fruiting body, which does not occur for the shiitake mycelia. MycoTechnology, Inc. therefore concludes that the differences in gene expression between shiitake mycelia and mature fruiting body tissues of the shiitake mushroom are of little to no consequence to the safety of consumption of shiitake mycelia.

6.2.3 Absence of Fungal Toxins in FPP

Cultivation of shiitake mycelia in solid culture to produce shiitake mushrooms as culinary mushrooms for use in food is a well-known practice (Van der Molen et al., 2017). Shiitake culture is grown in solid-state as mycelial tissue (“spawn”) usually on grain or wood chips. After running out of nutrient substrate, the mycelial tissue fruits mushrooms (basidiocarps) which produce spores (basidiospores) (Przybylowicz & Donoghue, 1988). Neither shiitake mushrooms nor shiitake mycelia are known to produce mycotoxins during the growth of the mycelia or during the fruiting phase (production of mushrooms) (Han et al., 2014).

Shiitake mycelia may also be grown in aqueous culture. In aqueous culture, shiitake mycelia are not known to produce mushrooms, instead propagating as mycelia only (Tsivileva et al., 2005; Aminuddin et al., 2013; Aminuddin et al., 2007). During growth of shiitake mycelia in aqueous culture, no known mycotoxins were produced (Van der Molen et al., 2017; EFSA, 2010). An exhaustive literature search also failed to identify any scientific report in which *L. edodes* or closely related fungal species (*Schizophyllum commune*, *Gymnopus luxurians*) have been associated with the production of mycotoxins or other toxic compounds. Inspection of the *L. edodes* genome identified a total of 32 metabolite gene clusters, none of them seem to be involved in the production of known fungal toxins (Chen et al., 2011).

As described in GRN 848, an analysis of organic compounds was performed on pea and rice protein fermented by shiitake mycelia compared with a sham fermentation control (pea and rice protein subjected to identical processing as FermentIQ™ protein but lacking a shiitake mycelia inoculation step) using LCMS-APCI-QTOF (MycoTechnology; unpublished data on file). This analysis did not identify the presence of any fungal toxins, corroborating the findings of the literature search discussed above. The pea and rice protein fermented by shiitake mycelia was further evaluated via comparison to National Biotechnology Center for Information (NCBI)’s databases “NCBI Fungi” and “NCBI green plant” (MycoTechnology; unpublished data on file). The results showed that less than 1% of the sample matched the identity of a fungal protein according to the database, and no toxic fungal proteins were identified.

From this information, MycoTechnology, Inc. concluded that shiitake mycelia grown under the conditions described under the manufacturing conditions as described in section 2.2 is not expected to produce mycotoxins or toxic metabolites during the production of FPP.

6.2.4 Safety of Consumption of Shiitake Mycelia

6.2.4.1 Safety of Consumption of Shiitake Mycelia at Estimated Dietary Exposures in FPP (Van der Molen et al (2017))

The estimated dietary exposure to shiitake mycelia from consumption of FPP at conservatively estimated mean and 90th percentile levels are 22 mg/person/day and 54 mg/person/day, respectively (see section 3.3). The safety of this exposure level for shiitake mycelia was evaluated using a weight-of-evidence approach as described by Van der Molen et al. (2017) for the safety assessment of mushrooms in dietary supplements by combining analytical data with *in silico* toxicology evaluation.

Van der Molen et al. (2017) assessed the safety of seven fungal raw materials including shiitake (*L. edodes*) consisting primarily of mycelium. Consumption of shiitake mycelia in dietary supplements at a maximum dose of 1,500 mg and a median dose of 50 mg was evaluated by a decision tree driven weight-of-evidence approach consisting of five key principles as outlined below. MycoTechnology has also addressed these five key principles in its weight-of-evidence approach to the safety assessment of the shiitake mycelia used to produce FPP.

- 1) Identification by sequencing the nuclear ribosomal internal transcribed spacer (ITS) region (commonly referred to as ITS barcoding)
 - Van der Molen et al. (2017) verified, by ITS barcoding, that an obtained fungal raw material analyzed was shiitake mycelia
 - MycoTechnology similarly verified, by ITS barcoding, that the microorganism used in the manufacturing process to produce FPP is *L. edodes* which is grown in aqueous culture as the vegetative form (mycelia) (see section 2.2.1.2)
- 2) Screening an extract of each fungal raw material against a database of known fungal metabolites
 - Van der Molen et al. (2017) screened the shiitake mycelia 1:1 MeOH-CHCl₃ extract against a database of 300 known cytotoxic metabolites. Table 9 of Van der Molen et al. (2017) confirms that shiitake mycelia in commerce produced no unique detectable toxins.
 - MycoTechnology similarly confirmed that no unique fungal toxins were detected in FPP (see Section 6.2.3 below).
- 3) Similarity of the shiitake mycelia extract to culinary mushroom extracts
 - Van der Molen et al. (2017) performed UHPLC-PDA-ELS-HRMS analysis to assign individual peaks for the shiitake mycelia extract a percent similarity or difference to grocery store-bought shiitake culinary mushroom. Van der Molen et al. (2017) showed that there was a 98% similarity of shiitake mycelia to grocery store-bought shiitake mushrooms, per UHPLCPDA-ELS-HRMS analysis (2% unevaluated) and that the shiitake mycelia is “[c]hemically very similar to food.” (see Van der Molen et al., 2017; Table 9).
 - As described above (Section 6.2.2), MycoTechnology concludes that the shiitake mycelia used to produce FPP is substantially equivalent to shiitake culinary mushrooms (fruiting body).
- 4) Review of the toxicological and chemical literature for each fungus
 - Van der Molen et al. (2017) performed a literature review of the current toxicological and chemical literature for shiitake mushrooms. The authors concluded that shiitake has a long history of use as food (fruiting body) and numerous toxicological studies were available showing minimal toxicity.
 - MycoTechnology also performed a review of the available literature regarding the safety of shiitake mycelia (see section 6.2.4.2, below). MycoTechnology concluded that the

available literature supports the safety of shiitake mycelia for use in the production of FPP.

5) Evaluation of data establishing presence in-market.

- Van der Molen et al. (2017) reviewed in-market data using the Dietary Supplements Labels Database (DSLDB) maintained by the National Institutes of Health (NIH) for a total of 223 shiitake products with the most common ingredients being “shiitake” (98 products) and “shiitake Mushroom” (43 products). Most products did not report the dose of the ingredient, instead listing only the dose of a proprietary blend in which shiitake was included (assumed to be shiitake mycelia since commercial fungal raw materials predominantly consist of mycelia because of its rapid growth characteristics compared to the mushroom / fruiting body). The maximum dose was 1500 mg, and the median dose was 50 mg.
- MycoTechnology concluded that the current market use of shiitake mycelia in the form of a dietary supplement supports the safety of shiitake mycelia at a lower range of exposure levels in FPP (i.e. mean and 90th percentile levels are 22 mg/person/day and 54 mg/person/day, respectively). In contrast to shiitake mycelia dietary exposure in the form of a dietary supplement, in FPP the shiitake mycelia is dispersed, i.e. it is intermixed with all other components (primarily pea protein). In both cases, the dietary exposure results from a heat-treated and killed shiitake mycelia.

Based on the above analysis, Van der Molen et al. (2017) concluded that:

“Shiitake and Maitake are commonly eaten as foods, and shiitake, at least, has a wealth of available toxicological data supporting its safe use. The apparent prevalence in the marketplace, the lack of reported adverse events, as determined by the literature review and the very high degree of similarity between their mycelial growths (the raw materials investigated) and the culinary fruiting bodies to which they were compared give confidence that these materials are safe for consumption at doses consistent with dietary intakes of culinary mushrooms.”

MycoTechnology, using the weight-of-evidence safety assessment approach of Van der Molen et al. (2017) concluded that a dietary exposure to shiitake mycelia of 50 mg to 1,500 mg (assumed daily) is safe, considering the existing history of use and available toxicological data indicating no greater risk than culinary mushrooms (Van der Molen et al., 2017). Of note, approximately 700 branded shiitake mushrooms products are available in the US market indicating widespread culinary use and consumption (USDA, 2022). Using the approach described by Van der Molen et al. (2017), including ITS barcoding results, demonstrated absence of fungal toxins, confirmed similarity of shiitake mycelia to culinary mushrooms, a review of toxicology and clinical safety literature, and comparison to in-market use of dietary supplements containing shiitake ingredients, MycoTechnology, Inc. also concluded that the dietary exposure to shiitake mycelia in FPP at lower exposure levels is safe.

6.2.4.2 Safety Assessment of *L. edodes* (Shiitake) Mycelia

In order to assess the safety of oral intake of *L. edodes* mycelia used to produce FPP, a comprehensive search of the scientific literature through March 2022 was conducted using the U.S. National Library of Medicine (NLM) PubMed and TOXLINE databases. Search terms to identify relevant literature on the mycelia included “*lentinula edodes*” / “shiitake mushroom” AND “mycelium” / “mycelia”. Search terms to identify relevant literature on the fruiting body included “*lentinula edodes*” / “shiitake mushroom” and the additional keywords (PubMed search only) “safety” / “toxicity” / “carcinogenicity” / “genotoxicity” / “adverse effect” / “tolerability” / “consumption” / “allergen” / “allergy”. Relevant literature regarding the safety of dietary consumption of shiitake mycelia is discussed below. Compared to the literature review summarized in GRN 848 (search performed in January 2019), the recent search identified one publication reporting a lack of reproductive or embryofetal developmental effects following oral dosing of *L. edodes* powder (Camargo et al., 2020; summarized below), and several publications reporting human case reports of flagellate dermatitis from shiitake mushroom consumption (references cited below) which is considered very unlikely to occur with FPP consumption due to heat treatment steps during the manufacturing process and low dietary exposure to shiitake mycelia in FPP. Therefore, the previous conclusions regarding the safety of *L. edodes* mycelia (as outlined in GRN 848) remain valid and support the current GRAS conclusion for FPP.

Yoshioka et al. (2010) assessed the safety of an aqueous suspension of a powdered extract of *L. edodes* mycelia (L.E.M.) when administered to male and female Wistar rats (10 animals/sex/group) via gavage at 2,000 mg/kg bodyweight/day (single dose level evaluated) for 28 days. The study was performed according to OECD testing guideline 407. Cultured *L. edodes* mycelia together with the solid medium were extracted with hot water (temperature not reported) and the L.E.M. extract was prepared by filtration, concentration, sterilization and lyophilization of the raw extract. Although an L.E.M. extract does not contain insoluble portions of shiitake mycelia cells, and is therefore not identical in composition to the shiitake mycelia present in FPP, both the L.E.M. extract and shiitake mycelia present in FPP will contain the same water-soluble (presumably bioavailable) components. Thus, the Yoshioka et al. (2010) L.E.M. extract repeated-dose toxicity data are relevant to the safety assessment of shiitake mycelia present in FPP.

Yoshioka et al. (2010) did not report any unscheduled deaths or clinical signs suggesting toxicity. Body weight and food consumption were slightly decreased () compared to the control groups, particularly for males. The lower body weights were statistically significant at Day 14 through 28 for males and Day 7 through 11 for females. Lower food consumption was statistically significant at Day 0 through 21 for males and not statistically significant for females. At the study termination, male body weights were only 8% less than control groups (associated with slightly [not statistically significant] decreased food consumption) and female body weights were only 5% less than control groups. These minor differences were not considered adverse. None of the hematological parameters were statistically significantly different from respective controls after the 28-day dosing phase. Serum biochemistry revealed very few statistically significantly different parameters compared to respective controls, including increased phosphorus in both sexes; however, all values were reported as being within the laboratory’s normal reference ranges. Although females had slightly increased organ weights relative to bodyweight (thyroid gland, kidneys, adrenals, uterus/ovaries) as did males (thyroid gland, adrenals), these differences were minor and without histopathological correlates. There were no pathological alterations in any examined tissues or organs. The no observed adverse effect level (NOAEL) of L.E.M. extract determined in this study was 2,000 mg/kg/day, the only dose tested. This rat NOAEL is the

equivalent to a dietary exposure of 120 g of L.E.M. extract per day for a 60 kg human. As discussed above in section 3.3, the worst-case estimated dietary exposure of shiitake mycelia from FPP consumption for proposed uses, at the 90th percentile is 54 mg per day (or 0.9 mg/kg bw/day). Therefore, a more than 2222-fold difference exists between the estimated dietary consumption of shiitake mycelia in FPP and L.E.M. extract tested in Yoshioka et al. (2010). MycoTechnology, Inc. concludes that this 28-day oral repeated-dose study with Wistar rats provides an adequate Margin of Safety for FPP at the estimated levels of consumption.

Yoshioka et al. (2009) evaluated the safety of foods containing an extract of cultured *L. edodes* mycelia (L.E.M.) in healthy adult volunteers. The publication is in Japanese with a limited English abstract, so details of the methods and results are difficult to discern. Yoshioka et al. (2009) evaluated a lyophilized hot water extract of *L. edodes* mycelia (LEM) material administered to subjects in granular food. Although an L.E.M. extract will not contain insoluble portions of shiitake mycelia cells and is therefore not identical in composition to the shiitake mycelia present in FPP, both the L.E.M. extract and shiitake mycelia present in FPP will contain the same water-soluble components. Thus L.E.M. extract clinical data is useful to evaluate the safety of FPP. Eleven healthy subjects (8 males and 3 females, ages 33.4 ± 9.4 years) consumed the test foods containing 5,400 mg L.E.M. extract per day for 4 weeks. No adverse effects were reported, except for mild gastrointestinal symptoms such as soft stool in one subject who had a “hypersensitive” intestine. The authors concluded that food containing L.E.M. extract is safe for healthy adults at up to 5,400 mg per day. As discussed above in section 3.3, the estimated dietary exposure of shiitake mycelia from FPP consumption at the 90th percentile of intake is 54 mg/day. This dietary intake would be about 100-fold less than the dietary exposure to L.E.M. extract administered daily for 4 weeks in Yoshioka et al. (2009). From this information, MycoTechnology, Inc. concludes that the Yoshioka et al. (2009) study provides an adequate Margin of Safety for shiitake mycelia present in FPP.

Additional human clinical studies addressing the safety of shiitake mycelial extract are summarized in Table 6.2.5-1. Although these studies were performed to assess the possible therapeutic effects of shiitake mycelial extract on quality of life and immune function, no adverse events from treatment with shiitake mycelial extract were reported. Therefore, these studies support a conclusion of safety for shiitake mycelia present in FPP. The worst-case theoretical estimate of dietary exposure to shiitake mycelia from FPP consumption (i.e. 54 mg/day at the 90th percentile of intake) is more than 33-fold lower than the doses of *L. edodes* mycelia extract (L.E.M.) that were evaluated for effects on immune function (i.e. 1800 mg/day [Okuno, 2011; Nagashima et al., 2013 & 2017; Suzuki, 2013], and the heat treatment steps during the manufacturing process of FPP protein are expected to render any immunomodulatory compounds inactive. Therefore, MycoTechnology, Inc. concludes that the putative or potential bioactive constituents of *L. edodes* are not a safety concern under the intended conditions of use of FPP.

Table 6.2.4.2-1. Shiitake Mushroom Mycelia, Safety Evidence from Human Studies

| Study Title (reference) | Study Design | Study Details | Results & Conclusions |
|---|---|--|--|
| Consuming <i>L. edodes</i> (Shiitake) Mushrooms Daily Improves Human Immunity (Dai, 2015) | A randomized dietary intervention study; to determine whether consumption of whole, dried <i>L. edodes</i> (shiitake) mushrooms could improve human immune function. | Fifty-two healthy males and females (21-41 years), participated in a 4 weeks parallel group study, consuming either 5 or 10 g of shiitake mushrooms daily. | Conclusion: Dosage was well tolerated. Safety and adverse events were not reported in the study. |
| Safety of orally administered <i>L. edodes</i> mycelia extract for patients undergoing cancer chemotherapy: a pilot study. (Yamaguchi, 2011) | Observational study to investigate safety of <i>L. edodes</i> on quality of life (QOL) and the immune response in patients undergoing cancer chemotherapy. | Seven patients were studied in total. The patients were undergoing post-operative adjuvant chemotherapy for breast cancer (n = 3) or gastrointestinal cancer (n = 2), or were receiving chemotherapy to prevent recurrence of gastrointestinal cancer (n = 2). The first course of treatment was chemotherapy alone and the second was chemotherapy plus concomitant administration of <i>L. edodes</i> extract. Outcome measures: Adverse events and changes in the QOL score were evaluated during the study period. | Conclusion: Treatment with <i>L. edodes</i> extract with chemotherapy is safe and no adverse events were attributable to <i>L. edodes</i> extract. |
| Oral Administration of <i>L. edodes</i> Mycelia Extract for Breast Cancer Patients Undergoing Postoperative Hormone Therapy. (Suzuki, 2013) | This was a 12-week, single-arm, open-label study. All subjects first entered a 4-week observation period, followed by an 8-week period of oral <i>L. edodes</i> extract | This study investigated the influence of <i>L. edodes</i> on the quality of life (QOL) and immune response in breast cancer patients undergoing postoperative adjuvant hormone therapy. | Conclusion: No subjects reported any serious adverse events. Safety of oral administration of <i>L. edodes</i> Mycelia Extract was supported by this study. |

| Study Title (reference) | Study Design | Study Details | Results & Conclusions |
|--|---|--|---|
| | <p>ingestion at 1800 mg daily.</p> <p>Preparation: <i>L. edodes</i> mycelia were cultivated in a solid medium composed of sugar-cane bagasse and defatted rice bran. Medium containing the mycelia was incubated in hot water, and then the soluble fraction was dried and used as <i>L. edodes</i> extract.</p> | <p>Twenty patients* were studied in total. They received only hormone therapy in the first 4 weeks followed by hormone therapy and <i>L. edodes</i> (1800 mg/day) during the next 8 weeks.</p> <p>*As subjects are breast cancer patients, this suggests strongly that all subjects are female.</p> | |
| <p>Efficacy of Orally Administered <i>L. edodes</i> Mycelia Extract for Advanced Gastrointestinal Cancer Patients Undergoing Cancer Chemotherapy: a Pilot Study.</p> <p>(Okuno, 2011).</p> | <p>This study was conducted as an 8-week single-group open label study. During the study period, each subject took two courses of chemotherapy. <i>L. edodes</i> extract was orally ingested during the second course at a dose of 1800 mg/day for 4 weeks.</p> <p>Preparation: <i>L. edodes</i> mycelia were cultivated in a solid medium composed of sugar-cane bagasse and defatted rice bran. Medium containing the mycelia was incubated in hot water, and then the soluble fraction was dried and used as LEM</p> | <p>This study investigated the influence of <i>L. edodes</i> mycelia extract (LEM), an oral immunomodulator, on immune function and adverse events from chemotherapy. Subjects comprised 1 gastric (male) and 7 colorectal (5 females, 2 males) cancer patients. Ages ranged from 52 to 71. The first course of treatment was chemotherapy alone and the second was chemotherapy plus concomitant administration of LEM. Adverse events and interferon (IFN)-γ production by CD4+ T, CD8+ T and CD56+ NK/NKT cells were evaluated at the end of each course.</p> | <p>Conclusion: Concomitant use of <i>L. edodes</i> Mycelia Extract with chemotherapy can decrease the incidence of adverse effects from cancer chemotherapy among patients with advanced cancer. Safety of <i>L. edodes</i> is supported by this study.</p> |

| Study Title (reference) | Study Design | Study Details | Results & Conclusions |
|--|--|--|--|
| <p>Dietary supplementation with rice bran fermented with <i>Lentinus edodes</i> increases interferon-γ activity without causing adverse effects: a randomized, double-blind, placebo-controlled, parallel-group study.</p> <p>(Choi, 2014)</p> | <p>A randomized, double-blind, placebo-controlled, and parallel-group investigated the hypothesis that dietary supplementation with rice bran fermented with <i>Lentinus edodes</i> (rice bran exo-biopolymer, RBEP), a substance known to contain arabinoxylan, enhances natural killer (NK) cell activity and modulates cytokine production in healthy adults.</p> | <p>Dosage: 80 healthy (non-pregnant/lactating adults, aged 25-70 years old comprised of 31 females and 49 males) participants were randomly assigned to take six capsules per day of either 3g RBEP or 3g placebo for 8 weeks.</p> | <p>Conclusion: This well designed RCT demonstrates the safety of rice bran fermented with <i>Lentinus edodes</i>. No adverse events were reported.</p> |
| <p>Evaluation of host quality of life and immune function in breast cancer patients treated with combination of adjuvant chemotherapy and oral administration of <i>L. edodes</i> mycelia extract</p> <p>(Nagashima et al., 2013)</p> | <p>Ten breast cancer patients with nodal metastases receiving surgery were enrolled in this study. This was an open-label trial with a single group. Subjects were treated with two courses of FEC75 chemotherapy for 3 weeks as one course. The first course comprised FEC75 chemotherapy alone, whereas the second course used LEM in combination with FEC.</p> | <p>Dosage: <i>L. edodes</i> mycelia extract (LEM; 1800 mg/day by mouth) was administered for 21 days during the second course.</p> | <p>The authors concluded that concomitant use of <i>L. edodes</i> mycelia extract with FEC75 therapy can maintain host QOL and immune function, and offer important implications for an application of LEM as a useful oral adjuvant to anthracycline-based chemotherapies. No adverse events associated with LEM treatment were reported.</p> |

| Study Title (reference) | Study Design | Study Details | Results & Conclusions |
|---|--|--|--|
| <p><i>L. edodes</i> mycelia extract plus adjuvant chemotherapy for breast cancer patients: Results of a randomized study on host quality of life and immune function improvement.</p> <p>(Nagashima et al., 2017)</p> | <p>A randomized double-blind study was conducted to evaluate the effectiveness of <i>L. edodes</i> mycelia extract (LEM), which is an oral biological response modifier (BRM) medicine for cancer patients as such an adjuvant. A total of 47 breast cancer patients who were scheduled to receive postoperative adjuvant anthracycline-based chemotherapy were enrolled in the study.</p> | <p>Dosage: <i>L. edodes</i> mycelia extract (LEM; 1800 mg/day by mouth) was ingested daily over two 3-week courses, for a total of 6 weeks</p> | <p>The authors concluded that LEM appears to be a useful oral adjuvant for patients receiving anthracycline-based chemotherapy. No adverse events associated with LEM treatment were reported.</p> |

6.2.4.3 Safety of Fruiting Bodies (Mushrooms) at Estimated Levels of Consumption of FPP

The fruiting bodies of *L. edodes*, also known as shiitake mushroom, are a common food, particularly in Asia. Shiitake mushroom is the second most popular edible mushroom in the global market (Bisen et al., 2010). Relevant literature regarding the safety of dietary consumption of shiitake mushroom (fruiting body) is discussed below.

Pregnant Wistar rats (6/group) were gavaged daily with *L. edodes* powder (100 mg/kg bw/day) before implantation from gestation days 1 to 19 days or after implantation from gestation days 9 to 19 (testing guideline not reported). A control group received daily gavage doses of 0.9% saline. On gestation day 20, cesarean sections were performed, blood was collected and hematological parameters (hemoglobin, hematocrit, white and red blood cells and platelets) were analyzed. Additionally, albumin, calcium, creatine kinase, alkaline phosphatase, transferases, creatinine, urea, triglycerides, cholesterol, lipase, glucose, and insulin were assessed in serum. Organs were collected and weighed, and the fetuses were analyzed morphologically by body measurements. There were no changes in maternal weight, biochemical and hematological parameters, organ weight, or reproductive capacity (as assessed by preimplantation loss percentage, postimplantation loss percentage, offspring vitality percentage, fetus weight, placenta weight, placental index, and ovary weight), and no morphological changes in the fetuses' body measurements (Camargo et al., 2020).

Frizo et al. (2014) reported on the effects of reconstituted shiitake mushroom powder consumption in a rat developmental toxicity study at daily gavage doses of 400 mg/kg and 800 mg/kg (0.53 g β -glucan per 100 g mushroom) from the 1st to the 20th day of gestation (testing guideline not reported). Saline was administered to the control group. Only an abstract is available so additional details of the methods and quantitative results cannot be discerned. The fetuses were removed on the 21st day of gestation by caesarean section. Maternal kidney and liver toxicity were assessed and oxidative stress was determined by measurement of glutathione (GSH). The corpora lutea, implantations, resorptions, live and dead fetuses were counted. The placentae and fetuses were weighed. External and visceral morphological examinations of fetuses were performed following fixation with Bouin solution. Skeletal evaluations were performed following diaphonization and staining with alizarin red-S. Although there was an absence of maternal toxicity, the glutathione (GSH) plasma ratio was reduced at 400 and 800 mg/kg/day, suggesting antioxidant properties of shiitake mushroom at the relatively high dose levels used in this study. No changes were reported in urea plasma ratio [sic], creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). There was an increase in post implantation loss, reduced body weight and external measurements of fetuses. However, no visceral or skeletal abnormalities of the fetuses were reported. The estimated dietary exposure of shiitake mycelia in FPP is 54 mg/day at the 90th percentile of intake, corresponding to 0.9 mg/kg/day level of consumption at a mean body weight of 60 kg. The shiitake mushroom dietary exposure in this study is more than 444-fold higher than that from FPP. From this study, MycoTechnology, Inc. concludes that the level of dietary exposure to shiitake mycelia in FPP is significantly less than the dietary exposure levels of shiitake mushroom powder reported to show developmental toxicity in fetal rats following *in utero* exposure for 20 days, providing an adequate Margin of Safety.

Mus musculus NIH/S mice (n = 6/group) received to 0, 3, 6, or 9 g dry *Lentinus edodes*/kg body weight/day (fresh mushroom equivalents 19.4, 41.9, and 61.4 g/kg bw/day) as a dietary admixture (1.8%, 3.6% or 5.4% of feed) for 5 days (Nieminen et al., 2009). These were high doses, i.e. with human equivalent doses of fresh shiitake mushrooms for a 60 kg person being 1164, 2514, and 3684 g/day. Food and water consumption, plasma clinical chemistry and liver and muscle histopathology were evaluated.

Although there were statistically significant decreases of HDL/total cholesterol (mid- and high-dose groups), and increases of total protein (low-, mid- and high-dose groups), creatinine kinase (high-dose group) and total bilirubin (low-, mid- and high-dose groups) following 5 days of consumption in the diet, no adverse histopathological findings were reported. The worst-case estimated dietary exposure of 54 mg per day (0.9 mg/kg body weight/day) to shiitake mycelia (section 3.3) from consumption of FPP is approximately 3333 times less than the low-dose level of dry shiitake mushroom (i.e. 3 g/kg body weight/day) showing minimal toxicity in mice in the Nieminen et al. (2009) study, providing an adequate Margin of Safety.

Four groups (6/group) of male Wistar rats received dry and powdered *L. edodes* (shiitake mushroom) reconstituted in water at daily gavage doses of 100, 400, or 800 mg/kg for 30 days. Reductions in hemoglobin concentration and leukocytes were reported at 400 and 800 mg/kg compared to controls; only the leukocyte differences were dose-dependent. The authors concluded that the NOAEL of *L. edodes* determined in this study was 100 mg/kg. The human equivalent dose of *L. edodes* for a 60 kg person would be 6000 mg/day; therefore, the worst-case estimated dietary exposure of 54 mg per day (0.9 mg/kg body weight/day) to shiitake mycelia (section 3.3) from consumption of FPP is more than 111 times less than the safe daily intake level of *L. edodes* identified in this study. (Grotto et al., 2016).

Levy et al. (1998) reported the effects of ingestion of shiitake mushroom powder on eosinophilia, changes in eosinophil-active cytokines and eosinophil proteins in blood and stool, or gastrointestinal symptoms. In this study, 10 normal persons (9 males and 1 female; average age 40.6 years; range, 31 to 63 years) were studied. Exclusion criteria were a history of allergy to mushrooms, disease associated with significant eosinophilia, and gastrointestinal disease. Additional exclusion criteria included baseline blood eosinophil counts greater than 500/mm³, abnormal serum IgE levels, use of prescription medication (except oral contraceptives), and pregnancy. Four (4) g shiitake mushroom powder (open label) was ingested daily by each subject for 10 weeks (trial 1), and the same protocol was repeated in these subjects after 3 to 6 months (trial 2). The investigators defined responders as subjects having peak eosinophil counts four or more times their average baseline counts. Each trial had four responders, and trial 2 had one new and three repeat responders. Responders had increased blood eosinophils, serum major basic protein, stool eosinophil-derived neurotoxin, and factors that enhanced eosinophil viability. Anti-shiitake IgE was not detected, but anti-shiitake IgG was increased in two responders. Gastrointestinal symptoms coincided with eosinophilia in two subjects. Gastrointestinal symptoms and eosinophilia resolved after discontinuing shiitake ingestion. The authors stated that eosinophilic response to shiitake does not appear to be a typical allergic reaction because of the inability to detect anti-shiitake IgE and by the delayed and gradual time-course of the response. However, the response is likely immune-mediated because it is associated with cytokines that enhance eosinophil viability and elevations in anti-shiitake IgG in two of the five responders.

During a 10-week clinical study of the cholesterol-lowering effect of 4 g/day shiitake mushroom powder ingestion (Unpublished Personal Communication, D. Jacobson, J. O. Hill, University of Colorado, 1994 summarized by Levy et al., 1998), 17 of 49 subjects (no demographic details provided) in the treatment arm withdrew from the trial because of either rash (seven subjects) or abdominal discomfort (10 subjects). Two subjects had marked peripheral blood eosinophilia at the time they stopped ingesting mushrooms. However, their eosinophilia resolved after discontinuation of mushrooms.

Although the results in these clinical trials reported by Levy et al. (1998) show transient effects for consumption of shiitake mushroom powder at 4 g per day for 10 weeks, this intake level is at least 74-fold higher than the 90th percentile daily estimated intake of 54 mg of shiitake mycelium from FPP

dietary consumption (§ 3.3). Therefore, MycoTechnology, Inc. concludes that similar adverse effects from FPP consumption are highly unlikely.

Nguyen et al. (2017) reported on the results of their review of published studies on the clinical features of shiitake dermatitis. They identified 50 total reported patient cases (38 males, 12 females; mean age: 44.58 years) of this “rare” cutaneous reaction and noted that “the majority” of cases resulted after consumption of raw mushrooms (93% of cases were associated with raw, lightly or undercooked mushrooms; Table 2). They further note that shiitake dermatitis “is self-limiting, resolving in approximately 12.5 d without treatment.” Additional case reports of flagellate dermatitis after ingestion of shiitake mushrooms were reported by Balasuriya and Goel (2021), Browning et al. (2021), Gomez et al. (2021), Albuscheit et al. (2020), Heer et al. (2020), Mills and Walker (2020), Mulhall et al. (2020), and Ribeiro et al., (2019). Nguyen et al. (2017) postulated that a heat-labile beta-glucan in the cell walls of shiitake mushrooms (lentinan) may be responsible for the clinical dermatitis. Corazza et al., 2015 (cited in Nguyen et al., 2017) reported a presumed association between lentinan exposure and dermatitis by demonstrating a cutaneous response to the consumption of shiitake mushrooms cooked at 100 °C but not to those cooked at 150 °C. Since lentinan would be expected to degrade at 150 °C, heat processing of shiitake mycelia would seem likely to minimize shiitake dermatitis from FPP. As noted in section 2.2, the FPP manufacturing process is concluded with thermal deactivation (65°C for 60 minutes) and heat treatment (80°C for 1 minute) before spray drying step (air inlet 250 °C; powder outlet 75 °C), which should be sufficient to denature and deactivate lentinan. MycoTechnology, Inc. concludes that the rare cutaneous shiitake dermatitis effect is very unlikely to occur with FPP consumption due to heat treatment steps during the manufacturing process, as well as due to the low dietary exposure to shiitake mycelia in FPP (54 mg per day at the 90th percentile of intake).

Additional human clinical studies reporting shiitake dermatitis are summarized in Table 6.2.4.3-1. MycoTechnology, Inc. concludes that the rare cutaneous shiitake dermatitis effect is very unlikely to occur with FPP consumption due to heat treatment steps during the manufacturing process, as well as due to the low dietary exposure to shiitake mycelia in FPP (54 mg per day at the 90th percentile of intake).

Miyaji et al. (2004) reported on the *in vitro* genotoxic and antigenotoxic effects of aqueous extracts of shiitake mushroom using the Comet assay with Hep-2 cells at high concentrations (0.5, 1.0, and 1.5 mg/ml) and three temperatures (4°, 22° and 60°C). They reported a “low level” of genotoxic activity at all aqueous extract test concentrations prepared at 22 ± 2 and 60°C and two concentrations (1.0 and 1.5 mg/mL) of extract prepared at 4 °C using the *in vitro* Comet assay. Since cytotoxicity data was not reported for this test and the validation status of the performing laboratory is unknown, the results of this study are of limited reliability. The International Workshop on Genotoxicity Testing (IWGT) has repeatedly concluded that cytotoxicity could be a confounder of Comet assay results, adding that Comet assay results are more reliable if obtained in laboratories with demonstrated proficiency (IWGT, 2015). It should also be noted that a standardized and validated regulatory testing guideline for the *in vitro* Comet assay is not available and the OECD guideline for the *in vivo* mammalian alkaline comet assay did not exist until recently (i.e. OECD Testing Guideline 489; Adopted 29 July 2016). Therefore, Miyaji et al. (2004) predated a standardized and validated Comet assay protocol. Furthermore, the Comet assay is not among the standard battery of *in vitro* tests for genetic toxicity assessment of food ingredients or pharmaceuticals recommended by regulatory authorities (e.g. ICH S2(R1), 2011; EFSA, 2012; FDA, 2007).

The same investigators have reported the possible “antigenotoxicity” effects of shiitake mushroom extracts via modulation of micronuclei induction after treatment with alkylating agents *in vitro* or *in vivo*

(de Lima et al., 2001; Miyaji et al., 2006). These studies are of limited relevance to the current safety assessment.

In an Ames test performed prior to standardized testing guidelines or Good Laboratory Practice (GLP) regulations, a crude ethanol extract of *L. edodes* was reported to have mutagenic activity on tester strains TA100 and TA1535, which are sensitive to base-pair substitutions (von Wright et al., 1982). As neither statistical analysis nor cytotoxicity data were reported, the results of this study are limited. Additionally, the crude ethanol extract used as the test article is not representative of the *L. edodes* mycelia used in the production of FPP. Based on this information, MycoTechnology, Inc. concludes that a genotoxic hazard is not likely to occur upon exposure to shiitake mycelia in FPP.

In its Scientific Opinion on the safety of “Lentinus edodes extract” (Lentinex®) as a Novel Food ingredient, the EFSA Panel on Dietetic Products, Nutrition and Allergies concluded that “owing to the fermentative production of the novel food ingredient [Lentinex®] from the mycelium and the final application of a heat-induced sterilization step, adverse effects reported after the consumption of the fruiting body of the shiitake mushroom are not considered relevant” (EFSA, 2010). MycoTechnology, Inc. similarly concludes that any adverse effects reported after the consumption of the fruiting body of the shiitake mushroom are not likely to occur upon exposure to low amounts (<0.1%) of heat-inactivated shiitake mycelia in FPP.

Table 6.2.4.3-1. Human Clinical Evidence of Shiitake Mushroom Dermatitis

| Study Title (reference) | Study Design | Study Details | Results & Conclusions |
|--|---|---|---|
| Shiitake (<i>Lentinus edodes</i>) dermatitis (Nakamura, 1992). | Retrospective study (from 1974 to 1991) to examine 51 patients with shiitake dermatitis due to the intake of half-baked raw shiitake. | Shiitake dermatitis in 41 men and 10 women (15-76 years) was analyzed retrospectively. Dosage: varies and not reported. | All patients (n=51) had truncal involvement of shiitake dermatitis. Extremities, neck, face and head were involved in decreasing order of frequency. No patients had digestive or nervous system symptoms, nor were the mucous membranes affected. Conclusion: Shiitake dermatitis can be avoided by eating sufficiently boiled raw shiitake. |
| Flagellate dermatitis after consumption of shiitake Mushrooms. (Czarnecka et al, 2014). | Case report, investigated Flagellate dermatitis occurrence in patients who had eaten shiitake mushrooms. | A 55-year-old German patient (male) was diagnosed with Flagellate dermatitis after eating shiitake mushroom at a restaurant. Dosage: not reported. | Examination revealed severely-itching parallel, striped whiplash-like infiltrated erythema with severe itching on the trunk and upper extremities. In addition, there were papulovesicles on urticarial erythemas on the shoulders. Conclusion: Flagellate dermatitis could be avoided by eating adequately cooked shiitake mushrooms. |
| Flagellate mushroom (shiitake) dermatitis and photosensitivity. (Hanada, 1998). | Case report, investigated Flagellate skin lesions in a patient after eating the mushroom <i>Lentinus edodes</i> . | A 44-year-old man was diagnosed with Flagellate dermatitis after eating shiitake mushroom. | This patient was diagnosed with flagellate skin lesions on his trunk after eating <i>L. edodes</i> . The patient also developed photosensitive lesions on skin exposed to sunlight . Analysis of the case histories of 94 Japanese |

| Study Title (reference) | Study Design | Study Details | Results & Conclusions |
|---|---|---|---|
| | | | <p>patients with shiitake dermatitis revealed that 44 (47%) cases developed dermatitis on the skin exposed to sunlight.</p> <p>Conclusion: Despite the high consumption of Shiitake mushrooms, the incidence of severe allergic reactions appears to be very low.</p> |
| <p>Systemic allergic contact dermatitis due to consumption of raw shiitake mushroom.</p> <p>(Kopp, 2009).</p> | <p>Case report, investigated the effect of raw shiitake mushroom (<i>Lentinus edodes</i>) on contact dermatitis.</p> | <p>A 52-year-old man who developed a generalized pruritic papulovesicular eruption 2 weeks after daily consumption of uncooked shiitake mushrooms. Prick-to-prick and scratch tests with uncooked mushrooms resulted in an eczematous reaction at 24 h that peaked at 72 h and persisted for 1 week.</p> | <p>This patient had systemic allergic contact dermatitis due to consumption of raw shiitake mushroom.</p> <p>Conclusion: Shiitake dermatitis could be avoided by eating adequately cooked or processed shiitake mushrooms.</p> |
| <p>Eosinophilia and gastrointestinal symptoms after ingestion of shiitake mushrooms.</p> <p>(Levy, 1998)</p> | <p>An open label, observational study; investigated whether ingestion of shiitake mushroom powder (freeze dried powder) induces eosinophilia or symptoms.</p> | <p>Dosage: Each capsule contained 250 mg of shiitake mushroom powder (freeze dried). 10 Subjects (9 men and 1 woman*, with an average age of 40.6 years, range of 31 – 63 years) ingested 4 grams (16 capsules = 4 medium sized mushrooms) of freeze dried shiitake powder daily for up to 10 weeks (trial 1) or 3 to 6 months (trial 2). *The woman was of child-bearing age and she was requested to use contraception to prevent pregnancy during the study.</p> | <p>Conclusion: At 4g per day of raw shiitake mushrooms some abdominal cramping and eosinophilia were reported.</p> |

6.2.5 Safety of Fungal Enzymes

The use of fungal enzymes to modify and improve food products is well established in the food industry. *Aspergillus*, a genus of filamentous fungus closely related to the filamentous fungus genus *Lentinula*, has been identified as a source of a number of enzymes used in industrial food processing applications, several of which are recognized as GRAS for use in food (Soares et al., 2012; FDA 2018a; FDA, 2018b). *L. edodes* is also known to secrete a number of these fungal enzymes with GRAS status, such as pectinase, cellulase, amylase, laminarinase (beta-glucanase), and xylanase (Mata et al., 2016; Soares et al., 2012). In particular, it is known that shiitake mycelia constitutively express laccases, and expression of laccases in shiitake mycelia may be upregulated or stimulated by the presence of lignin-derived phenols and/or polymeric lignin materials (Matjuškova et al., 2017). MycoTechnology concludes that there are no safety concerns associated with these enzymes commonly used for food processing that may be present during the manufacturing process for FPP.

Testing by MycoTechnology has confirmed the secretion of endogenous laccase during the manufacturing process for pea and rice protein fermented by shiitake mycelia, which is attributed to the improved organoleptic properties (via oxidation of volatile taste and aroma compounds) of the input pea and rice protein raw materials (see GRN 848). However, the manufacturing conditions used for concentration and spray-drying steps of the manufacturing process as described in section 2.2 are consistent with conditions that are known to denature and deactivate enzymes (i.e. the fermentation process is terminated at 65°C for 60 minutes followed by an evaporator/concentration step; a heat treatment step is carried out at 80°C for 1 minute, followed by spray drying (air inlet 250 °C; powder outlet 75 °C)). In GRN 848, testing for residual laccase enzyme activity in pea and rice protein fermented by shiitake mycelia after termination of fermentation was performed according to published methodology² and no residual laccase enzyme activity was detected (MycoTechnology; unpublished data on file). The production process for FPP is identical to pea and rice protein fermented by shiitake mycelia (less the input of rice protein as a primary raw material), therefore the result is applicable for FPP. From this information, MycoTechnology, Inc. concluded that any enzymes secreted by the shiitake mycelia during the manufacturing process are inactivated in the finished FPP product.

6.3 Allergenicity Assessment for FPP

MycoTechnology Inc. uses an allergen control program to ensure that the facility has evaluated processes and the premises to mitigate with proper use, storage and labeling any risk of allergen related food safety incidents. MycoTechnology Inc. also maintains a complete HACCP program including personnel training as it relates to allergens in its facility.

Although at least 170 foods have been reported to cause allergic reactions, there are only nine major food allergens – milk, egg, peanut, tree nuts, wheat, soy, sesame, fish and crustacean shellfish are responsible for most of the serious food allergy reactions in the US (FARE, n.d.; FDA CFSAN, 2022). It is estimated that up to 32 million Americans have food allergies, including 5.6 million children under the age of 18 (FARE, n.d.). Each year in the U.S., it is estimated that anaphylaxis to food results in 30,000 emergency room visits, 2,000 hospitalizations, and 150 deaths (USDA, 2019).

² <https://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-laccase.html>

MycoTechnology, Inc. acknowledges that FPP does not contain any of the nine food allergens (milk, egg, fish, crustacean shellfish, tree nuts, peanuts, soybeans, wheat, sesame) considered to be major food allergens under the U.S. Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). On April 23, 2021, the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act was signed into law, declaring sesame as the 9th major food allergen recognized by the United States. For a more extensive review of potential allergens which may be present in FPP please refer to Table 6.3-1

FPP is primarily composed of pea protein. The following discussion is limited to the potential allergenicity of pea protein.

Peas are part of a family of plants called legumes, which also include alfalfa, clover, beans, lentils, mesquite, carob, soybeans, peanuts, tamarind, and wisteria (Grains & Legumes Nutrition Council, 2017). Allergenic response to legumes may range from mild skin reactions to life-threatening anaphylactic reactions (Verma, 2013). Legumes have been reported to be a cause of food allergies, especially peanut allergy (Sicherer, 1999). Peanuts and soybeans are the major legume allergies in the United States, United Kingdom, and Japan, while lentils, chickpeas and pea allergies are more common in the Mediterranean area and India (Sanchez-Monge, 2004). Peanut and/or Tree Nut allergy affects approximately 1.1% of the general population, or about 3 million Americans (Sicherer, 1999). Legume cross-reactivity varies by region - while extensive cross-reactivity among lentil, chickpea and pea were reported in the Mediterranean area, only minimal cross-reactivity among legumes (mainly reported between peanut and soy) have been reported in North America (Abrams, 2015). The available information for allergenicity of pea proteins indicates that persons with peanut allergies may be sensitive to peas; however, allergic reactions to peas appear to be rare and may fluctuate among different populations. For example, Lavine and Ben-Shorshan (2019) described a Canadian pediatric case series in which 6 children presented with allergic reactions to foods that were confirmed to contain pea ingredients. Of the 6 patients, 4 had confirmed peanut allergy with either known clinical reactivity or with strongly reactive skin tests; 2 were able to eat peanuts and tree nuts freely. Pea protein allergy has not been extensively studied although the prevalence of allergy to pea protein in adults was reported to be identical to the prevalence of soy allergy according to FDA Food Safety Surveys (Messina and Venter, 2020). A search of the literature did not locate any additional information related to the prevalence of pea protein allergy in children. Additionally, it is expected that boiling or roasting decreases the IgE-binding capacity for legume allergens (Lavine and Ben-Shorshan, 2019); therefore, the heat treatment steps during the processing of FPP are also expected to decrease the potential for allergenicity of this ingredient.

The low anticipated allergenicity concern with pea protein in FPP can be mitigated by the listing of the common name of this product on a label, which is pea protein fermented by shiitake mycelia. Appropriate labelling by use of the common name of FPP does not hinder the safety and GRAS status that is the subject of this notification.

Table 6.3-1. Absence of Allergens in FPP

| Component | Present in the product | Present in other products produced on the same line | Present in the same plant |
|---|------------------------|---|---------------------------|
| 1. Barley, Rye, Oats | NO | NO | NO |
| 2. Celery (not including seeds) | NO | NO | NO |
| 3. Corn | NO | NO | NO |
| 4. Egg or egg product | NO | NO | NO |
| 5. Fish | NO | NO | NO |
| 6. Mille & Mille by-product | NO | NO | NO |
| 7. Monosodium Glutamate (MSG) | NO | NO | NO |
| 8. Peanuts or peanut products | NO | NO | NO |
| 9. Seeds (Poppy, Sunflower, Cottonseed) | NO | NO | NO |
| 10. Sesame Seeds | NO | NO | NO |
| 11. Shell Fish & Crustaceans | NO | NO | NO |
| 12. Soybean Oil (excluding refined soy oil) | NO | NO | NO |
| 13. Soybean (not including oil) | NO | NO | NO |
| 14. Sulphites (enter maximum ppm) | NO | NO | NO |
| 15. Tree Nuts | NO | NO | NO |
| 16. Wheat or wheat products | NO | NO | NO |
| 17. Gluten <10 ppm | NO | NO | NO |
| 18. Yellow 5 (Tartrazine) | NO | NO | NO |
| 19. Animal Fat | NO | NO | NO |
| 20. Grains containing gluten | NO | NO | NO |
| 21. Mustard | NO | NO | NO |
| 22. Lupin | NO | NO | NO |
| 23. Lactose | NO | NO | NO |

6.4 Safety Narrative Summary

Pea protein fermented by shiitake mycelia (FPP) is a product manufactured with fermentation technology composed of proven safe food ingredients with a long history of common use and regulatory acceptance in the worldwide food supply. MycoTechnology Inc. has determined the Generally Recognized as Safe (GRAS) status of FPP based on the following:

- FPP is manufactured within a British Retail Consortium (BRC) inspected facility under current Good Manufacturing Practices (cGMPs) and meets appropriate food grade specifications.
- The identity of FPP has been clearly defined and confirmed through scientific data and information.
- Pea protein, the main constituent of FPP, has been consumed for centuries through the consumption of peas and through the consumption of the protein products as affirmed GRAS (GRNs 581, 608, 788, 803, 804,851, and 948).
- All ingredients included in FPP, including shiitake mycelia, are concluded to be safe for use in food at inclusion levels and food categories proposed.
- No FPP raw materials are listed as major allergens according to Food Allergen Labeling and Consumer Protection Act of 2004 and Food Allergy Safety, Treatment, Education, and Research (FASTER) Act that expands the definition of major food allergen to include sesame.
- The fermentation organism used to produce FPP, *L. edodes* (shiitake mushroom), is commonly consumed as food and there are no identified hazards associated with the use of shiitake mycelia described herein.
- Following fermentation, the absence of live shiitake mycelia or fungal enzymes in the final FPP is achieved through multiple heat treatment steps and thermal deactivation.
- An estimated daily intake of FPP from intended uses was conservatively calculated for the U.S. population. In summary, the worst-case mean and 90th percentile intake of FPP for “all users” is estimated to be 22 g/person/day and 54 g/person/day, respectively.
- FPP will substitute for other protein sources in the diet, and thus will not increase the overall consumption of protein in the diet.
- The exaggerated, highly unrealistic protein consumption estimates from FPP at the 90th percentile of intake would not pose any human safety concern.
- The mean, but still exaggerated estimate of protein intake from FPP consumption is similar to or below current FDA and IOM recommendations for protein in the diet further supporting a conclusion of safety.
- The weight of evidence from reliable published toxicological and human clinical studies using the same or closely-related (e.g. Shiitake mycelial extracts, reconstituted powdered shiitake mushroom) test materials as those components included in FPP support a conclusion that no adverse health effects are expected at dietary intake levels which are proposed for FPP.

6.5 Conclusion of the GRAS Panel

At the request of MycoTechnology, Inc., a panel of experts, (the “GRAS Panel”), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, independently and collectively critically evaluated the information summarized in this GRAS dossier on the safety of the proposed uses of pea protein fermented by shiitake mycelia (FPP). The GRAS Panel also considered other published data and information deemed appropriate. The GRAS Panel consisted of Michael W. Pariza, Ph.D. (Chair), Joseph Borzelleca, Ph.D., and Madhusudan Soni, Ph.D.

Following its independent and collective critical evaluation of the available information, the GRAS Panel convened by telephone, summarized its findings, and unanimously concluded that the proposed uses of MycoTechnology’s pea protein fermented by shiitake mycelia, produced consistent with current Good Manufacturing Practice (cGMP) and meeting the food ingredient specifications described herein, are safe and GRAS based on scientific procedures.

The GRAS Panel opined that other qualified experts would concur with these conclusions.

The signed opinion of the GRAS Panel is provided as Appendix A.

Part 7: §170.255 List of Supporting Data and Information

All the references used in this GRAS including animal and human studies are generally available and listed below.

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Appendix A GRAS Panel Consensus Statement

Appendix B Measurement of Viable Mycelium in FPP

Appendix C Intake Assessment for FPP