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Supporting document 1

Risk and technical assessment – Application A1279

Lentinula edodes (shiitake mushroom) mycelia as a processing aid

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Executive summary

Food Standards Australia New Zealand (FSANZ) received an application from MycoTechnology, Inc. to vary the Australia New Zealand Food Standards Code (the Code) to permit the use of a fermented preparation of *Lentinula edodes* (shiitake mushroom; *L. edodes*) mycelia as a processing aid in the fermentation of pea and rice protein for the manufacturing of fermented pea and rice protein (FPRP).

The fermented preparation of *L. edodes* mycelia performs its technological purpose during processing of FPRP and does not perform its technological purpose in the food for sale, therefore functioning as a processing aid for the purposes of the Code.

Based on the risk assessment, there are no public health safety concerns associated with use of a fermented preparation of *L. edodes* mycelium as a processing aid. *L. edodes* has a long history of safe consumption as a food and the mycelium has been determined to be neither pathogenic nor toxigenic. Given the history of safe consumption as food, FSANZ considered that no toxicological, including genotoxicity studies, were required. A review of the available animal and genotoxicity studies from the public literature did not indicate any adverse effects. FSANZ did not consider such studies suitable to determine a no observed adverse effect level (NOAEL) or establish an acceptable daily intake (ADI).

Using a budget method approach, the dietary exposure assessment calculated the theoretical maximum daily intake (TMDI) of the processing aid to be 7.5 mg/kg bw/day. For the Australian and New Zealand population groups assessed, the mean and 90th percentile consumption of all mushrooms were estimated to be 200 – 600 mg/kg bw/day and 400 – 1400 mg/kg bw/day respectively. These results demonstrate that the exposure to the processing aid is well below the estimated consumption of mushrooms for the population groups assessed.

Overall, FSANZ concludes that there are no safety concerns from the use of a fermented preparation of *L. edodes* as a processing aid.

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1 Introduction

MycoTechnology, Inc. (MycoTechnology) is seeking to amend Schedules 3 and 18 of the Australia New Zealand Food Standards Code to include a fermented preparation of *Lentinula edodes* (shiitake mushroom) mycelia (referred to herein as *L. edodes* mycelia) as a processing aid. Specifically, the *L. edodes* mycelia is used for fermentation of pea and rice protein in the manufacture of fermented pea and rice protein (referred to herein as FPRP).

The FPRP was considered by the FSANZ Advisory Committee on Novel Foods[1]. The committee formed a view that FPRP was a non-traditional food and not a novel food. No safety concerns were identified. However, the Committee noted that the *L. edodes* appears to function as a processing aid and there is no current permission for *L. edodes* as a processing aid in the Code.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is a solely technological purpose and that the preparation achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate any potential public health and safety concerns that may arise from the use of the processing aid.

2 Food technology assessment

2.1 Identity of the preparation

The fermentation preparation that is the subject of the application is based on the mycelia[2] of a non-genetically modified shiitake mushroom, called *L. edodes.*

The applicant stated that the strain of *L. edodes* was originally obtained from Pennsylvania State University, ID No. WC 1008) and is not genetically modified.

A glycerol stock is produced by propagating the mycelia on a supporting media for growth before being stored at -80°C until required, which can be up to one year. The *L. edodes* fermentation is conducted using standard fermentation processes as explained in section 2.2. The glycerol stock is used to commence the staged fermentations, where nutrients are added to support growth during the fermentation steps. The final fermentation preparation is achieved when the appropriate biomass is reached for it to transferred as the fermentation aid to the secondary main fermentation to produce the FPRP. This final volume of the completed fermentation of *L. edodes* mycelia is the processing aid. It is not isolated and processed further but is used in situ as it is transferred to the fermenter tank as part of the inputs to assist in the main secondary fermentation.

The taxonomic information for the strain of the mushroom mycelia is provided in Table 1 (and more detail is provided on the microbiological assessment in section 3.1).

[1] Novel food - Record of views formed in response to inquiries | Food Standards Australia New Zealand

[2] Mycelia is the plural term of mycelium. Mycelium is a root-like structure of a fungus consisting of a mass of branching threads. For mushrooms, the fruiting body (i.e. the mushroom) can sprout from the mycelium which often grow underground.

Table 1: Taxonomic information for the Lentinula edodes

Kingdom	Fungi
Phylum	Basidiomycota
Class	Agarcomycetes
Order	Agaricales
Family	Ompalotaceae
Genus	*Lentinula*
Species	*edodes*
Strain	WC 1008

2.2 Manufacturing process

The *L. edodes* mycelia preparation is the result of a fermentation of the *L. edodes* (shiitake mushroom) mycelia. The production strain is grown under the conditions of submerged fermentation. Under these conditions *L. edodes* grows as its vegetative form, known as mycelia (Tsivileva et al., 2005; Aminuddin et al., 2007; Aminuddin et al., 2013).

Standard fermentation processes are used which involves multi-stage successive fermentations starting from the first fermentation of the initial glycerol stock of *L. edodes* mycelia in a small shake flash. The successive fermentations of larger volumes of 'seed development' fermentations build up to an appropriate amount of pure *L. edodes* mycelia biomass that can be subsequently used for the secondary fermentation of the pea and rice protein.

All stages of the manufacturing process are conducted in conformance with the USA Code of Federal Regulations, Title 21 section 117 "Current Good Manufacturing Practice, Hazard Analysis, And Risk-Based Preventative Controls for Human Food" (US FDA, 2022). The various fermentation substates and sources are appropriate and considered safe for food fermentations.

All the multi-stage fermentations are conducted using appropriate processes (e.g. time, temperature, agitation, air flow). They are also checked using normal fermentation parameters (e.g. pH, aerobic plate count, appearance and microscopy) to ensure compliance with process and quality requirements before the secondary fermentation with the pea and rice protein is commenced.

Because the *L. edodes* mycelia preparation is not isolated and analysed as a discrete preparation there are not detailed data on the chemical and physical properties or a specification for it.

However, what is known and analysed is the starting glycerol stock of the *L. edodes* mycelia which is discussed in the specification section below.

An internal method is used to demonstrate that there is no viable processing aid (*L. edodes* mycelium) in the final FPRP product

2.2.2 Specifications for identity and purity of the glycerol stock of *L. edodes* mycelia

The Code does not contain a relevant specification for *L. edodes* mycelia. Therefore, a specification is required.

As discussed in section 2.2 – Manufacturing process, the actual *L. edodes* mycelia preparation is not isolated as a standalone product. However what is known and identified as a standalone product is the initial glycerol stock of the *L. edodes* mycelia. It is therefore appropriate to consider and develop an identity and purity specification for this glycerol stock preparation.

A summary of this information along with analytical results for such a specification are provided within the application. An appropriate specification is provided in Table 2, to be added into the Code.

Table 2 Proposed specification for the glycerol stock of *Lentinula edodes* (Shiitake mushroom) mycelia

Parameter	Specification
Name of the species	*Lentinula edodes*
Arsenic	< 10 µg/kg
Cadmium	< 5 µg/kg
Lead	< 5 µg/kg
Mercury	< 5 µg/kg
Aerobic plate count	<10 CFU/g

2.3 Technological function and justification

The preparation of *L. edodes* mycelia is proposed by the applicant to be used as a processing aid, with its purpose as a fermentation aid, to improve the organoleptic properties of a subsequent fermentation of FPRP. These altered properties may be due to the action of the various enzymes (proteases and phytases, including specifically laccases) secreted from the fermented L. edodes mycelia preparation.

The organoleptic properties of volatile odours of the final protein preparations were assessed using olfactory techniques comparing the untreated protein preparations to the fermented preparations produced using the processing aid. Analytical methods using gas chromatography with mass spectroscopy (GC-MS) and GC with olfactory and Combined Hedonic Aroma Response Measurement (CHARM) were used and the results reported. As well as using analytical results, human sensory perceptions using a trained sensory panel were also used.

These two approaches supported the conclusions that the fermentation with the preparation of *L. edodes* mycelia reduced some of the unpleasant off-odours of the untreated pea and rice protein preparations. There was a decrease in the earthy, beany, potato and mustard off-odours with fatty and musty notes increased. Overall there was an improvement in the odour of the fermented pea and rice protein preparation compared to the unfermented product.

Additionally the fermented *L. edodes* mycelia preparation used in the subsequent fermentation of pea and rice protein produced improvements in digestibility, reduced the

concentration of antinutrient compounds phytates and protease inhibitors, and solubility (Clark et al., 2022).

2.3.1 Allergen considerations

Information provided as Confidential Commercial Information on the manufacturing process to produce the fermented *L. edodes* mycelia preparation did not indicate the likely presence of allergens that require mandatory labelling due to the Code requirements within Standard 1.2.3. Testing on the rice and pea protein products showed that the levels of gluten (wheat, rye and barley), peanut and soy were below the limit of detection. It is also noted that the processing aid is not sold as such but is used to produce the final pea and rice protein preparations.

The applicant noted that the final fermented pea and rice protein ingredient has been sold in many countries for a number of years without any reports of allergenicity.

2.4 Food technology conclusion

FSANZ concluded that the applicant's preparation of a fermentation of *L. edodes* mycelia that is subsequently used in the fermentation of pea and rice protein raw material concentrate is technologically justified as a processing aid.

It's function is as a fermentation aid, and it does not have any further technological purpose in the final pea and rice protein preparation. The purpose of the final fermentation is to improve the organoleptic and other additional properties of the pea and rice protein product which is used as an ingredient to be added to other foods.

The method of production of the processing aid preparation is conducted using closed fermentation systems and standard fermentation processes. It involves multi-stage successive fermentations of a primary culture of *L. edodes* mycelia to build up to an amount of pure *L. edodes* mycelia biomass that can be subsequently used for the secondary fermentation of the pea and rice protein.

Since it is produced and used in situ, and not as an isolated product that can be analysed, it was impractical to draft a specification for the preparation. Therefore, it is proposed to include a specification for the glycerol stock of the *L. edodes* mycelia in the Code.

3 Safety assessment

3.1 History of use

L. edodes is the second most cultivated edible mushroom in the world and an important medicinal fungus (Yu et al., 2021). The strain of *L. edodes* used in the fermented preparation was originally obtained from Pennsylvania State University (ID No. WC 1008), and is not genetically modified. The production strain was genotyped and its species identity confirmed as *L. edodes* by internal transcribed spacer (ITS) sequencing data (28S DNA).

The production strain is grown under the conditions of submerged fermentation. Under these conditions *L. edodes* grows as its vegetative form, known as mycelia (Tsivileva et al., 2005; Aminuddin et al., 2007; Aminuddin et al., 2013). It has been reported that *L. edodes* mycelium can contain pyrenocine A and pughiinin A (VanderMolen et al., 2017). These compounds can be toxic to plants, but no human toxicity has been shown. *L. edodes* is also reported to not produce mycotoxins (VanderMolen et al., 2017). The applicant provided additional genotypic and phenotypic data confirming the production strain's absence of

mycotoxin production.

Following fermentation the production organism is heat-killed, with less than 0.1% of the *L. edodes* remaining in the final FPRP product. The applicant also demonstrated that any remaining *L. edodes* was non-viable, indicating a successful heat-kill procedure.

The microbiological risk assessment undertaken by FSANZ has not identified any public health and safety concerns associated with the use of *L. edodes* as a processing aid. *L. edodes* mycelium has been determined to be neither pathogenic nor toxigenic.

3.2 Toxicity studies

3.2.1 Animal Studies

Considering the history of safe consumption of the *L. edodes* fruiting body as a food, and that comparison between *L. edodes* extracts of the fruiting body and mycelium have been shown by high-resolution mass spectrometry (*m/z* 100-2000) to not be substantially different (VanderMolen et al., 2017), no toxicological studies were required for the use of the fermented preparation of *L. edodes* mycelium as a processing aid.

FSANZ did however review three repeat-dose animal studies on the safety of orally administered *L. edodes*, which were available in the public literature (Appendix 1). None of the identified studies examined *L. edodes* mycelia directly, nevertheless there were no effects in the reviewed studies that FSANZ would consider adverse, in rats or mice, related to oral exposure to *L. edodes* fruiting bodies.

However, given the low quality, the type and duration of the available animal studies, and the specific nature of the *L. edodes* test item used in those studies, FSANZ did not consider the reviewed evidence was appropriate for determining a no observed adverse effect level (NOAEL), nor to establish an acceptable daily intake (ADI) for *L. edodes* mycelium as a processing aid.

3.2.2 Genotoxicity

Consistent with not requiring animal testing, FSANZ considered that no genotoxicity studies were required for *L. edodes* mycelium as a processing aid. However, two non-guideline genotoxicity studies were available in the public literature on the *L. edodes* fruiting body and were reviewed by FSANZ (Appendix 1).

The reviewed results from both studies did not suggest that exposure to the *L. edodes* mycelium would be genotoxic in mammalian cells.

3.2.3 Clinical trials

Two clinical trials were available in the public literature that examined the effects of *L. edodes* on the immune system when consumed orally. While these clinical trials were not tolerance studies, they were relevant to the body of evidence supporting the safety of *L. edodes* as a processing aid and were reviewed as part of the safety assessment (Appendix 1).

The reviewed results from both studies do not suggest that repeated dose oral exposure to the *L. edodes* represents a health and safety risk in humans.

3.3 Potential for allergenicity

In extremely rare cases, consumption of the *L. edodes* fruiting body (shiitake mushrooms) can cause dermatitis in sensitive individuals (Nakamura, 1992; Nguyen et al., 2017). Shiitake dermatitis presents as linear pruritic erythema that resembles a flagellate (whiplike) pattern 24-48 hours after ingestion of shiitake mushrooms, appearing predominantly on the torso of sufferers, but can also appear on the arms and face (Hanada and Hashimoto, 1998).

The exact cause of shiitake dermatitis is unclear, but the evidence supports a rare hypersensitivity. There is some evidence this idiosyncratic hypersensitivity is related to the presence in *L. edodes* of lentinan, a 1,3 beta-glucan polysaccharide with beta-1,6 branching (Corazza et al., 2015; Nguyen et al., 2017).

Furthermore, there are cases in the scientific literature documenting cases of IgE-mediated allergic reactions to the *L. edodes*, which appear to be rare adverse reactions that are unrelated to shiitake dermatitis. These events have occurred in occupational environments, as well as arising from the consumption of shiitake mushrooms as food (Aalto-Korte et al., 2005; Goikoetxea et al., 2009; Kopp et al., 2009; Pravettoni et al., 2014; Tarvainen et al., 1991).

Considering the worldwide availability of shiitake mushrooms as a food, the rare occurrence of shiitake dermatitis or shiitake allergic reactions in the population, and the low quantity of *L. edodes* in the final product when used as a processing aid, the risk of shiitake dermatitis or allergenicity from the use of *L. edodes* as a processing aid is not expected to be greater than the current risk associated with *L. edodes* in the food supply.

3.4 Safety of the FPRP preparation

The Applicant's FPRP is not the subject of the application. Both pea and rice have a history of safe use in food, and are not considered novel in Australia and New Zealand. The use of *L. edodes* mycelia to improve organoleptic characteristics of the pea and rice protein is not expected to create a health and safety risk for consumers of FPRP.

3.4.1 Overseas assessments.

The FPRP was assessed by the European Food Safety Authority (EFSA) Panel on Nutrition, Novel Foods and Food Allergens. The panel concluded that, under the proposed conditions of use, there were no safety concerns associated with the consumption of FPRP (EFSA, 2022). The panel noted that the FPRP has the potential to sensitise individuals, or to induce allergic reactions in individuals allergic shiitake mushroom. However, the Panel determined that the risk is not expected to be higher than normal consumption of shiitake mushrooms in the population.

The Applicant states that the FPRP has been generally recognised as safe (GRAS) in the United States since 2020, following the submission of an expert opinion and subsequent 'No Questions' from the U.S. Food and Drug Administration (FDA; GRN: 000848). FSANZ notes that GRAS notifications are not assessments by the FDA, and are not accepted by FSANZ as an assessment by other international agencies.

The applicant also stated that the FPRP is approved or registered in India, Brazil, Canada, Japan, South Korea, Singapore, Malaysia, Indonesia, the Philippines, Thailand, Chile, Ecuador and Hong Kong. The assessments associated with these approvals and registrations were not provided to support FSANZ's risk assessment.

3.5 Dietary exposure assessment

3.5.1 Introduction and purpose

The purpose of the dietary exposure assessment was to estimate the levels of chronic dietary exposure to the processing aid (*L. edodes* mycelia) for the Australian and New Zealand populations. Chronic dietary exposure estimates are used to represent the long term, usually life-long, dietary exposure for the population from the range of foods containing the chemical (or preparation) of interest.

3.5.2 Approach to estimating dietary exposures and results

Dietary exposure assessments at FSANZ are conducted using a tiered approach. The first assessment is conducted using the most conservative assumptions and the least amount of resources, with refinements made following this assessment if needed. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009).

The safety assessment did not identify any population sub-groups or at-risk groups for which there were specific safety considerations or where separate chronic dietary exposure estimates were needed. Hence, the budget method calculation was used as a 'worse-case scenario' approach to estimating likely levels of dietary exposure to the processing aid from all general purpose foods, assuming 0.1% of the *L. edodes* mycelia will remain in the FPRP ingredient. Estimation of mushroom consumption (the fruiting body) for the Australian and New Zealand populations was considered for comparison purposes.

Budget method

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass et al., 1997). Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO, 2020). This method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO, 2021) for dietary exposure assessments for processing aids. The calculation is based on physiological food and liquid requirements, the processing aid concentration in foods and beverages, and the proportion of foods and beverages that may contain the processing aid.

In this budget method calculation, FSANZ made the following assumptions that are conservative and reflective of a first tier in estimating dietary exposure (FAO/WHO, 2009):

- the maximum physiological requirement of solid foods (including milk) is 50 g/kg body weight/day. This is the standard level used in a budget method calculation where there is a potential for the processing aid to be present in baby foods or general purpose foods that would be consumed by infants (Hansen 1966).

- the maximum physiological requirement for liquids is 100 mL/kg body weight/day. This is the standard level used in a budget method calculation.

- the processing aid (*L. edodes* mycelia) remains in the FPRP at 0.1% (w/w).

- 12.5% of solid foods and 25% of non-milk beverages contain the FPRP as an ingredient. These are commonly used default proportions noted in FAO/WHO, 2009.

- the maximum FPRP contained in 100 g of solid foods (including milk) is 40 g. This is the highest level of the range of maximum use levels proposed for solid foods (meat alternatives) in Table D.1-1 in the application.

- the maximum FPRP contained in 100 g of non-milk beverages is 20 g. This is the highest level of the range of maximum use levels proposed for non-milk beverages (vegetable juices or smoothies) in Table D.1-1 in the application [3].

Based on these assumptions, FSANZ calculated the TMDI of the processing aid (*L. edodes* mycelia) to be 7.5 mg/kg bw/day. The calculated TMDI will be an overestimate of the dietary exposure to the processing aid given the conservatisms in the budget method. This includes that it was assumed that the processing aid remains in the FPRP at 0.1% (w/w) and 12.5% of solid foods and 25% of non-milk beverages contain the FPRP as an ingredient at the highest level of the range of maximum use levels proposed for these types of foods.

Estimation of mushroom consumption

Consumption of mushrooms (the fruiting body) was estimated for the Australian and New Zealand populations using consumption data of all mushrooms reported in the most recent national nutrition surveys[4] (ABS, 2015; MoH, 2005; MoH, 2011a; MoH, 2011b). In these surveys, Shiitake mushrooms are not listed as a specific survey food, therefore estimated consumption of all mushrooms was assumed to be equal to the consumption of Shiitake mushroom for this assessment.

For this estimation, the Harvest[5] 'raw commodity model' was used that considered consumption of fresh and dried mushrooms eaten 'as is' (e.g. 'mushroom, common, boiled or steamed') and from mixed dishes, such as mushroom on a pizza, in stir fries etc. Mean and 90th percentile (P90) consumption of all mushrooms were estimated to be 0.2 – 0.6 g/kg bw/day (10 – 37 g/day) and 0.4 – 1.4 g/kg bw/day (24 – 98 g/day) respectively for the Australian and New Zealand population groups assessed. See Table 2 for details.

Table 2 Estimated consumption of all mushrooms for the Australian and New Zealand populations

Country	Age group	Proportion of consumers to respondents (%)	Estimated consumption of mushroom (for consumers only)			
			g/day		g/kg bw/day	
			Mean	P90	Mean	P90
Australia*	2 years and above	63.1	10	24	0.2	0.4
New Zealand#	5-14 years	12.4	22	58	0.6	1.4
	15 years and above	19.1	37	98	0.5	1.3

*Based on data from Day 1 and 2. Two day average data better reflects longer term estimates of consumption.
#Based on data from Day 1 only.

4 Conclusion

The proposed use of a fermented preparation of *L. edodes* mycelia as a processing aid in the quantity and form proposed to be used poses no safety concerns. The fermented preparation

[3] The proposed maximum use level for 'ready-to-mix beverage powder' was not considered as it is not provided 'as consumed' and dilutions occur before consumption.
[4] The design of these nutrition surveys and the key attributes, including survey limitations, are set out on the FSANZ website.
[5] Harvest is FSANZ's custom-built dietary modelling program that replaced the previous program, DIAMOND, which does the same calculations just using a different software program.

of *L. edodes* mycelia performs its technological purpose during processing of FPRP and does not perform its technological purpose in the food for sale, therefore functioning as a processing aid for the purposes of the Code.

L. edodes has a history of safe consumption as a food and the mycelia has been determined to be neither pathogenic nor toxigenic. The FSANZ safety assessment did not establish an ADI for *L. edodes* mycelium as a processing aid. Overall, FSANZ concludes that there are no safety concerns from the use of a fermented preparation of *L. edodes* mycelia as a processing aid.

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6 Appendix 1

Study summaries - Animal Studies

5-day repeated dose oral toxicity study in mice (Nieminen et al., 2009). Regulatory status: not stated

Female NIH/S mice (age: 82-118 days; 6 per treatment group) were fed dried shiitake mushroom powder in feed at 0% 1.8, 3.6% and 5.4 % (equivalent to 0, 2.9, 6.3 9.2 g/kg bw/day dried mushroom) for 5 days. Animals were killed immediately after the test period. Body weight was recorded at the beginning and end of the test period. Food and water consumption was recorded (frequency of recoding not stated), liver, kidney, spleens and adrenal glands were weighed, with liver and muscle (left hindlimb quadricep) undergoing histopathological examination.

There was a possible treatment dose-related increase in group mean total serum bilirubin, recorded above that of controls, but no changes in ALT, AST, liver weight or body weight. There were statistical increases in total serum protein in all treatment groups above controls, but it is unclear if this was related to the test item. Creatine kinase was significantly increased in the high-dose group, but not significantly in the low- or medium-dose groups. Historical values for the test laboratory were not stated, nor were the histopathology findings shown or discussed.

A NOAEL or lowest observed adverse effect level (LOAEL) was not stated by the study authors.

Considering the low number of test animals in the study, the short 5-day administration period, limitations in reporting, and the lack of clinical chemistry results to support a conclusion of hepatotoxicity, FSANZ determined that this study was not appropriate for the purpose of establishing a NOAEL or LOAEL.

28-day repeated dose oral toxicity study in rats (Yoshioka et al., 2010). Conducted according to OECD TG 407.

Wistar rats (10 /sex/group) were dosed by oral gavage with 0 or 2000 mg/kg bw/day for 28 days. Test item was *L. edodes* mycelia, grown in sugar-cane bagasse and defatted rice bran, then extracted using hot water and filtration. Final test item was sterilised and lyophilized. Animals were observed daily for signs of toxicity. Body weights were recorded on days 0, 1, 3, 7, 11, 14, 18, 21 and 28. Animals were killed on day 28 and organ weights recorded for all animals. Haematology, biochemistry and histopathology were undertaken on all animals.

No mortality was recorded during the study. There was a statistically significant difference in body weight gain in the male test animals, which was associated with a decrease in food consumption. A significant difference was not observed in female test animals. The authors noted that the food consumption in the males began to recover towards the end of the test period. FSANZ notes that this body weight gain difference was less than 10%, was only observed in males, and there were no additional test doses to confirm a dose relationship.

No changes in haematological or biochemical parameters were noted outside reference ranges. It was unclear if historical values were available for the test facility. There were no treatment-related observations in histopathology or organ weights.

The NOAEL was taken to be 2000 mg/kg bw/day by the study authors, the only dose tested.

30-day repeated dose oral toxicity study in rats (Grotto et al., 2016). Regulatory status: not stated

Male Wistar rats (age: 45 ± 3 days; 6 per treatment group) were dosed with the test item by oral gavage daily for 30 days. Test item was dehydrated and powdered *L. edodes* fruiting body. Test dosages were 0, 100, 400 and 800 mg/kg bw/day (equivalent to 0, 1000, 4000, and 8000 mg/kg bw/day of wet-weight *L. edodes* fruiting body, prior to processing). Vehicle control was water.

Food and water consumption was recorded daily, and body weights recorded weekly. Animals were killed at study termination and biochemical and haematological parameters were measured. Survival was not explicitly noted. No findings from gross necropsy, organ weights, nor histopathology results were described.

There were no changes in body weight, food and water intake, or serum biochemistry. There was a small (< 5%), but statistically significant decrease in hemoglobin (HGB) in the medium- and high-dose groups without a clear dose-response relationship. There was a statistically significant decrease in white blood cells (WBC; 30 %) in the high dose group only. Neither was considered by FSANZ to be adverse.

The NOAEL was taken to be 100 mg/kg/bw/day, based on the reduction of HGB and WBC at and above 400 mg/kg bw/day. However, due to the low animal numbers used in the study and limitations in either reporting or measured outcomes, FSANZ found this study to be of low quality for establishing adverse effects of shiitake mushroom consumption in humans.

Study summaries - Genotoxicity

Bacterial reverse mutation test (von Wright et al., 1982). Regulatory status: not stated

The potential mutagenicity of *L. edodes* and other edible mushrooms was evaluated in *Salmonella enterica* ser. Typhimurium strains TA100, TA98, TA1535, TA1537 and TA1538, with and without metabolic activation using mouse liver homogenate (S9). Mutation tests were conducted in triplicate. No positive control data were reported.

The test item was an *L. edodes* extract produced by collecting the supernatant following centrifugation of blended fruiting bodies. 100g of mushrooms produced 60ml of the test extract, which was added to the test cultures at 10, 25, 50, 75 and 100 ul/plate.

The TA100 test strain showed a concentration-related increase in revertant colonies with and without metabolic activation. Interestingly, this was consistent for all other edible mushroom species tested (*Lactarius necator, Lactarius torminosus, Lactarius helvus, Lactarius rufus, Boletus edulis,* and *Agaricus bisporus*). Additional experimentation showed that boiling of the test item for up to 20 min did not decrease mutagenic effects of the *L. edodes* extract on TA100.

The bacterial reverse mutation test uses prokaryotic cells, which differ from mammalian cells, and cannot provide direct information on the mutagenic and carcinogenic potency of a substance in mammals. Considering the history of consumption of shiitake mushrooms, the result in a single tester strain, and the observed effect in other edible mushroom species, FSANZ does not consider this result to be a direct indicator of mutagenic potential of *L. edodes*.

In vivo micronucleus test (de Lima et al., 2001). Regulatory status: not stated

Study design was to compare the interaction between *L. edodes* fruiting body and alkylating agents *in vivo*. However, the results for *L. edodes* alone, compared with vehicle control, was judged to be relevant for risk assessment. Only these results have been summarised.

Male Swiss mice (age: 7-8 weeks; 6 animals per treatment) were dosed with the test item by oral gavage daily for 14 days. Powdered *L. edodes* fruiting body was dissolved in water (2.5 % w/v) at 4 °C, 21 °C and 60 °C and filtered. Test dosage of each temperature test item was administered in 0.6 ml water per animal at approx. 125 mg/kg bw/day of dried *L. edodes*. Vehicle control was water. Cyclophosphamide, administered by intraperitoneal injection (25 and 50 mg/kg) on day 15, was examined in the assay and served as a positive control for induction of micronuclei.

Either 24 or 48 hours after final dosage with test item, mice were killed and bone marrow extracted. There was no increase in the frequency of micronuclei in animals treated with *L. edodes* for any of the test items, compared to untreated controls. There was an increase in micronuclei in cyclophosphamide-treated animals.

The *L. edodes* test item did not induce micronuclei *in vivo*, under the conditions of this test.

Study summaries – clinical trials

28-day repeated dose clinical trial (Dai et al., 2015).

Human clinical trial to examine the effects of oral consumption of *L. edodes* on the immune system. Healthy volunteers (26 per group) consumed 5 or 10 g of dried *L. edodes* fruiting body (equivalent to 5 or 10 medium sized shiitake mushrooms) in the diet daily for four weeks. Blood and saliva samples were taken before the first intervention and at study termination. Self-reported questionnaires were undertaken to monitor study compliance, adverse indications and any confounding illness.

Two participants dropped out in the 5g group due to developing shiitake dermatitis and three dropped out of the 10g treatment group due to developing nausea and GI distress. One participant and 3 participants in the 5 g and 10 g groups respectively, did not return for the second blood draw and their data were not reported.

Differences in cell surface markers of *ex vivo* γδ-Natural Killer T (NKT) cells were noted, but only in the pooled samples (i.e. treatment vs. initial). Treatments were pooled because no significant changes were observed in all but one measurement (Interleukin-4 expression).

This study is of limited use for determining *L. edodes* safety due to the lack of concurrent controls, the lack of information on the method for data analysis, and because it is unclear if the effects on γδ-NKT cell parameters constitutes an adverse reaction in humans.

48-day repeated dose double-blind parallel-group clinical trial (Choi et al., 2014).

Human clinical trial to examine the effects of oral consumption of *L. edodes* on the immune system. Healthy volunteers (40 per group) consumed 150 g of an alkali and ethanol extract from *L. edodes* mycelium grown in rice bran, in a capsule form daily for 8 weeks.

No significant adverse effects were observed during the study. There was a mild, but statistically significant decrease in WBC and a statistically significant increase in interferon-γ in test item participants, compared to controls, but this was not accompanied by significant concomitant changes in other haematological parameters.