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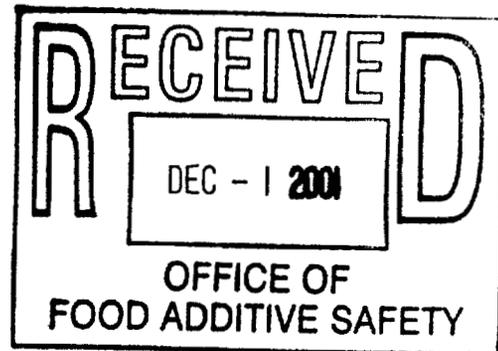
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November 30, 2001

Stuart M. Pape
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VIA HAND DELIVERY

Dr. Linda Kahl
Office of Food Additive Safety (HFS-206)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W.
Washington, DC 20204



Re: NOTIFICATION OF GRAS DETERMINATION FOR MYCOPROTEIN

Dear Dr. Kahl:

On behalf of Marlow Foods Ltd., a wholly owned subsidiary of AstraZeneca Ltd., and pursuant to the rule proposed at 62 Fed.Reg. 18960 (April 17, 1997) (proposed 21 C.F.R. §170.36), I hereby submit this notification to the FDA that mycoprotein is generally recognized as safe (GRAS) for general food use. The notification consists of a GRAS exemption claim and a detailed summary of the basis for the GRAS determination. Please find three copies enclosed.

As you know, Marlow Foods Ltd. is the sponsor of a food additive petition for mycoprotein that has been pending with the agency since 1986 (Food Additive Petition No. 6A3930). We understand that FDA has completed its technical review of the petition and that FDA expects to issue a food additive regulation for mycoprotein in the future. However, given the agency's limited resources and the changing priorities at the agency, we recognize that there continues to be delay in the issuance of the food additive regulation. Therefore, because Marlow Foods has determined that mycoprotein has become GRAS during the time in which the food additive petition has been pending, we are submitting this GRAS notification to inform the agency of Marlow Foods' determination and its intention to begin marketing products containing mycoprotein.

Please note that Marlow Foods does not intend for this GRAS notification to replace the mycoprotein food additive petition, and Marlow Foods is not withdrawing the petition. We look forward to continuing to work with the agency as necessary to ensure that the agency may issue a mycoprotein food additive regulation in the near future.

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PATTON BOGGS LLP
ATTORNEYS AT LAW

Dr. Linda Kahl
November 30, 2001
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Thank you for your consideration of this matter. If you have any questions, please do not hesitate to call me.

Sincerely, |

Stuart M. Pape
Counsel, Marlow Foods Ltd.

SMP/jjs

Enclosure

GRAS NOTIFICATION

for

MYCOPROTEIN

Submitted by:

Marlow Foods Ltd.
Station Road Stokesley
North Yorkshire TS9 7AB
United Kingdom

November 30, 2001

Stuart M. Pape, Esq.
Patton Boggs LLP
2550 M St. NW
Washington, DC 20037
Counsel to Marlow Foods Ltd.

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GRAS Exemption Claim

Pursuant to the policy described at 62 Fed.Reg. 18938, 18960 (April 17, 1997) (proposed 21 C.F.R. § 170.36), Marlow Foods Ltd. hereby notifies the Food and Drug Administration that it has determined that the use of mycoprotein is generally recognized as safe (GRAS) and is therefore exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

The data and information that are the basis for the GRAS determination will be made available upon request for FDA review and copying at reasonable times at the Contact's address below, or will be sent to FDA upon request.

The following information is provided pursuant to the proposed rule:

Notifier: Marlow Foods Ltd.
Station Road Stokesley
North Yorkshire TS9 7AB
United Kingdom

Contact: Stuart M. Pape
Patton Boggs LLP
2550 M Street, N.W.
Washington, D.C. 20037
(202-457-5240)

Notified Substance: Mycoprotein (detailed information about the identity of the notified substance appears in the document following)

Conditions of Use: General food use, excluding use in infant formula (detailed information regarding levels of use -- including any self-limiting levels -- and purpose of use appears in the document following)

Basis for GRAS Determination: Scientific procedures

A detailed summary of the basis for the determination that the above use of mycoprotein is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the use is GRAS is set forth in the document below.

Stuart M. Pape
on behalf of Marlow Foods Ltd.

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1. SUMMARY

Mycoprotein is a food ingredient that can be used in whole foods or as a whole food itself. It is composed of the hyphae of a fungal organism that is grown through a continuous fermentation process. Mycoprotein's very favorable nutrient profile (high in protein and fiber; low in fat; no cholesterol) and its excellent taste characteristics make it suitable and beneficial for use in a variety of products. For example, mycoprotein can be used in dairy and cereal products, and its structure and texture make it a particularly suitable alternative to traditional sources of protein in the human diet.

Numerous scientific studies have been conducted demonstrating that the general use of mycoprotein in human foods is safe. Furthermore, mycoprotein has a significant history of use in Europe, where it has been sold under the trade name Quorn™ for fifteen years. In Europe, millions of consumers use Quorn™ products, and this use has provided an impressive record of safety. This safety has been generally recognized, as is evidenced by the scientific studies, the general use and recognition of mycoprotein as an ingredient in Europe, the recognized manufacturing process, the publication of information about mycoprotein, and the evaluation of available safety information by an expert panel.

A food additive petition for mycoprotein has been pending with the Food and Drug Administration (FDA) since 1986.¹ While Marlow Foods Ltd.² (Marlow Foods) anticipates that FDA will issue a food additive regulation for mycoprotein in the near future, during the time in which the petition has been pending with the Agency, Marlow Foods has self-determined that mycoprotein is generally recognized as safe (GRAS) for general food use. Therefore, while Marlow Foods is not withdrawing the food additive petition for mycoprotein that is pending with the Agency, and, as noted, fully expects a food additive regulation to issue, Marlow Foods has decided to submit this notification to declare the GRAS status of mycoprotein for general food use.³

2. INTRODUCTION

2.1 GRAS Determination

Marlow Foods hereby notifies the FDA that, pursuant to the policy set forth in the proposed rule at 62 Fed. Reg. 18938, 18960 (April 17, 1997), Marlow Foods has determined that mycoprotein is GRAS for general use in foods.⁴ General recognition of the safety of mycoprotein has been determined through scientific procedures.

The GRAS determination for mycoprotein meets the technical safety and common knowledge elements of a GRAS determination based on scientific procedures. Sections 3-8 of this

¹ Food Additive Petition No. 6A3930.

² Marlow Foods Ltd. is a wholly owned subsidiary of AstraZeneca Ltd. For ease of reference, although other predecessor companies may have been involved in particular instances, all references in this notification will be to Marlow Foods Ltd.

³ Throughout this notification, reference to the "general food use" of mycoprotein is not intended to include use in infant formula.

⁴ Pursuant to the policy, the use of mycoprotein is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act.

notification provide technical evidence of safety (including nutritional information, manufacturing procedures, and scientific studies) that is augmented by extensive evidence of safety derived from the broad high level human consumption of mycoprotein in Europe. Sections 9 and 10 (in conjunction with the preceding sections) provide a basis to conclude that the evidence of safety is generally known and accepted. This GRAS determination therefore meets the requirements of §201(s) of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 C.F.R. §§170.3 and 170.30, and the amendments to these rules proposed at 62 Fed. Reg. 18960.

2.2 Mycoprotein Analysis

Mycoprotein is the name of a food product that is derived from *Fusarium venenatum*, PTA-2684,⁵ in which the ribonucleic acid (RNA) content has been reduced. It is produced by an axenic fermentation process, using a food-grade carbohydrate substrate and other appropriate safe and suitable ingredients. Mycoprotein has a variety of uses as a protein source, including, due to its physical properties, as an alternative to meat in a multitude of products. Mycoprotein has a favorable nutrient profile; when used as a food ingredient, 100g of mycoprotein typically contains about 11.25g of protein, 6.25g of fiber, 3.25g of fat, 2.5g of carbohydrate, and 85kcal of energy.

As a whole food, mycoprotein presents a somewhat different framework for safety evaluations when compared to typical food additives. In contrast with conventional food additives, mycoprotein is a food that may form a significant part of the diet and contribute substantially to the nutrition of those who consume the product. Therefore, traditional food additive safety evaluation models, such as studies using substantial multiples of the anticipated human exposure, are not feasible. Furthermore, because mycoprotein is composed of common nutrients, there is no adverse effect of consumption and therefore, safe levels cannot be set by using a fraction of the no effect level. Therefore, the safety assessment of mycoprotein reviewed in this notification is based on a broad analysis that includes not only the results of extensive toxicology studies and use data, but that utilizes all available information about mycoprotein including factors such as physical properties, nutrient composition, production methods and quality assurance, and consumer exposure in Europe.

2.3 History of Mycoprotein Development

Mycoprotein was originally discovered and developed in the 1960s due to concerns about a potential world shortage in food protein (*see* Rodger 2001, attached). In response to these concerns, food companies sought alternate sources of protein and the food industry began investigating the use of by-products to grow single-cell systems (such as bacteria, yeast, and fungi) to harvest and use as new protein sources. During this research, mycoprotein was discovered. Mycoprotein is derived from a member of the mushroom family that was originally discovered growing naturally in Buckinghamshire, United Kingdom. It became apparent that mycoprotein had physical and nutritional properties that enabled the development of mycoprotein as a human food.

Throughout the mid-1970s, mycoprotein was tested to determine if it was fit for human consumption and if it could be grown in large scale for production and marketing. Ranks Hovis

⁵ Deposited in the American Type Culture Collection, Washington, D.C., as PTA-2684.

McDougall (RHM) of Windsor, England, entered into a joint venture with ICI of London, England, a major international company with experience in fermentation, to further develop the production processes. Extensive safety testing was implemented and, in the 1980s, development work focused on perfecting the texture and taste of mycoprotein as a food ingredient. The development of mycoprotein as a human food is described in various detail in several published articles (Trinci 1992; Marsh, *et al* 1985; Anderson, *et al* 1984).

In 1985, the Government of the United Kingdom gave approval for mycoprotein to be used in food and subsequently issued a certificate for its free sale. The product has been sold in the U.K. since January 1985. In 1991, sales in other countries began. Mycoprotein now may be sold lawfully in all the countries of the European Community, Switzerland, Norway, and Taiwan. Mycoprotein is sold in textured and flavored formats under the trademarked name Quorn™. Currently, mycoprotein is sold in prepared convenience products (e.g., meat-free burgers and fillets), as the central component of prepared ready-meals (e.g., stir-fries, curries, pasta dishes), and as a food/ingredient for home use. Mycoprotein products are sold in a range of flavors and in both chilled (fresh) and frozen forms. While use as an alternative to meat is common, mycoprotein can be used in a variety of food products, including dairy and cereal products.

On March 11, 1986, RHM submitted a petition to the FDA for food additive approval of mycoprotein in the United States as a meat alternative in frozen entrees. The petition was accepted for filing and notice was published in the *Federal Register* on May 30, 1986, as petition No. 6A3930. In 1990, responsibility for the petition was taken over by ICI, then by Zeneca Ltd. of London, England in 1993, and subsequently in 1999 by Marlow Foods. The food additive petition was amended in November, 1996, to broaden the proposed intended uses of mycoprotein to general food use.

The food additive petition for mycoprotein is still pending with the Agency. Marlow Foods has remained in close communication with the Agency during the Agency's review of the petition, and Marlow Foods recognizes that shifting priorities and limited resources have continuously increased the timeframe for FDA approval of the food additive petition. Therefore, Marlow Foods has completed a self-determination of the GRAS status of mycoprotein. Marlow Foods is submitting this notification to demonstrate the basis for the GRAS determination and to apprise the Agency of its intent to market products containing mycoprotein in the U.S. during the Agency's completion of the food additive approval. This notification is not intended to replace the food additive petition, and Marlow Foods continues to anticipate the Agency's approval of the petition in the near future.

3. CHEMICAL IDENTITY AND COMPOSITION

Mycoprotein is a food that is derived from *Fusarium venenatum*, PTA-2684, in which the RNA content has been reduced by a process that also renders the organism non-viable. The filamentous organism is grown axenically in a continuous fermentation system on a medium comprising food-grade carbohydrate together with other safe and suitable ingredients.

3.1 Physical Properties

As a member of the class Fungi-Imperfecti, *F. venenatum*, PTA-2684, has a structure and composition typical of micro-fungi (except for a higher protein content) (Miller and Dwyer 2001, attached; Miller, *et al* 1999, attached). The cell wall, which constitutes approximately one-third of the cell dry weight, is composed of chitin (poly N-acetyl glucosamine) and β -glucans (β -1:3 and β -1:6 glucosidic linkages). The lipid content is about 13 percent. The fatty acids contain an even number of carbon atoms. Significant quantities of ergosterol are present, but no cholesterol is present. Typical analyses of mycoprotein are given in Table 1 (note that the true protein content is based on amino nitrogen multiplied by 6.22 rather than the conventional 6.25, because of the content of non-protein nitrogen).

The physical characteristics of the hyphae of mycoprotein make it suitable for a variety of applications. The hyphae are filamentous with a high length/diameter ratio and thus are morphologically similar to animal muscle cells. Mycoprotein is not soluble and it does not give rise to any soluble components. Appropriate applications include as a muscle fiber replacer in meat alternative products, a fat replacer in certain dairy products, and a cereal replacer in products such as breakfast cereals or puffed snacks.

Table 1 - Typical Mycoprotein Analyses (Miller, *et al* 1999, attached)

Analyses (gm/100gm dry cell wt)		
True Protein (Amino Nitrogen x 6.22)	42-50	
Crude Protein (Total Nitrogen x 6.25)	52-59	
RNA	0.5-2	
Total lipid	12-14	
Dietary Fiber	22-28	
Ash	3-4	
Mineral Analysis mg/kg		
Phosphorus	9000-12000	
Zinc	300-450	
Copper	10-30	
Magnesium	1700-2000	
Calcium	1600-1800	
Sodium	100-300	
Potassium	3000-5000	
Manganese	100-400	
Heavy Metals mg/kg		
Cadmium, Lead, Arsenic, Mercury: Total 0.1 ppm		
Fatty Acid Composition g/kg		
Palmitic	16:0	13
Stearic	18:0	2
Oleic	18:1	14
Linoleic	18:2	43
Linolenic	18:3	9
Polyunsaturated/saturated ratio		3.5/1
Triglycerides and diglycerides		65% total lipid
Sterols and unsaponified		5%
Phospholipids		30%
Vitamin Analysis mg/kg		

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Thiamine B1	0.4
Riboflavin B2	9
Niacin B5	14
Pyridoxine B6	5
Pantothenic Acid	10
Folic Acid	0.4
Biotin	0.6

Amino-acid Analysis of Mycoprotein (g/100g protein)

Lysine	8.3	Histidine	3.5
Methionine	2.1	Arginine	7.3
Cystine	0.8	Tyrosine	4.0
Threonine	5.5	Aspartic Acid	10.3
Tryptophan	1.6	Serine	5.1
Valine	6.2	Glutamic Acid	12.5
Leucine	8.6	Proline	4.5
Isoleucine	5.2	Glycine	4.3
Phenylalanine	4.9	Alanine	6

Carbohydrate

The carbohydrate composition of the cell wall is:

	g/100gm cell dry weight
Chitin (poly-N-acetyl glucosamine)	12
β-1:3 and β-1:6 glucans	22
Glycogen	2
Other polysaccharides (galactomannans)	trace
TOTAL	36

Fiber:

Chitin and much of the β-glucans are conventionally analyzed as 'fiber'; mycoprotein contains about 25% fiber on a dry weight basis.

Lipid:

The lipid content is about 13%, much of which occurs as phospho-lipid in the cell membranes. The fatty acids contain an even number of carbon atoms, since they are synthesized from glucose through the normal metabolic pathways.

3.2. Food-grade Specifications

Table 2 displays the food-grade specifications established for mycoprotein.

Table 2 - Mycoprotein Specification Definition

Definition

Mycoprotein shall mean the cellular mass of the fungus ATCC PTA-2684, *Fusarium venenatum*, processed to reduce the level of ribonucleic acids and to meet the specifications below.

Process

Mycoprotein is manufactured by the continuous pure culture fermentation of the fungus *Fusarium venenatum* ATCC PTA-2684 in a nutritionally balanced defined glucose medium. The specific growth rate of the fungus is maintained at a level of at least $0.17h^{-1}$. The culture is subsequently heated to kill the fungus and reduce the level of ribonucleic acids in the cellular mass.

Mycoprotein meets the following specifications when testing using the methods described in the document entitled, "Methods for Specification Tests for Mycoprotein," prepared by Marlow Foods Ltd., Station Road, Stokesley, North Yorkshire, U.K., TS9 7AB.

Composition (calculated on a dry weight basis)

- i. **Protein** - Not less than 41%.
- ii. **Ribonucleic Acid** - Not more than 2% .
- iii. **Ash** - Not more than 5%.

Contaminants (calculated on a dry weight basis)

- i. **Metals** - Lead, arsenic, mercury and cadmium , each not more than 0.1 mg/kg.
- ii. **Myco-toxins** – Not detectable.

3.3 Nutrition Information

The chemical analysis of mycoprotein indicates that it contains a wide spectrum of nutrients. Typically, the dry matter in 100g mycoprotein contains 45g protein, 25g fiber, 13g fat, 10% available carbohydrate, and a range of vitamins and minerals. When used as a food ingredient having a solids content of about 25%, 100g of mycoprotein typically contains about 11.25g protein, 6.25g fiber, 3.25g fat, 2.5g carbohydrate, and 85kcal of energy. The general nutrition properties of mycoprotein have been the subject of various publications (Edwards 1993; Wheelock 1993; Sadler 1991; Sadler 1990; Edelman, *et al* 1983). The results of various nutritional studies are reviewed below (each was conducted using RNA-reduced mycoprotein).

In sum, mycoprotein is a source of good quality protein which is combined with low energy and high fiber content. Its fat consists of largely unsaturated fatty acids, predominantly ω -6 and ω -3, linoleic and linolenic acids respectively. The fiber is a mixture of chitin and beta-glucans that performs physiologically as fiber and does not have any adverse effect on mineral status.

Mycoprotein also may exhibit physiological effects that provide health benefits in the context of the human diet. Clinical studies have examined the potential ability of mycoprotein to reduce

blood cholesterol (total and LDL), slow glucose absorption, and induce satiety following the consumption of mycoprotein. Many of these studies are in the published literature (Nakamura, *et al*, 1994; Turnbull, *et al* 1998, 1995, 1993, 1992, 1991, 1990; Burley, *et al* 1993; Homma, *et al*, 1995; Ishikawa, *et al* 1995).

3.3.A. Protein

The intended use of mycoprotein in products that form the center of a meal requires that mycoprotein provide high quality protein. The amino acid composition shows that mycoprotein contains all of the essential amino acids. Bioassays in chicks have shown that methionine (the sulphur amino acids, methionine and cystine, are the first limiting ones) and lysine (which is particularly important for growth) are highly available. Rat bioassays have demonstrated that the protein efficiency ratio (PER) and net protein utilization (NPU) are both in excess of 85% of the values for casein (Jonker 1995). Slope ratio assays in rats gave similar or better results to these in comparison with casein.

The results of a human volunteer study (Udall, *et al* 1984) confirmed that the results of the animal assays could be extrapolated to man. In comparison to skimmed milk protein, mycoprotein had the same biological value (BV), while the NPU was calculated to be slightly lower as a result of the somewhat lower digestibility (D) and the content of non-protein nitrogen.

Table 3 - Measurement of Protein Quality

	RAT			MAN	
	<u>PER</u>	<u>NPU</u>	<u>D</u>	<u>BV</u>	<u>NPU</u>
Mycoprotein	2.4	61	78	84	65
Mycoprotein + methionine	3.4	82	79	92	73
Casein	2.5	70	-	-	-
Skimmed Milk	-	-	95	85	80

3.3.B. Fat

While 55 percent of the energy content of mycoprotein comes from the protein, one-third of the energy content comes from fat. Typically, the fat content is 13g/100g on a dry basis, but as mycoprotein would normally have a solids content of around 25%, the fat content as consumed is typically close to 3g/100g. The fat in mycoprotein is much more like vegetable fat than animal fat, with a low proportion of saturated fat and high proportion of mono- and polyunsaturated fatty acids (Miller and Dwyer 2001, attached). Furthermore, within the polyunsaturates, 4.3g/100g of the ω -6 linoleic acid (c.18:2) is present together with a relatively high concentration of ω -3 linolenic acid (c.18:3).

3.3.C. Fiber

The cell wall components contribute a dietary fiber content of about 25g/100g of mycoprotein dry matter, which is over 6g/100g on a 25% solids basis, giving mycoprotein the unusual combination of being a source of protein which is not only low in fat, but also rich in fiber. The amount and composition of the fiber was confirmed in research undertaken at the Dunn

Laboratory, Cambridge, U.K. (Cummings 1990). It is largely insoluble fiber, consisting of approximately one-third chitin (poly-N-acetyl glucosamine) and two-thirds β -glucans. The study in ileostomy patients demonstrated that the fiber was poorly digested in the small intestine, and *in vitro* microbiological investigations indicated that it would be fermented in the large intestine. Thus, both analytically and physiologically the cell wall components have been confirmed to possess characteristics consistent with their classification as dietary fiber.

Table 4 - Analysis of Mycoprotein Non-Starch Polysaccharides

	<u>g/100g dry matter</u>	
	Soluble	Insoluble
N-acetyl galactosamine	0.25	0.09
N-acetyl glucosamine	0.43	9.31
Arabinose	0.16	0.06
Mannose	0.63	2.67
Galactose	0.37	0.77
Glucose	0.58	8.22
Uronic Acid	0.43	2.00
TOTAL	3.22	23.12
TOTAL FIBER		26.34

3.3.D. Minerals

As background consumption of the fiber in mycoprotein is limited because intakes of similar sources such as mushroom fiber and arthropod chitin are generally low, the question of possible effects on mineral absorption has been investigated in two rat studies and in one study in humans. The first rat study showed no adverse effect on mineral balance with regard to calcium, phosphorus, magnesium, iron, copper and zinc. As this was conducted with mature rats that were beyond the rapid growth phase, a further study was undertaken in young, fast-growing rats. The same minerals plus manganese were examined and mineral availability was judged on the basis of mineral balance and organ analysis. In comparison with pectin, known to affect some minerals, levels of dried mycoprotein up to 300g/kg in the diet produced no effects considered to be of significance in the human diet.

The research cited above (Cummings 1990) included an examination of mineral absorption during the digestibility study involving ileostomy patients. There was no significant effect on the apparent absorption of calcium, magnesium, phosphorus, iron and zinc in comparison with a polysaccharide-free diet (PSF) and wholemeal bread (see Table 5).

Table 5 - Apparent Absorption of Minerals in Man (%)

	<u>PSF</u>	<u>BREAD</u>	<u>MYCOPROTEIN</u>
Calcium	31.5	28.3	29.5
Phosphorus	80.3	74.4	73.5
Magnesium	48.4	42.8	42.1
Iron	46.7	40.5	49.8
Zinc	19.3	23.8	24.4

3.4 Stability

Mycoprotein is suitable for incorporation into a wide variety of foods, including cereals, snack foods and main dish items. Mycoprotein may be stored indefinitely at or below -18°C and can be stored at 0-4°C for at least 72 hours. The storage conditions common in the food industry for moist products with a protein content are appropriate for mycoprotein.

During typical processing into food products, mycoprotein is combined with binding agents such as egg albumin, and natural flavorings and colorings, as necessary. Other food ingredients also may be incorporated. The product is steam heated to denature the protein binder, and hence to 'set' the texture of the product. Some product forms may be heated in sauce in order to absorb flavor systems for a particular recipe, while others may be barbecued or otherwise cooked. None of these processes produces any significant further change within the mycoprotein.

4. PRODUCTION METHODS

4.1 Manufacturing Process

The manufacturing process for mycoprotein is set forth in the Expert Panel Report (see section 9) (Miller, *et al* 1999, attached) and a July 2001 *Food Technology* article (Rodger 2001, attached). Mycoprotein is produced by the process outlined in Table 6.

Table 6 - Manufacturing Process



4.2 Quality Assurance

The manufacture of mycoprotein is one in which the key parameters which have a direct effect on product quality have been identified and limits have been set such that in-specification product can be produced reliably in continuous operation. The product is tested routinely against the criteria set down in the specification, and the finished mycoprotein meets all these criteria.

A quality system has been developed which incorporates interdependent elements of quality control and quality assurance based on accumulated experience. Changes are controlled within the quality system. Marlow Foods has extensive experience in the consistent production of mycoprotein, and scale-up of production through two levels of pilot plants to commercial production has not resulted in any significant change in protein quality.

4.2.A. Process Controls

Most raw materials used to process mycoprotein are made up as batches of mixed solutions, and written specifications are established with the supplier. Standard operating procedures are followed to ensure integrity, but samples are kept for analysis should process deviations be

observed. The flows of all nutrients are controlled into the fermenter to ensure adequate availability. The temperature of heat sterilization is continuously monitored and controlled.

For the key process parameters identified to have a direct effect on product quality, limits have been set such that in-specification product can be produced reliably in continuous operation. For parameters that directly affect product quality, online measurement and automatic control preferably are used. Where this is not practicable, routine analysis and standard operating procedures are used. Any operations outside the preset operating limits lead to a deviation report.

For example, fermentation parameters that determine the growth environment of the organism are continuously monitored. Organism growth rate is also estimated by methods such as measuring CO₂ in exhaust gas or cell concentration. Where practicable, online control is used, such as temperature and pH. Otherwise, routine sampling and standard operating procedures are followed for purposes such as assuring nutrient concentrations.

Other key parameters are also closely monitored. RNA reduction temperature is continuously controlled and monitored to ensure that the product's RNA specification is achieved. The separation and cooling steps are controlled to achieve the desired final solids content and temperature.

Automatic rejection limits are also set. If product measurements are between the non-conformance and rejection limits, the product is quarantined until an investigation is completed by a competent person into the acceptability of the product. This investigation considers all information and normally calls for additional product analyses. For product parameters other than key parameters, measurements also are taken and recorded to enable corrective action before product quality is jeopardized. Deviations sufficient to raise concern about product quality are rare and result in a non-conformance report.

4.2.B. Quality Control

Mycoprotein is manufactured to a rigorous and demanding specification (see Table 2). Although mycoprotein is obtained from an organism that has the potential to produce trichothecene mycotoxins and fusarin mycotoxins under certain extreme conditions, the growing conditions required for mycoprotein are not compatible with those needed for mycotoxin production. Therefore, when mycoprotein is grown and harvested correctly, mycotoxins are not produced during the mycoprotein production process. The currently accepted view is that mycotoxins are only produced when mycelial growth is limited by imbalanced nutritional or physical conditions such as high C/N ratios, low oxygen tension, and incomplete nutrient requirements; these conditions are completely unsuitable for the production of mycoprotein (Nigam, *et al* 1999; Beremand, *et al* 1992; Demain 1992; Jackson, *et al* 1989).

Although the manufacturing conditions for mycoprotein are not suitable for mycotoxin growth, Marlow Foods elaborated and validated a method for monitoring mycoprotein prior to marketing to confirm that contamination with trichothecene or fusarin mycotoxins at detectable levels could not occur. The mycotoxin limits that were developed reflect analytical sensitivity, not anticipated content. Notably, in depth analysis has demonstrated that, even at the specification

level, the intake of tricothecenes would be significantly less than that expected from other commodities such as cereal grains.

Analysis methods are continuously refined to reflect current best practice. The current tricothecene detection method is based on one for cereals and spices published by Patel, *et al*, in *Food Additives and Contaminants*, and is applicable to the following tricothecene mycotoxins: deoxynivalenol (DON), 3-acetoxydeoxynivalenol (3-AcDON), nivalenol (NIV), diacetoxyscirpenol (DAS), fusarenone X (FUSX), and neosolaniol (NEO). Detection limits for individual tricothecenes must be no greater than 2×10^{-6} g/kg (as harvested, on a wet basis). Methods also have been implemented and validated for detecting fusarin mycotoxins. Fusarin mycotoxins are detected by high performance liquid chromatography with mass spectrometric detection. Detection limits for fusarin mycotoxins are at no greater than 5×10^{-6} g/kg (as harvested basis) for total fusarins (wet basis).

Full scale commercial operation with purpose designed fermenters commenced in 1994. Since then, there have been 88 operating campaigns. These campaigns have generated more than 6,000 samples for mycotoxin analysis, and no production of mycotoxins has ever been detected.

5. INTENDED USE IN FOOD

Mycoprotein is a food ingredient with high protein (ca 45%) and fiber content (ca 25%), a lipid content of 13% on a dry weight basis, and that has a high ratio of unsaturated to saturated fatty acids (at 3.5:1). Because of its textural and nutritional characteristics, mycoprotein may be processed into products used as an alternative to meat in a range of dishes. For example, mycoprotein may be used in frozen entrees as well as for the central component of a meal as a fillet, as pieces, mince, cold cuts, etc. As mycoprotein is a whole food, there are no technological self-limiting factors.

Mycoprotein provides good nutrition, convenience, and an appropriate texture, by virtue of its inherent hyphal structure, which is 'set' in use by a mixture with small quantities of a suitable binding agent. The texture created enables a wide range of properties to be achieved, including meat-like properties, fat-like properties, or cereal-like properties; addition of natural coloring and flavoring as necessary facilitates the production of product which can be used in a wide range of dishes. Selection of suitable binders ensures these properties are maintained throughout whatever types of further preparation and cooking processes the product undergoes during its preparation for consumption, either by the food processor, or by the consumer. Mycoprotein can be offered chilled (fresh), frozen, or in shelf stable ambient formats, given appropriate use of preservatives and packaging.

Mycoprotein will be present in final products in varying amounts - from about 85% in products like ingredients for home cooking, to about 40-50% in convenience products like meat-free burgers. The final dish in which mycoprotein is consumed will typically contain about 20-25% mycoprotein, such as in ready-prepared meals. In Europe, mycoprotein is currently sold in over 40 different retail formats.

6. CONSUMER EXPOSURE

6.1 Use in Europe

Mycoprotein, as a food ingredient under the branded line of Quorn™ products, was first test marketed regionally in the U.K. in 1985, and the full national launch took place when commercial scale production facilities came on stream in 1994. Current estimates are that overall, more than 10 million people in the U.K. consume mycoprotein products an average of 7 times per year. Of these, 3-4 million consume mycoprotein products more than 12 times per year. Additionally, about 2 million other Europeans, mainly from Switzerland, Sweden, Belgium, and Holland, consume mycoprotein products at a similar frequency. Market research shows that the product is consumed by all age ranges and social groups.

In the original 1986 food additive petition it was reported that the tolerance of mycoprotein during the human volunteer studies was extremely high. This was affirmed in the test marketing phase prior to full U.K. approval, and has been further demonstrated and confirmed during the last 15 years of regular and significantly increasing commercial product consumption. In a study of mycoprotein tolerance, Udall, *et al* (1984) reported an absence of reactions to mycoprotein.

Since marketing began, many communications have been received from consumers about a wide range of product issues, such as product availability, packaging, requests for background information, and positive comments about product quality. In addition, a small number of communications related to alleged adverse reactions to products containing mycoprotein have been reported. For example, Marlow Foods received 92 communications regarding adverse events in 1999, and 89 such communications in 2000 (see Table 10 for number and incidence of reported reactions). These figures equate to an incidence rate per the estimated number of consumers of 1 in 130,000 and 1 in 146,000. By comparison, adverse reactions in the U.K. to soy have been estimated at 1 in 350 and to fish/shellfish at 1 in 35 (Young *et al*, 1994).

Marlow Foods has carefully considered each adverse reaction report that it has received. Communications regarding alleged adverse reactions caused by mycoprotein are referred directly to the technical team and, if available, residual samples of the products are tested to determine if microbiological contamination has occurred. If the testing is negative, or if testing could not be done and there is no obvious reason to believe the reported incident is caused by contamination, then the incident is recorded as a potential adverse reaction. Thus, the number of alleged adverse reactions reflect the upper level of potential occurrence of such events. In personal communications to Marlow Foods, experts in the field have stated that the incidence of alleged adverse reactions in mycoprotein products is much smaller than for other protein foods.⁶

The reactions to mycoprotein that have occurred are primarily gastrointestinal (approximately 90%) with a much smaller incidence of rashes and edema. It is believed that only a very small percentage of these reactions (approximately 5%) are true food allergies (IgE-mediated), with the rest being food intolerances (see Tee, *et al* 1993). There is also evidence that, in some cases, the reactions are caused by other ingredients in the final food consumed (such as egg or milk proteins).

⁶ Dr. E. Young, Royal Amersham Hospital, U.K.; Prof. S. Taylor, University of Nebraska; Emeritus Prof. M. Lessof (ret'd), London.

Table 10 details the rates of occurrence of reported reactions.

Table 10 - Experience in use data

Year	Estimated # of consumers millions	# reported reactions	Incidence rate Consumers per response
1994	2.25	27	1 in 83,000
1995	4.25	60	1 in 71,000
1996	7.5	98	1 in 76,000
1997	10	111	1 in 90,000
1998	11	115	1 in 96,000
1999	12	92	1 in 130,000
2000	13	89	1 in 146,000

The extensive reported experience and performance data, when examined relative to other common foods indicates that, as part of a regular human diet, mycoprotein is very well tolerated.

6.2 Estimated Daily Intake

Calculating the Estimated Daily Intake (EDI) for a whole food, such as mycoprotein, presents different issues from a standard food additive EDI calculation. For example, for food additives, it can be determined how much of the additive is needed in each type of food to achieve the desired effect (i.e., how much sweetener is needed in a type of food to produce a sweetened effect) (Miller and Dwyer 2001, attached; Miller, *et al* 1999, attached). It can also be determined from food consumption surveys what is the quantity of consumption of a particular food category consumed by different subgroups within the population at the 90th or 95th percentile consumption level. An exaggerated consumption level can then be calculated from these data.

When calculating whole food consumption, it cannot be assumed that all foods in a certain category will contain the additive at issue (Miller, *et al* 1999, attached). For example, if mycoprotein is consumed as a protein source in a meat alternative product, it cannot be assumed that no other sources of protein will be consumed. Thus, traditional assumptions for the EDI calculation will result in unreasonably high and unrealistic estimates of consumption that do not indicate the safety of the ingredient.

A similar consideration influences the Acceptable Daily Intake (ADI) against which the EDI is usually compared to determine acceptability. The ADI is derived from the NOAEL (No Observed Adverse Effect Level) established in toxicological studies. With whole foods there is often no meaningful toxicological effect, even at the maximum achievable inclusion rate. Thus, any NOAEL quoted is inherently conservative, and the usual 'safety' factor applied to the NOAEL to derive the ADI is not available. This is not unusual with whole foods, which require a different assessment process.

Therefore, to determine EDI, the range of consumption must be estimated, and an inference must be drawn, based on the body of evidence available about the ingredient, as to whether the ingredient is safe (Miller, *et al* 1999, attached). Table 11 presents the results of the calculation of

EDIs using the three sources of data. The first estimates EDI of mycoprotein for the anticipated use as a meat substitute, using Pennington, *et al* (1983), as a data source. The second two, for homemade recipes and ready-to-eat meals, compare use to U.K. experience. The third EDI reflects the estimated consumption of vegetarians.

Table 11 - Calculation of Estimated Daily Intakes

Summary of Estimated Daily Intakes (EDI) for Mycoprotein						
		Estimated Intake per Serving (g)		Estimated Intake per serving (g/kg for 60kg adult)	Frequency of Consumption	EDI (g/kg/day for 60kg adult)
Source	Applicable Population	Wet Weight	Dry Weight	Dry Weight	Per Unit Time	Dry Weight
Pennington et. al. (1983)	All US Consumers	26-46	6-11	0.10-0.18	1 serving/day	0.10-0.18
British Market Data (Homemade Recipes)	All US Consumers	75	18.8	0.31	1 serving/4 weeks	0.01
British Market Data for (Ready-to-Eat Meals)	All US Consumers	26-70	6.5-17.5	0.11-0.29	1 serving/1 week	0.02-0.04
NLSMB (1994)	US Vegetarians and Meat Avoiders	EDI + 34	EDI + 8.5	EDI + 0.14	1 serving/day	0.24-0.46

The EDI for mycoprotein ranged from 0.01 - 0.18g dry weight/kg/day for the general US population and 0.24 - 0.46g dry weight/kg/day for the meat avoider/vegetarian population.

7. SAFETY STUDIES

An extensive database, consisting of thorough analytical data on identity and composition, manufacturing process, analysis for possible impurities, animal studies, digestibility and nutritional evaluation, and human studies is used to support the safety assessment. With regard to safety studies, animal toxicology studies have indicated that there are no health concerns from acute or chronic exposure and that mycoprotein supports normal growth and development in animal species. A reasonable assurance of safety is established from the analysis of all these data and subsequent clinical studies with human subjects that verified availability of nutrients and assessed tolerance.

The evaluation of the substantial body of safety data on mycoprotein supports the conclusion that the proposed uses would not be expected to produce any acute or chronic adverse effects. Therefore, the evaluation of all available analytical, animal and human safety data, as well as market information on typical levels and frequency of consumption of mycoprotein, leads to the conclusion that the proposed intended use of the additive will not be expected to produce any acute or chronic adverse effects in individuals consuming these food products under the intended conditions of use. The studies summarized below were reviewed by the the Expert Panel (Miller,

et al 1999, attached) and discussed in a July 2001 *Food Technology* article based on the Expert Panel's review (Miller and Dwyer 2001, attached).

7.1 Background

Due to the fact that mycoprotein is a whole food, several conventional components of food additive evaluation are not relevant to the safety evaluation of mycoprotein. For example, because mycoprotein is a whole food, there was not a need to study the absorption, metabolism, and other actions of its components. Instead, studies measured the digestibility and protein quality, identified the nature of the fiber, etc. Furthermore, while areas such as the effects of fat soluble dietary constituents and blood levels of constituents are studied for traditional food additives, as a whole food, these studies were not necessary for mycoprotein. However, to the extent they were relevant, a variety of *in vitro*, acute, subchronic, and chronic studies were conducted to verify the safety of mycoprotein.⁷

7.2 In Vitro Tests

In vitro testing showed that mycoprotein is nonmutagenic (Miller, *et al* 1999, attached; *see* Miller and Dwyer 2001, attached). Due to the tendency of high protein materials to cause false positive results, a modified *Salmonella* reverse mutation test was performed in liquid suspension. Mycoprotein was compared to chicken meat and both were nonmutagenic.

7.3 Acute Studies

Mycoprotein exposure showed no significant acute reactions in skin testing (Miller, *et al* 1999, attached; *see* Miller and Dwyer 2001, attached). Mycoprotein provoked no reaction when applied to intact or abraded skin of rabbits as a 5% or 10% aqueous suspension, twice daily for 14 days. Guinea pigs showed only mild erythema after similar treatment with the 10% suspension. Intra-dermal injections of mycoprotein induced a granulomatous response in both rabbits and guinea pigs associated with the insoluble component; however, the reaction was indistinguishable from that provoked by mushroom or other food- grade microorganisms. In addition, the estrogenic potential of mycoprotein was tested in the mouse and non-RNA reduced mycoprotein was tested in the Landrace pig, and no estrogenic potential was observed in either species.

7.4 Subchronic Toxicity

Four subchronic studies in rats were performed. The first study, of 22 weeks duration, tested mycoprotein at dietary levels of 26-52% in rats. The second study, of 13 weeks duration, tested dried cooked mycoprotein at dietary levels of 17.5% and 35% against casein controls. The third study was similar, but tested an undried form of mycoprotein. Growth, blood parameters, organ weights, histopathology, and mineral balances were examined. With the exception of caecal enlargement attributed to the high fiber content of the food, no significant findings were observed in the studies (*see* Miller and Dwyer 2001, attached).

⁷ Unless otherwise noted, the mycoprotein used in the studies was RNA-reduced.

The fourth study used a similar protocol and the diets consisted of 20% and 40% mycoprotein for 90 days. The study included in-utero exposure and 90-day studies in the offspring, and ophthalmoscopic examinations. Aside from the expected decrease in plasma cholesterol and triglycerides (a consistent feature of mycoprotein in all species tested), body weights (and consequently, liver weights) were marginally lower in the test group. This was attributed to reduced food consumption (*see* Miller and Dwyer 2001, attached). These subchronic studies supplement the chronic studies and indicate that the intended use of mycoprotein is not expected to product any adverse effects (Miller, *et al* 1999, attached).

A 13-week study was performed in which baboons were fed a diet containing mycoprotein that had not been RNA reduced. Experimental diets contained 26% or 51.5% mycoprotein and there was a casein control group. All baboons survived the study in good condition and there were no effects on growth, food and water intake, or hematology. Serum alkaline phosphatase activity was increased in the groups fed mycoprotein and serum alanine transaminase and aspartate transaminase activities were increased from normal levels in the casein control group. There were no changes in electrocardiograms. The test groups showed marginally higher liver weights than the casein control and colon and rectum weights were higher, but this reflected the increased contents. No histopathological changes were seen. It was concluded that mycoprotein was without adverse effect, but the casein diet produced a minimal degree of liver damage.

7.5 Chronic Studies

Three chronic studies evaluated the safety of mycoprotein, including a two-year carcinogenicity study in rats with an in-utero phase, a one-year dog study, and a two-generation study in rats with a teratology phase. The two-year study used freeze-dried mycoprotein equivalent to the commercial product, and a casein control. The mycoprotein was fed at 21% and 41% of the diet; at 41%, all of the dietary protein was derived from mycoprotein. Satisfactory growth and reproduction were observed in the F-0 generation, and sufficient litters were produced to permit the selection of rats for the 2-year study. Although minor intergroup variations were observed in some parameters, no significant adverse effects were noted in either phase of the study on growth, survival, incidence or onset of tumors or in hematological, urinary or histopathological examinations (Edwards, *et al* 2001; Milburn, *et al* 2001; *see* Miller and Dwyer 2001, attached), indicating that mycoprotein is not toxic or carcinogenic (Miller, *et al* 1999, attached).

A one-year study in beagle dogs fed diets containing 20% and 40% mycoprotein examined growth, clinical condition, urine, hematology, and histopathology. All diets supported good growth and no clinical signs that were diet-related were reported. As in the rat, dogs fed diets containing mycoprotein showed lower plasma cholesterol and triglycerides; females showed marginally greater thyroid weight. No gross or microscopic pathological findings were made that were diet related (Hodge, *et al* 2001; *see* Miller and Dwyer 2001, attached; *see* Miller, *et al* 1999, attached).

A two generation study demonstrated that mycoprotein can sustain normal reproduction and development. Rats were fed diets of mycoprotein at 12.5%, 25%, and 50% of the protein in the diet, and casein as 50% of the protein in the diet. The mycoprotein diets supported good growth and maturation in both parental generations and offspring, and fertility was within normal limits. There were no changes in reproductive function that were attributable to mycoprotein, and there

were no microscopic or pathological findings (Miller, *et al* 1999, attached; *see* Miller and Dwyer 2001, attached).

Finally, a series of studies performed in rats and rabbits to study teratogenesis and embryo toxicity showed no evidence of occurrence with mycoprotein-containing diets. Seven teratology studies on rats used a variety of mycoprotein, some being cooked, some not freeze dried, some RNA-reduced and some not RNA-reduced. The only effects observed were attributed to imbalances in the diet, and not to the mycoprotein component of the diet. A rabbit teratology study used diets with 25% mycoprotein, 50% mycoprotein, a control diet with soya as the protein source, and a commercial diet. The mycoprotein diets showed no teratogenic or embryotoxic effect (Miller, *et al* 1999, attached; *see* Miller and Dwyer 2001, attached).

7.6 Human Studies

Four studies were performed to assess human tolerance to mycoprotein. Mycoprotein, with levels ranging from 10 to 40 g/day, was fed to human volunteers for periods ranging from 1 to 30 days. Subjects who recorded adverse responses were rechallenged under controlled conditions. The reported events did not recur in any of the cases, except those in which the subject was shown to be atopic to fungus-derived foods. The results indicated that mycoprotein is well-tolerated by humans and has extremely low allergenic potential (Miller, *et al* 1999, attached; *see* Miller and Dwyer 2001, attached).

A significant history of use in Europe has also demonstrated that humans tolerate mycoprotein well, and human use has been examined in published studies (Tee, *et al* 1993; Udall, *et al* 1984). In addition, in the studies listed in section 3.3 that investigated the clinical nutrition effects of mycoprotein consumption, there were no reports of any adverse effects in the test subjects.

8. RISK ASSESSMENT

Because mycoprotein is a whole food, risk assessment cannot be conducted as with a traditional food additive under the techniques described in the FDA's "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food" (Redbook II). The traditional safety assessment of food additives uses the application of a 100-fold safety factor to the highest dose in an appropriate chronic animal toxicity study that is associated with a NOAEL to calculate the ADI (Pauli 1994; Borzelleca 1992; Vershcuren 1988). The safety factor is used to reflect differences between and within species and should also reflect the most sensitive human subpopulations (Borzelleca 1992). Chronic ingestion of the additive at the ADI is considered consistent with a reasonable certainty of no harm (Redbook II). The size of the safety factor used in the calculation of the ADI may vary; the more extensive and relevant the available toxicity data are, the smaller the safety factor can be (Kroes and Hicks 1990). This approach is not applicable for new foods because the similarity of composition of novel and conventional foods means that toxicity relating to the major nutrients is unlikely. Thus, toxicity studies are unlikely to yield effects and no effect levels as commonly required for food additives. Therefore, it is appropriate to establish a safety case for new foods by a customized approach.

Evaluation of the safety of mycoprotein has been accomplished through a review of the extensive data base of information available regarding mycoprotein, including detailed analysis of



composition, manufacturing process, impurities or contaminants, nutritional evaluation, and acute and chronic animal testing. A reasonable assurance of safety is established from the analysis of all these data, and subsequent clinical studies with human subjects as well as practical experience verified the availability of nutrients and assessed tolerance. The evaluation of the substantial body of safety data on mycoprotein supports the conclusion that the proposed uses would not be expected to produce any acute or chronic adverse effects. All available information about the potential market for mycoprotein as an alternative ingredient to meat in both home-cooked foods and ready-to-eat meals indicates that no acute or chronic adverse effects are expected in individuals consuming these food products.

9. FINDINGS OF EXPERT PANEL



In 1998, Marlow Foods convened an expert panel to review relevant data and information on the safety and suitability for use of mycoprotein (Miller, *et al* 1999, attached). The "Expert Panel on Mycoprotein" (Expert Panel) was chaired by Sanford A. Miller, Ph.D., Dean of the Graduate School of Biomedical Sciences at the University of Texas Health Sciences Center in San Antonio, Texas and formerly the director of the FDA Center for Food Safety and Applied Nutrition. The Expert Panel also included: Johanna T. Dwyer, D.Sc., R.D., Professor of Medicine (Nutrition) at Tufts University and Director of the Frances Stern Nutrition Center, New England Medical Center in Boston, Massachusetts; Eric A. Johnson, Sc.D., Professor, Department of Food Microbiology and Toxicology, Food Research Institute, University of Wisconsin-Madison, Wisconsin; Marcus Karel, Ph.D. Professor Emeritus of Food Science at Rutgers State University of New Jersey, and of Chemical and Food Engineering at Massachusetts Institute of Technology in Cambridge, Massachusetts; and Vernon R. Young, Ph.D., D.Sc., Professor Nutritional Biochemistry, Laboratory of Human Nutrition, School of Science, Massachusetts Institute of Technology in Cambridge, Massachusetts. Collectively, the members of the Expert Panel have expertise in toxicology, nutrition, food technology, engineering and production, food safety, and the regulatory requirements that apply to the determination of whether a food ingredient is safe and suitable.

The Expert Panel met in Washington, D.C., on April 6, 1998. In the advance of that meeting, the Panel members were provided with a detailed summary of the data and information submitted to the FDA in the 1986 food additive petition (and as subsequently amended) to demonstrate the safety and suitability of mycoprotein for food use. The panelists were advised that they could have access to any of the underlying data and information that were described in the summary.

The panel found as follows:



Mycoprotein is a well-characterized and well-studied novel food produced by the fermentation of a *Fusarium* strain of fungus. The edible food material that results from the fermentation possesses numerous desirable nutritional properties for a food: it contains high quality and readily digested protein, is low in fat and saturated fat and is cholesterol-free, and is a good source of fiber. The food material is extremely versatile, which permits it to be used in a variety of useful food forms: it can be fabricated to resemble a "burger," a fillet, a chicken breast and can be used as an ingredient in numerous other food applications.

The method of manufacture of mycoprotein is sound and complies with good manufacturing practices. The manufacturing process is well-characterized and controlled. The process has been demonstrated to a reasonable certainty to produce a consistent product that is free of contaminants that might pose a risk to human health. The manufacturing process for mycoprotein does not produce mycotoxins.

Mycoprotein has been well tested in a variety of appropriate models. There exists a substantial body of toxicological information about the ingredient derived from traditional toxicological studies, including those that examined the potential for chronic toxicity. Mycoprotein has also been tested for reproductive toxicity and teratogenicity. The Panel concludes based on these studies that mycoprotein is not a reproductive toxicant nor is it a teratogen. The Panel further concludes that mycoprotein does not cause chronic toxicity.

The safety of mycoprotein is further demonstrated by the extensive body of data available from clinical nutrition studies and its use in the United Kingdom over a substantial period of time. It is notable that over 15 million consumers consumed the product in the United Kingdom over 13 years (more than 400 million meals)⁸ without any evidence of intolerance. Moreover, the level of allergic reactions to the product is extraordinarily low and well below that of the products which are mainstays of the human diet.

Estimates of consumption of mycoprotein provide useful insight into potential levels of consumption in the United States.

Based on an evaluation of all available information about mycoprotein, the Expert Panel concludes that:

Mycoprotein is a safe and suitable ingredient for use in food as a source of protein in the diet and as a partial replacement for meat-derived protein in the human diet. Mycoprotein has been demonstrated to be safe for use in food to a reasonable scientific certainty.

10. BASIS FOR CONCLUDING THAT THERE IS COMMON KNOWLEDGE OF THE SAFETY OF MYCOPROTEIN

10.1 Introduction

GRAS status requires both technical evidence of safety (demonstrated in prior sections of this notification) and a basis to conclude that this technical evidence of safety is generally known and accepted. 62 Fed. Reg. at 18940. GRAS status achieved through scientific procedures is based on generally available and accepted scientific data, information, methods, or principles, which ordinarily are published and may be corroborated by unpublished scientific data, information, or methods. *Id.* at 18960; proposed 21 C.F.R. § 170.30(b). The common knowledge element of the GRAS standard is established by demonstrating that (1) the data and information relied on to

⁸ To end 1998, circa 500 million including sales onto mainland Europe.

establish the technical element are generally available; and (2) there is a basis to conclude that there is consensus among qualified experts about the safety of the substance for its intended use. 62 Fed. Reg. at 18940.

10.2 General Availability

Sections 3 – 8 of this notification contain information referring to the identity and composition of mycoprotein, production methods, intended use, consumer exposure, and scientific studies, that was considered in determining the safety of mycoprotein. This information is generally known and available through published literature and other publically available information regarding mycoprotein. Section 7 specifically addresses the studies that resulted in the safety determination, which are either published or otherwise generally available (see section 12 for citations). Therefore, with reference to the sections mentioned above, Marlow Foods determines that the scientific information establishing the safety of mycoprotein is generally available.

10.3 Consensus Among Qualified Experts

In the *Federal Register* notice concerning the proposed GRAS notification policy, FDA states that consensus among qualified experts of the safety of mycoprotein has been established by publication of data in peer-reviewed scientific journals, and that this information has been supplemented by: (1) publication of data and information in secondary scientific literature; (2) documentation of the opinion of an “expert panel”; or (3) the opinion or recommendation of an authoritative body. 62 Fed. Reg. at 18941. Not only has the scientific safety data and information regarding mycoprotein been published as reviewed in this notification, but numerous articles have been published regarding mycoprotein as a food, its production and its various nutritional and other properties, and its history of use in Europe.⁹ Furthermore, an expert panel of independent experts has documented the safety of mycoprotein. The panel concluded that mycoprotein is a “safe and suitable ingredient for use in food as a source of protein in the diet and as a partial replacement for meat-derived protein in the human diet,” and that the safety of mycoprotein for use in food has been demonstrated to a reasonable scientific certainty. Therefore, Marlow Foods concludes that there is consensus among qualified experts regarding the safety of mycoprotein.

11. GRAS DETERMINATION

Based on the information summarized in this notification, Marlow Foods concludes that mycoprotein for general use in foods is generally recognized as safe within the meaning of 201(s) of the Federal Food, Drug, and Cosmetic Act, 21 C.F.R. § 170.30, and the proposed rules described at 62 Fed. Reg. 18960.

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⁹ While a citation list of mycoprotein articles is not included in this notification, we would be happy to provide one upon request to demonstrate the general acceptance of mycoprotein and its safety.

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Myco-Protein

Report of the Expert Panel

June 1999

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MYCO-PROTEIN: REPORT OF THE EXPERT PANEL

I. Introduction

This document summarizes the conclusions of an expert panel convened at the request of Zeneca Group PLC to review relevant data and information on the safety and suitability for use of a novel food material known as "myco-protein." Myco-protein is a new whole food made from the cells of a *Fusarium* strain (ATCC # 20334), a fungus discovered growing in the soil in a field in Buckinghamshire in the United Kingdom in 1972. A food additive petition for the use of myco-protein is pending before the United States Food and Drug Administration (FDA). Foods made with myco-protein are available and widely consumed in the United Kingdom, Belgium, Holland, Ireland, and Switzerland.¹

The "Expert Panel on Myco-protein" (Expert Panel) was chaired by Sanford A. Miller, Ph.D., Dean of the Graduate School of Biomedical Sciences at the University of Texas Health Sciences Center in San Antonio, Texas and formerly the director of the FDA Center for Food Safety and Applied Nutrition. The Expert Panel also included: Johanna T. Dwyer, D.Sc., R.D., Professor of Medicine (Nutrition) at Tufts University and Director of the Frances Stern Nutrition Center, New England Medical Center in Boston, Massachusetts; Eric A. Johnson, Sc.D., Professor, Department of Food Microbiology and Toxicology, Food Research Institute, University of Wisconsin—Madison, Wisconsin; Marcus Karel, Ph.D. Professor Emeritus of Food Science at Rutgers State University of New Jersey, and of Chemical and Food Engineering at Massachusetts Institute of Technology in Cambridge, Massachusetts; and Vernon R. Young, Ph.D., D.Sc., Professor Nutritional Biochemistry, Laboratory of Human Nutrition, School of Science, Massachusetts Institute of Technology in Cambridge, Massachusetts. Collectively, the members of the Expert Panel have expertise in toxicology, nutrition, food technology, engineering, and production, food safety, and the regulatory requirements that apply to the determination of whether a food ingredient is safe and suitable.

The Expert Panel met in Washington, D.C., on April 6, 1998. In the advance of that meeting, the Panel members were provided with a detailed summary of the data and information submitted to the FDA to demonstrate the safety and suitability of myco-protein for food use. The panelists were advised that they could have access to any of the underlying data and information that were described in the summary.

The first portion of the Expert Panel meeting in April was devoted to a series of presentations by representatives of Zeneca, Inc. The presentations covered these topics: basic characteristics of the organism that produces myco-protein; production of myco-protein and the formulation of food products from it; safety and toxicology tests that have been conducted on myco-protein; nutritional composition and clinical nutrition

¹ Quorn™ is the brand name of food products made with myco-protein marketed by Marlow Food Ltd., a subsidiary of Zeneca Group PLC.

studies; and experience derived from the use of myco-protein in other countries over a number of years. Thereafter, the Expert Panel convened in executive session to deliberate. The conclusions of the Panel are set forth in this document.

II. Background



² When the organism that produces myco-protein was first isolated, it was classified as *Fusarium graminearum*. Subsequent review of this classification, aided by advances in the taxonomy of fungi generally and *Fusaria*, in particular, have led to the current classification as *Fusarium venenatum*.

³ Operating procedures to support good manufacturing practice are included within ISO 9001 registration under which the production process operates, and against which biannual external audits are conducted by BSI (British Standards Institute).



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B. Properties and Composition of Myco-Protein

As a member of the class of Fungi-Imperfecti, the *Fusarium* strain has a structure and composition typical of microfungi, except for a higher protein content. Myco-protein cells have a general morphology as indicated in Figures 2a and 2b.

Fig 2a:

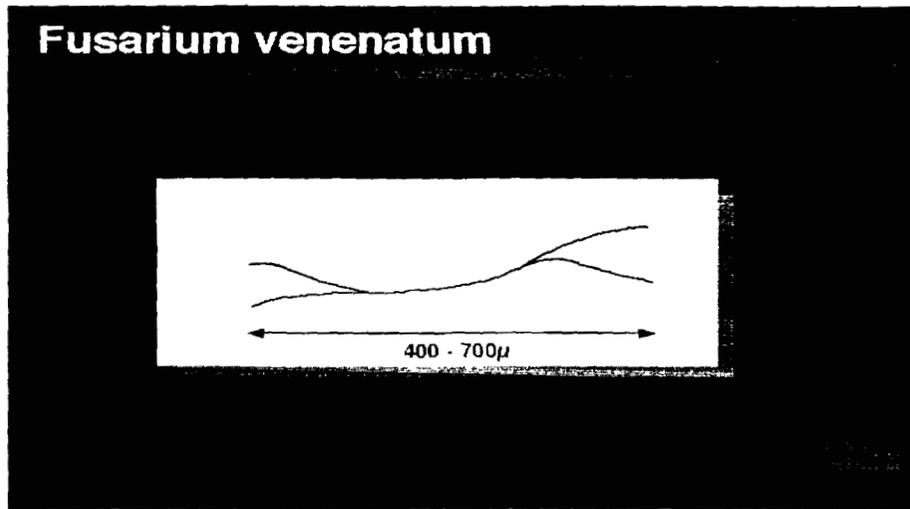
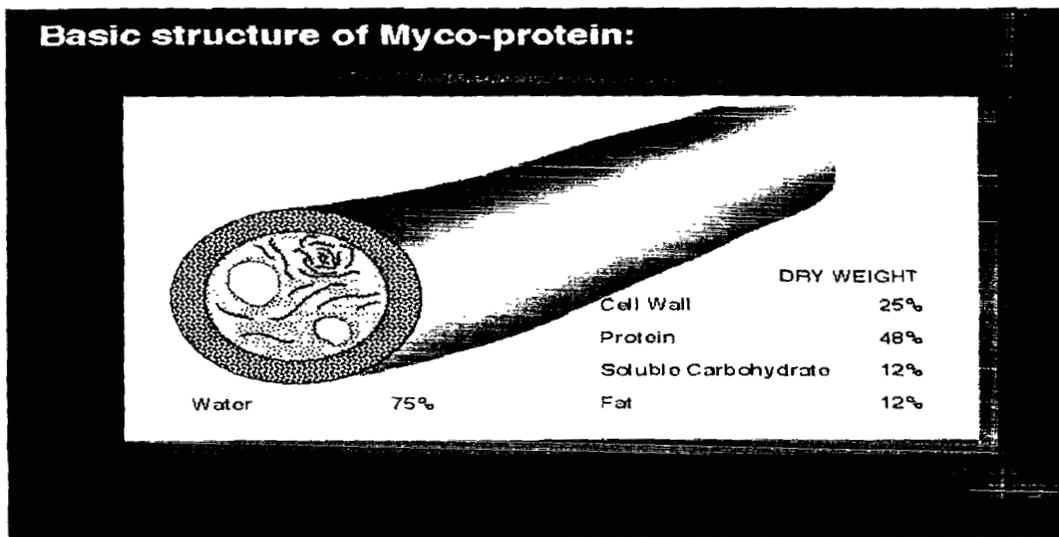


Fig 2b:



The cells are filamentous, which is responsible for the meat-like texture of myco-protein (see appended micrograph of myco-protein). Typical values for the physical characteristics of myco-protein are listed in Table 2.

Table 2: Physical Characteristics of Myco-protein

Physical Characteristics	Typical Values
Length	400-700 μ
Diameter	3-5 μ
Branch Frequency	1 per 250-300 μ

The myco-protein cell wall, which constitutes approximately one-third of the cell dry weight, is composed of chitin (poly-N-acetyl glucosamine) and β glucans (β -1:3 and β -1:6 glucosidic linkages). The lipid content is about 13 percent. The fatty acids contain an even number of carbon atoms. Significant quantities of ergosterol are present, but myco-protein is naturally cholesterol-free.

Typical analyses of myco-protein are presented in Table 3. Specifications for the microbiological quality and content of contaminants of myco-protein are presented in Table 4. Notably, sophisticated methodology is used to analyze for the presence of mycotoxins; mycotoxins have not been detected in myco-protein.

Table 3 – Typical Myco-protein Analyses

Analyses (gm/100 gm dry cell wt)

True Protein (α Amino Nitrogen x 6.22)	42-50
Crude Protein (Total Nitrogen x 6.25)	52-59
RNA	0.5-2
Total lipid	12-14
Dietary fiber	22-28
Ash	3-4

Mineral Analysis mg/kg

Phosphorus	9000-12000
Zinc	300-450
Copper	10-30
Magnesium	1700-2000
Calcium	1600-1800
Sodium	100-300
Potassium	3000-5000
Manganese	100-400

Heavy Metals mg/kg

Cadmium, Lead, Arsenic, Mercury : each not > 0.1

Fatty Acid Composition g/kg

Palmitic	16:0	13
Stearic	18:0	2
Oleic	18:1	14
Linoleic	18:2	43
Linolenic	18:3	9
Polyunsaturated/saturated ratio		3.5/1
Triglycerides and diglycerides		65% total lipid
Sterols and unsaponified		5%
Phospholipids		30%

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Table 3 – Con't.

Vitamin Analysis mg/kg

Thiamin B1	0.4
Riboflavin B2	9
Niacin B5	14
Pyridoxine B6	5
Pantothenic Acid	10
Folic Acid	0.4
Biotin	0.6

Amino-acid Analysis of Myco-protein (gm/100gm protein)

Lysine	8.3	Histidine	3.5
Methionine	2.1	Arginine	7.3
Cystine	0.8	Tyrosine	4.0
Threonine	5.5	Aspartic Acid	10.3
Tryptophan	1.6	Serine	5.1
Valine	6.2	Glutamic Acid	12.5
Leucine	8.6	Proline	4.5
Isoleucine	5.2	Glycine	4.3
Phenylalanine	4.9	Alanine	6.3

Carbohydrate

The carbohydrate composition of the cell wall is:

	gm/100gm cell dry weight
Chitin (poly-N-acetyl glucosamine)	12
β -1:3 and β -1:6 glucans	22
Glycogen	2
Other polysaccharides (galactomannans)	trace
TOTAL	36

Fiber:

Chitin and much of the β -glucans are conventionally analyzed as 'fiber'; myco-protein contains about 25% fiber on a dry weight basis.

Lipid:

The lipid content is about 13%, much of which occurs as phospho-lipid in the cell membranes. The fatty acids contain an even number of carbon atoms, since they are synthesized from glucose through the normal metabolic pathways.

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Table 4 – Myco-protein Specification

Definition

Myco-protein shall mean the mycelium of the fungus *Fusarium venenatum* (ATCC 20334), processed to reduce the level of ribonucleic acids.

Process

The fungus shall be grown axenically on a medium comprising food grade carbohydrate, together with other safe and suitable food grade or reagent grade ingredients using a process described in the manufacturing section. The mycelium shall be processed to reduce the RNA content.

Composition (calculated on a dry weight basis)

- i. **Protein** – Not less than 42% calculated as α -amino nitrogen x 6.22 where α -amino nitrogen is determined using the procedure of Moorhouse, Law & Maddix (1976), 7th Technicon International Congress, New York.
- ii. **Ribonucleic Acid** – Not more than 2% as determined by the procedure of Ogur and Rosen (1950), Analytical Biochemistry 25, 262.
- iii. **Ash** – Not more than 5% as determined by pre-ashing at 200°C and then heating at 650°C to constant weight.

Contaminants (calculated on a dry weight basis)

- i. **Metals** – Lead, arsenic, mercury and cadmium each not more than 0.1 mg/kg.
- ii. **Mycotoxins** – Myco-protein shall not contain mycotoxins in amounts which might constitute a hazard to health. Myco-protein shall be sampled periodically and analyzed for representative trichothecenes. Each trichothecene < 20 μ g/kg (as is basis).

Nutritional Value

The net protein utilization of myco-protein as determined in the rat shall not be less than 85% of that of casein calculated on the basis of dry weight and α -amino nitrogen content.

Microbiological Quality

- i. **Total Aerobic plate count** – Not more than 60% of samples to exceed 10,000/g. No sample to exceed 200,000/g.
- ii. **Yeasts and Moulds** – Not more than 100/g.
- iii. **Viable *Fusarium*** – Not detectable in 1 g.
- iv. **Coagulase positive *Staphylococci*** – Not detectable in 0.10g.
- v. ***Salmonellae*** – Not detectable in 25g.
- vi. **Fecal coliforms** – Not detectable in 0.1g.
- vii. ***Clostridium perfringens*** – Not detectable in 0.1g.

Myco-protein is suitable for incorporation into a wide variety of foods. Its principal intended use is as a source of good quality protein in main meal items. Myco-protein may be stored indefinitely at or below -18°C and can be stored at 0-4°C for at least 72 hours. The storage conditions common in the food industry for moist products that contain protein are appropriate for foods made with myco-protein.

C. Nutritional Composition of Myco-Protein

Myco-protein is an excellent source of high quality and easily digested protein and a good source of fiber. Myco-protein is also low in total fat, saturated fat, and sodium, and contains no cholesterol.

Protein Content. Myco-protein is an excellent source of high quality protein because it contains all of the nine essential amino acids necessary to support growth (Table 5).

Table 5: Essential Amino Acid Content of Myco-protein Compared to Other Foods that Contain Protein (g amino acids per 100 g edible portion)

Essential Amino Acids	Myco-Protein	Cow's Milk (i)	Egg (ii)	Beef (iii)	Soybeans (dry) (iv)	Peanuts (v)	Wheat (vi)
Histidine	0.39	0.09	0.30	0.66	0.98	0.65	0.32
Isoleucine	0.57	0.20	0.68	0.87	1.77	0.91	0.53
Leucine	0.95	0.32	1.10	1.53	2.97	1.67	0.93
Lysine	0.91	0.26	0.90	1.60	2.40	0.92	0.30
Methionine	0.23	0.08	0.39	0.50	0.49	0.32	0.22
Phenylalanine	0.54	0.16	0.66	0.76	1.91	1.30	0.68
Tryptophan	0.18	0.05	0.16	0.22	0.53	0.25	0.18
Threonine	0.61	0.15	0.60	0.84	1.59	0.88	0.37
Valine	0.60	0.22	0.76	0.94	1.82	1.08	0.59

(i) Whole fluid milk [3.3% fat] (ii) Raw fresh egg (iii) Ground beef (regular, baked-medium) (iv) Mature raw soybeans (v) Raw peanuts [all types] (vi) Durum wheat

Source: USDA Nutrient Data Base for Standard Reference, March 12, 1998

To evaluate protein quality, the FDA requires that the Protein Digestibility-Corrected Amino Acid Scoring (PDCAAS) method be used for most nutrition labeling purposes. This method takes into account the food protein's essential amino acid profile, its digestibility, and its ability to supply essential amino acids in amounts required by humans. It compares the essential amino acid profile of a food, corrected for digestibility, to the Food and Agricultural Organization/World Health Organization 2-5 year old essential amino acid requirement pattern. The 2-5 year old pattern is used because it is the most demanding pattern of any age group other than infants.

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The PDCAAS for myco-protein is 0.91 based on a digestibility factor of 78 percent for myco-protein. Table 6 shows how myco-protein compares to the PDCAAS of other food proteins.

Table 6: Protein Digestibility Corrected Amino Acid Score of Selected Food Proteins

Protein Source	PDCAAS	Data Source
Quorn pieces	1.0	(iv)
Casein	1.0	(i)
Egg white	1.0	(i)
Chicken (light meat-roasted)	1.0	(iii)
Turkey (ground-cooked)	0.97	(iii)
Fish (Cod-dry cooked)	0.96	(iii)
Soybean protein	0.94	(ii)
Beef	0.92	(i)
Myco-protein	0.91	(iv)
Pea flour	0.69	(i)
Kidney beans (canned)	0.68	(i)
Rolled oats	0.57	(i)
Lentils (canned)	0.52	(i)
Peanut meal	0.52	(i)
Whole wheat	0.40	(i)
Wheat gluten	0.25	(i)

- Sources:
- (i) FAO/WHO Joint Report 1989.
 - (ii) Sarwar and McDonough, 1990.
 - (iii) Calculated from amino acid data in USDA Nutrient Data Base for Standard Reference, March 12, 1998 (assumes a digestibility equivalent to beef = 94%).
 - (iv) Calculated from Marlow Foods data.

The protein quality of myco-protein is similar to soy protein. But when incorporated into Quorn™ products⁴, the small amounts of added egg albumen and milk proteins enhance protein quality, as shown in Table 6. Because consumers find the taste and texture of Quorn products exceptionally good, the high quality protein content of Quorn products is not a compromise for meat eaters, but an extremely acceptable alternative for part of the meat in the diet.

Fat Content. Myco-protein contains only 3 g of fat per 100 g. The fat is rich in mono- and polyunsaturated fatty acids, the latter being predominantly the essential fatty acids linoleic (C18:2, ω-6) and α-linolenic (C18:3, ω-3) (Table 7). Myco-protein contains no trans-fatty acids.

Table 7. Fatty Acid Profile of Myco-protein (Fat Content = 3 g/100 g)

Fatty Acid	Grams per 100 Grams Fat in	Grams per 100 Grams
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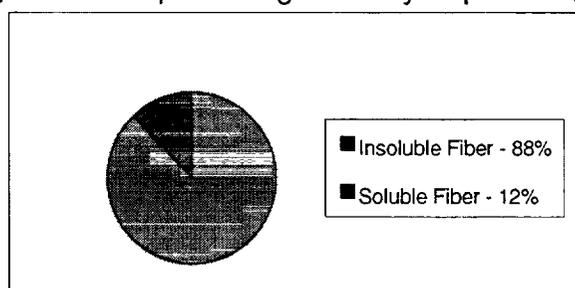
⁴Quorn™ is the brand name of food products made with myco-protein marketed by Marlow Foods Ltd., a subsidiary of Zeneca Group PLC.

		Myco-protein	Myco-protein
C16:	Palmitic	9.3	0.3
C18:0	Stearic	2.0	0.1
C18:1	Oleic	9.6	0.3
C18:2	Linoleic	29.8	1.0
C18:3	α -Linolenic	13.5	0.4

Fiber Content. Myco-protein contains 6 g dietary fiber per 100 g. The fiber consists of 35 percent chitin (poly-N-acetyl glucosamine) and 65 percent β glucans. The fiber is 88 percent insoluble and 12 percent soluble (Figure 3).

Figure 3. Fiber Content of Myco-protein

Total: 6 grams fiber per 100 grams myco-protein (wet basis)



Myco-protein does not contain any phytic acid or phytic salts that may interfere with mineral absorption. Research conducted at the Dunn Laboratory in Cambridge, England, showed myco-protein had no significant effect on the absorption of calcium, magnesium, phosphorus, zinc or iron in comparison with a polysaccharide-free diet.

Micronutrient Content. Myco-protein is low in sodium and contains small amounts of other minerals (Table 3). In comparison to meat, myco-protein in general is not a good source of vitamins, except for riboflavin.

III. Context for the Expert Panel's Evaluation of Myco-Protein for Food Use

A. Regulatory Framework

Although myco-protein is fundamentally a novel food, it is regulated in the United States as a "food additive."⁵ Under the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 301 et. seq. (FDC Act), a food additive is required to be shown to be safe ("reasonable certainty of no harm") before a regulation authorizing its use can be issued by FDA. Safety within the meaning of section 408 of the FDC Act, 21 U.S.C. §348, is typically demonstrated by generating data concerning the composition of the material, its method of manufacture, nutritional characteristics, and toxicology (based on a series of

⁵ FDA classified myco-protein as a "macroingredient"—"a class of food additives that are intended to replace conventional macronutrients such as fats, proteins, and carbohydrates and are intended for use at relatively high levels in foods." (FDA Draft Redbook II, 1993). The classification of myco-protein as a macroingredient is appropriate.

experiments in animals). In some instances, the safety evaluation is also based on clinical trials involving humans. The safety evaluation involves taking all of the available information that bears on the safety of the ingredient and forming a considered scientific judgment as to whether the ingredient will be safe under its intended conditions of use (the anticipated foods in which the ingredient will be used, the levels of anticipated use, and the consumption patterns of those foods in the population and in subgroups of the population).

B. Considerations in the Evaluation of the Safety of Myco-Protein

As a novel whole food, the safety evaluation of myco-protein presents a somewhat different set of issues than are present with conventional food additives. As with most foods, myco-protein is composed of numerous individual components and its complete characterization is not possible.⁶ Furthermore, myco-protein is a food that may form a significant part of the diet and contribute substantially to the nutrition of those who consume it.

The fact that myco-protein may form a significant part of the diet means that the standard approach to toxicological tests of potential new food ingredients -- animal studies in which the test material is fed to the animals at many multiples of the level of exposure expected in humans -- is not possible. As a result, the safety assessment of a macroingredient, such as myco-protein, relies less on inferences drawn from standard animal tests, and more on data and information about the nutrient composition, digestibility, possible presence of contaminants and by-products, and other available information about the ingredient.

The toxicological tests that were conducted on myco-protein were designed (in consultation with the FDA) to account for the unique issues that arise in the testing of novel foods. For example, the similarity in basic composition between the novel food and conventional foods means that toxicity relating to the major nutrients is improbable. Nevertheless, it is possible that a minor component or an unexpected interaction of nutrients, may suggest a toxic effect. Acute and chronic studies in animals (mainly rodents) are useful in helping to identify any unanticipated toxicity.

The design of chronic studies for a novel food presents a formidable challenge. In order to expose the test animals to a multiple of the amount of the material anticipated in human use, specially formulated diets must be used. The animals will be receiving substantially all of their nutrition from the test material as incorporated into the test diets. Great care must be taken to balance these diets for micronutrient content in order to minimize the occurrence of spurious results arising from imbalances in animal nutrition. Even when great care in diet formulation is taken, minor intergroup differences in parameters such as growth rate or mature body weight may still be expected. These types of differences reflect the different properties of the diets fed to the animals, as opposed to suggesting effects related to the test material. The probability of this type of effect increases with the proportion of the new food in the test

⁶ By contrast, a conventional food additive is typically fully characterized and described by a precise chemical formula.

diets, reflecting the generally observed pattern with traditional foods where overdependence on a single food for basic nutrition produces changes from the normal physiological state associated with a properly balanced and varied diet.

Once animal tests have established the absence of toxic effects associated with consumption of the novel food, clinical studies in human volunteers are conducted to assess palatability, allergenicity, nutritional effect, and overall tolerance. The information obtained from human clinical studies is especially important in the case of novel foods.

IV. Safety Assessment of Myco-Protein

A. Introduction

Myco-protein has been the subject of an extensive program of testing, including laboratory tests, both *in vitro* and in experimental animals, and in human clinical trials. In addition, there is substantial information on human exposure available about myco-protein based on many years of its use in other countries, notably in the United Kingdom. An assessment of the available information as a whole establishes that myco-protein is not toxic, carcinogenic, teratogenic, or genotoxic. The available information also establishes that myco-protein is well-tolerated in humans and has a low allergenic potential. Based on all of this information, the Expert Panel concluded that myco-protein is safe and suitable for use in food, subject only to the traditional limitation that its use be in accordance with good manufacturing practices.

B. Summary of Available Information

A brief summary of the information bearing on the safety of myco-protein that was relied on by the Expert Panel follows.

(1) Toxicological Data and Information

(a) *In vitro Studies:* *In vitro* tests showed that myco-protein was not mutagenic in a modified *Salmonella* reverse mutation assay. The Expert Panel concluded that myco-protein is non-genotoxic.

(b) *Acute Studies:* Aqueous solutions of myco-protein provoked no reaction when applied to rabbits; guinea pigs showed mild erythema. Intra-dermal injections of myco-protein induced a granulomatous response in both rabbits and guinea pigs, associated with the insoluble component. The reaction was indistinguishable from that provoked by mushroom or other food grade micro organisms. No estrogenic potential was observed in the mouse or Landrace pig. The Expert Panel concluded that animal studies show that there are no health concerns from acute exposure to myco-protein.

(c) *Preliminary Feeding Studies*⁷: Initial short-term studies in rats were conducted to assess the suitability of diets containing myco-protein. The studies showed that formulated diets in which myco-protein provided most or all of the protein content supported satisfactory growth. For example, in a study involving the use of purified ingredient diets in which myco-protein was included at 20 and 40 percent (with a casein control), myco-protein was shown to support normal growth and development. These studies also showed no significant differences in clinical pathology or histopathology between control and treated groups and consumption of myco-protein had no effect on the availability and balance of calcium, phosphorus, magnesium, iron, copper or zinc. A preliminary study in dogs showed that myco-protein consumption supported weight gain comparable to control diets. As in other preliminary studies, plasma cholesterol and triglyceride levels were reduced in the test animals fed myco-protein. The Expert Panel concluded that preliminary feeding studies supplement definitive chronic feeding studies and indicate that the proposed intended use of myco-protein will not be expected to produce any adverse effects.

(d) *Chronic Feeding Studies*: Definitive chronic feeding studies involving myco-protein included a 2-year carcinogenicity study in rats with an *in utero* phase, a 1-year study in the dog, and a two-generation study in the rat with a teratology phase. These studies show that no chronic effects are associated with the consumption of myco-protein.

(i) The 2-year study in rats with an *in utero* phase tested myco-protein at levels of 41 percent and 21 percent against a casein control and a commercial laboratory diet. All diets supported satisfactory growth and reproduction in the F₀ generation (76 pairs) and sufficient litters were produced to allow the selection of the main study rats (56 males and females each). There was no significant intergroup difference in the type or incidence of tumors, and diets containing myco-protein were not associated with abnormally early onset of any tumors seen. Pigmentation of various organs was more prominent in rats fed myco-protein and is considered to reflect the high levels of polyunsaturated lipids present. The Expert Panel concluded that myco-protein is not toxic or carcinogenic.

(ii) A 1-year study in dogs tested groups of four beagle dogs of each sex with diets containing 40 percent and 20 percent myco-protein against a casein control. A further group was fed a commercial diet. All diets supported growth at comparable rates, and there were no clinical signs that were diet-related. The Expert Panel concluded that myco-protein is without adverse effect in the beagle dog.

⁷ Several of the preliminary studies of myco-protein were conducted many years ago before the introduction of Good Laboratory Practice requirements and before full recognition of the importance of the calcium:phosphorus ratio as a factor in nephrocalcinosis in the rat. These early studies found a range of histological changes, especially in the kidney, which resulted from mineral imbalance in the diet. Later feeding studies corrected for this mineral imbalance. The Expert Panel concluded that there exists no basis to conclude that the effects seen in the early studies were due to myco-protein.

(iii) To study the ability of myco-protein to sustain normal reproduction and development, a multigenerational rat study was conducted. In consultation with FDA, a total of four diets were prepared: (1) Myco-protein as 50 percent of the dietary protein, with the remainder from cereal and animal sources (2) myco-protein as 25 percent of the total protein, 25 percent from casein, and the remainder from other ingredients (3) myco-protein as 12.5 percent of the protein and 37.5 percent as casein, and (4) casein control with 50 percent of total protein from casein. Two generations of rats were studied. The results showed all diets supported satisfactory growth and maturation in both parental generations and offspring. There were no significant intergroup differences in fertility, reproductive performance, litter size or survival to weaning.

This study was supplemented by specific studies designed to assess development toxicity, or teratology, in both rats and rabbits. These studies gave no evidence of teratogenic or embryotoxic effect of myco-protein.

The Expert Panel concluded that myco-protein does not adversely affect fertility, reproduction or post natal growth of the rat and there is no evidence of teratogenic or embryotoxic effect of myco-protein.

(iv) **Human Clinical Studies:** Four studies were conducted to assess acceptability of myco-protein as a food and any intolerance. The results indicated that myco-protein is well tolerated in human trials with an extremely low allergenic potential. The Expert Panel concluded that myco-protein is well tolerated in humans.

(v) **Toxicology Summary:** The Expert Panel concluded that the results of animal and human studies reviewed show that myco-protein is not toxic, carcinogenic, teratogenic or genotoxic. The Expert Panel also concluded that myco-protein is well tolerated in humans with a low allergenic potential.

(2) Experience in Use of Myco-Protein

Consumption of myco-protein in the form of Quorn food products has been shown to be extremely well tolerated. In the United Kingdom (UK) and Europe, over 15 million people have consumed Quorn, with over 10 million regular consumers currently in the UK.

Table 8 shows the number of reported adverse reactions to myco-protein from the years 1994 – 1997. The Expert Panel concluded that the incidence of intolerance of myco-protein appears to be below that reported for other foods in common use.

Table 8: Experience in Use - Adverse Reactions

Year	Estimated No. of Consumers (million)	No. of Reported Reactions*	Incidence Rate/Consumers	Incidence Rate Reports per million products sold
1994	2.25	27	1 in 83,000	5.7
1995	4.25	60	1 in 71,000	4.9
1996	7.5	98	1 in 76,000	3.6
1997	10.0	111	1 in 90,000	3.1

*The adverse reactions are typically mild and self-limiting intestinal discomfort.

(3) Estimated Daily Intake of Myco-Protein

Similar to the safety considerations discussed above, estimating the daily intake of a novel whole food presents a somewhat different set of issues than are present with conventional food additives.

Table 9 presents the results of the calculation of estimated daily intakes using four different approaches: (a) anticipated use as a replacement for meat using Pennington as a data source; (b) anticipated use as a replacement for meat using data from the 1994-96 Continuing Survey of Food Intakes by Individuals ("94-96 CSFII"); (c) comparable use to the UK experience; and (d) estimated consumption of meat avoiders/ vegetarians.

Table 9: Calculation of Estimated Daily Intakes

Summary of Estimated Daily Intakes (EDI) for Myco-protein						
Source	Applicable Population	Estimated Intake per Serving (g) Estimated Intake		Estimated Intake per serving (g/kg for 60kg adult)	Frequency of Consumption	EDI (g/kg/day for 60 kg adult)
		Wet Weight	Dry Weight	Dry Weight	Per Unit Time	Dry Weight
Pennington et al. (1981)	All US consumers	26-46	6-11	0.10-0.18	1 serving/day	0.10-0.18
94-96 CSFII	All US consumers	70-139	17-33	0.28-0.56	1 serving/day	0.28-0.56
British Market Data (Homemade Recipes)	All US consumers	75	18.8	0.31	1 serving/4 weeks	0.01
British Market Data for (Ready-to-Eat Meals)	All US consumers	26-70	6.5-17.5	0.11-0.29	1 serving/1 week	0.02-0.04
NLSMC (1994)	US vegetarians and meat avoiders	EDI + 34	EDI + 8.5	EDI + 0.14	1 serving/day	0.24-0.46

The calculation of an EDI for a whole food, such as myco-protein, is necessarily a less precise exercise than the comparable calculation for a functional food additive, such as a nonnutritive sweetener. In the later case, one can determine how much of the additive is needed in each specific food type to achieve the desired functional effect (e.g., how much of a sweetener is needed to achieve a known and specific level of sweetness). Further, one can determine from food consumption surveys the quantity of a food category (soft drinks, for example) that is consumed by different subgroups within the population at the 90th or 95th percentile consumption level. Using these data, and assuming that **all** of the food category of interest will contain the additive in question, one can postulate a possible (albeit exaggerated) consumption level. This approach is not possible for a whole food, such as myco-protein.

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In the case of a whole food, one cannot employ the assumption that all foods of a certain category will contain the additive at issue.⁸ Myco-protein is intended to be used as a substitute for meat or poultry products. Whereas a consumer of diet soft drinks could conceivably consume exclusively those diet soft drinks which contain a specific nonnutritive sweetener, consumers of myco-protein will not do so to the exclusion of other sources of protein in the diet. Even a consumer who is enamored of chicken, does not consume chicken to the exclusion of other meat or poultry sources. Likewise, meat avoiders (including vegetarians) do not exclusively rely on a single source of protein in their diet. Thus, using traditional assumptions in an EDI calculation for a whole food produces unreasonably high and unrealistic estimates of consumption that are not helpful in determining whether the ingredient is safe.

Moreover, the purpose of the EDI calculation is to be able to compare an estimate of consumption with the Acceptable Daily Intake ("ADI"), a value determined from traditional toxicological studies conducted on the ingredient. The calculation of an ADI requires that toxicological effects be seen in those studies and that a "No Observed Adverse Effect Level" or "NOAEL" be determined. In the case of most whole foods, and certainly in the case of myco-protein, the ADI calculation cannot be usefully made because there are no adverse effects seen in the toxicological studies.

Consequently, in the case of a whole food, the utility of the estimates of consumption is limited to suggesting a potential range of consumption of the ingredient and permitting an inference, based on an assessment of the entire body of available data on the ingredient, as to whether the use of the ingredient will be safe. The Expert Panel considered the estimates of consumption to be reasonable predictions of consumer exposure to myco-protein. Specifically, the Panel concluded that the estimates based on U.S. food consumption data were accurate and sufficiently inclusive of food categories in which myco-protein might be used to confidently identify the range of potential consumption by various population groups. The Panel also found it useful to compare these estimates with information based on actual use of myco-protein in the United Kingdom over several years, especially since food consumption patterns in the United Kingdom for meat and poultry and replacements for them are reasonably comparable to those in the United States. Overall, the data and analysis on potential consumption of myco-protein in the United States permit conclusions to be drawn about the safety of the product for use in food.

⁸ The calculation of estimated daily intake using the 94-96 CSFII data assumed that myco-protein would replace all meat in the diet. This assumption obviously results in an exaggerated consumption estimate.

V. Conclusions And Opinion of the Expert Panel

Myco-protein is a well-characterized and well-studied novel food produced by the fermentation of a *Fusarium* strain of fungus. The edible food material that results from the fermentation possesses numerous desirable nutritional properties for a food: it contains high quality and readily digested protein, is low in fat and saturated fat and is cholesterol-free, and is a good source of fiber. The food material is extremely versatile, which permits it to be used in a variety of useful food forms: it can be fabricated to resemble a "burger," a fillet, a chicken breast and can be used as an ingredient in numerous other food applications.

The method of manufacture of myco-protein is sound and complies with good manufacturing practices. The manufacturing process is well-characterized and controlled. The process has been demonstrated to a reasonable certainty to produce a consistent product that is free of contaminants that might pose a risk to human health. The manufacturing process for myco-protein does not produce myco-toxins.

Myco-protein has been well tested in a variety of appropriate models. There exists a substantial body of toxicological information about the ingredient derived from traditional toxicological studies, including those that examined the potential for chronic toxicity. Myco-protein has also been tested for reproductive toxicity and teratogenicity. The Panel concludes based on these studies that myco-protein is not a reproductive toxicant nor is it a teratogen. The Panel further concludes that myco-protein does not cause chronic toxicity.

The safety of myco-protein is further demonstrated by the extensive body of data available from clinical nutrition studies and its use in the United Kingdom over a substantial period of time. It is notable that over 15 million consumers consumed the product in the United Kingdom over 13 years (more than 400 million meals)⁹ without any evidence of intolerance. Moreover, the level of allergic reactions to the product is extraordinarily low and well below that of the products which are mainstays of the human diet.

Estimates of consumption of myco-protein provide useful insight into potential levels of consumption in the United States.

⁹ To end 1998, circa 500 million including sales into mainland Europe.

Based on an evaluation of all available information about myco-protein, the Expert Panel concludes that:

Myco-protein is a safe and suitable ingredient for use in food as a source of protein in the diet and as a partial replacement for meat-derived protein in the human diet. Myco-protein has been demonstrated to be safe for use in food to a reasonable scientific certainty.

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