Foreword

This manual marks an important milestone in the development of the mushroom industry in Australia. It is the culmination of several years of intense global scientific endeavour in which Australian scientists and mushroom industry personnel have played a significant role in developing vitamin D mushrooms that will be enjoyed and appreciated by consumers all around the world.

These ingenious Australians helped transform a chance discovery by a Finnish scientist just fifteen years ago into an affordable food that will make a significant and unique contribution to the health and well-being of most people. For Australians, vitamin D mushrooms will arguably be the most important food contribution to public health in this country since First Fleeters ate limes to overcome scurvy.

Vitamin D insufficiency and the increasing array of health problems associated with it, has received a lot of recent attention from medical researchers and public health authorities, particularly in North America. Vitamin D guru and author of the *"The Vitamin D Solution"*, Boston University Professor of Medicine, Dr Michael Holick, claims "Vitamin D deficiency is probably the most common nutritional and medical condition in the world, affecting more than 50 per cent of the world's population".

Unfortunately, sourcing adequate vitamin D from the diet has been difficult so the discovery that just 100 grams of vitamin D mushrooms (3 medium buttons) can supply 100% of the daily vitamin D need is a major public health breakthrough.

The purpose of this manual is to bring together the technical information needed to support the production of vitamin D mushrooms. It is primarily designed for growers who will produce the new mushroom but the range of scientific information provided will enable others in the supply chain to understand and support this exciting new addition to the mushroom category in Australia.

The manual is divided into Sections. The first Section explains the human health and nutrition aspects of vitamin D while Section 2 summarises the scientific underpinnings of the technology. It details the research findings and how they may be utilised commercially. Importantly, the research demonstrates how the technology mimicks nature to produce vitamin D_2 naturally in the mushrooms in the same way it occurs for mushrooms growing in the wild. A further Section recommends Quality Assurance and Food Safety protocols for use during the commercial production of vitamin D mushrooms to ensure the product consistently meets the vitamin D information stated on the package label.

A number of Critical Issues should be addressed by growers preparing to produce vitamin D mushrooms. They can be found at the beginning of the manual.

Although the manual is primarily written for commercial growers who intend to produce vitamin D mushrooms, it will also be of value to medical personnel, nutritionists, retail organisations and other interested parties.

Greg Seymour General Manager, AMGA

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CRITICAL ISSUES FOR INTENDING PRODUCERS OF VITAMIN D MUSHROOMS

***** Read this manual carefully.

* Decide between a pulsed UV lighting system and a flood UV lighting system for your situation. (2.3)

***** Users of both pulsed and flood UV lighting systems MUST consult the equipment supplier for advice/design of the specific system to ensure all factors are optimised to achieve required levels of vitamin D_2 . (2.4 and 2.5)

* Regularly check that UV light equipment is operating according the design specifications.

* Ensure that the external exhaust system is effectively removing hot air from around the lamp housing. (2.4 and 2.5)

* Ensure that all UV light generating equipment is effectively screened to protect personnel who operate in the area. (2.4 and 2.5 and 2.6)

* Personnel MUST wear protective clothing and UV light-blocking safety glasses when operating or in close proximity to the UV equipment. (2.6)

* Prior to commercial operation of UV light systems, trial punnets of mushrooms should be treated and samples for analysis sent to a National Association of Testing Authority (NATA)-recognised laboratory that is experienced in vitamin D_2 analysis using the methodology described by Koyyalamudi *et al.* (2009). (2.4.2 and 2.5.2 and 2.7)

* Critical attention must be shown to PRECAUTIONS FOR PERSONNEL WHO HAVE ACCESS/EXPOSURE TO ULTRAVIOLET LIGHT SOURCES. (2.6)

* Business owners must ensure adequate procedural training is provided for all personnel working with UV light equipment. (3.1.3 and 3.3.1)

There are Legal requirements that apply to nutrient claims made in labelling and advertising. Producers and marketers of vitamin D mushrooms should seek legal advice on these issues.

SECTION 1: VITAMIN D, HUMAN HEALTH AND NUTRITION

Vitamin D has become of great interest to health researchers and medical authorities around the world, especially since 2005. Research has linked adequate vitamin D to a rapidly increasing number of benefits beyond healthy bones and the prevention of rickets and osteoporosis. These benefits include a decreased risk of multiple sclerosis, diabetes, rheumatoid arthritis, high blood pressure, influenza, heart disease, bowel cancer, breast cancer, prostate cancer, ovarian cancer and depression (Lee 2008; Wang 2008; Dobnig 2008). There is a link between vitamin D deficiency and the risk of falls and bone fragility fractures (Nowson 2004).

1.1 Vitamin D and human health:

■ Vitamin D is required to maintain normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralisation of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body (WHO 2004).

• The term Vitamin D is often used to cover both vitamin D_2 and vitamin D_3 . The most common dietary form of vitamin D is vitamin D_3 (cholecalciferol) available from foods such as oily fish and table margarine. Vitamin D_2 (ergocalciferol) comes from the conversion of ergosterol in plants and mushrooms to ergocalciferol via the action of sunlight. When cholecalciferol (D_3) is further converted to 1, 25dihydroxycholecalciferol via the liver and kidneys it becomes a potent steroid hormone. When consumed, ergocalciferol (D_2) is also converted to 1, 25dihydroxycholecalciferol via the liver and kidneys. One role of this hormone is to enhance the absorption of calcium from the intestine.

The body can make adequate vitamin D providing it is exposed to enough sunlight. It does not have to rely on dietary sources until sunlight exposure is low. It is the action of sunlight that converts 7-dehydrocholesterol to cholecalciferol (vitamin D₃).

7-dehydrocholesterol	-> (sunlight)	-> c	holecalciferol
Pro-vitamin D ₃ (made in	n liver)		Vitamin D ₃ (inactive)
	,		
Cholecalciferol -> (live	r) -> 25-hydroxy D_3	-> (kidne	y) -> 1,25 dihydroxy D_3
Vitamin D ₃	Calcidiol		Calcitriol
(inactive)	(inactive)		(active)

- Vitamin D is transported around the body in a complex with a specific vitamin D-binding α-globulin protein (WHO 2004). Almost all of the vitamin D in circulation is 25-hydroxy D₃. Plasma levels of 25 (hydroxy) D₃ are measured to determine vitamin D status.
- There was a widespread assumption that the two forms of vitamin D (D₂ and D₃) are equivalent. The World Health Organisation (2004) states: "From a nutritional

perspective, the two forms are metabolized similarly in humans, are equal in potency, and can be considered equivalent."

• This assumption has been disputed recently. Armas (2004) suggests that dietary D_2 potency was less than a third of D_3 . Houghton (2006) reviewed the data to show that D_2 and D_3 are not equivalent and that in humans D_3 is around 2-3 times more potent than D_2 . However, a recent article claims that vitamin D_2 is just as effective as vitamin D_3 in maintaining plasma levels of vitamin D when given as a supplement (Holick 2008). Further research by the same group showed that both D_2 and D_3 were equal in their ability to raise plasma vitamin D (25(OH) cholecalciferol) when given as a supplement in orange juice (Biancuzzo 2010).

There is still not broad agreement whether vitamin D₂ is equally as effective as vitamin D₃ in maintaining plasma vitamin D levels. One advantage of ultra-violet (UV) light-exposed mushrooms is that high levels of vitamin D₂ can easily be attained to meet the expected needs of humans.

1.2 Populations at risk for vitamin D insufficiency:

The WHO report states that the main populations at risk for vitamin D deficiency are infants, adolescents, the elderly, and pregnant and lactating women. Infants have relatively large needs for vitamin D due to their high rate of skeletal growth. This can be further compounded by restricted sunlight exposure. Skeletal growth is rapid during puberty making adolescents more susceptible to vitamin D deficiency. With ageing there is a reduced amount of skin conversion to cholecalciferol, reduced conversion to calcitriol in the kidneys and a lesser response by target tissues such as bone. Again this effect is compounded if there is less sunlight exposure due to illness or reduced mobility. As human milk is a poor source of vitamin D, rickets can occur in breast-fed infants deprived of sunlight exposure (WHO 2004).

Others that are at risk for low vitamin D are those living far from the equator, people with dark skin (UV light cannot reach the appropriate layer of skin), those that completely cover the skin for medical, cultural, social or religious reasons, and the liberal users of sunscreens as they inhibit the skin production of vitamin D (WHO 2004).

1.3 Vitamin D insufficiency in Australia:

It is a fallacy that most people in Australia get enough vitamin D each day from the sun. Vitamin D insufficiency is prevalent in Australia. Many people are not exposed to the sun during the day due to work commitments or fear of skin cancer. Published research shows that vitamin D insufficiency (plasma < 50 nmol/L) is found in:

- 1. 30% of healthy women in Geelong (Pasco 2001)
- 2. 41% of people in south east Queensland (van der Mei 2007)
- 3. 58% of aged care residents in Melbourne (Woods 2009)
- 4. 67% of Tasmanian women (van der Mei 2007)
- 5. 70% of people living in hostels (position statement MJA 2005)
- 6. 74% of general inpatients in a Melbourne hospital (Chatfield 2007)
- 7. 80% of women in Royal Women's Hospital Melbourne (Erbas 2008)
- 8. 83% of dermatologists in the winter (Czarnecki 2009)
- 9. 87% of African refugees in Sydney (Benitez-Aguirre 2009)

Generally, low levels of vitamin D have been found in older people in residential care, older people admitted to hospital, people with hip fractures, dark skinned people and mothers of infants with rickets (Working Party ANZBMS, ESA, OA 2005). Vitamin D levels are lower during the winter months than in summer due to reduced sun exposure (Pasco 2001).

1.4 Recommended Daily Intakes:

The recommended vitamin D daily needs as stated in the Nutrient Reference Values for Australia and New Zealand (NHMRC 2005) are shown in Figure 1:

Figure 1.



Note: Sometimes vitamin D is expressed in International Units. One microgram is equal to 40 IU.

The minimal amount of vitamin D required each day is not exactly clear. The NHMRC use the expression Adequate Intake (AI). AI is used "when an (sic) RDI cannot be determined" and is based on "observed or experimentally-determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be accurate" (NHMRC 2005).

With ageing, there is diminished ability for the skin to produce vitamin D from sunlight exposure and the ability for the kidneys to produce Calcitriol. In addition the requirement for vitamin D increases to around 15mcg per day from the age of 70 years.

There has been conjecture that the AI for vitamin D is too low and needs to be raised (Yetley 2009, International Osteoporosis Foundation 2010). A position statement by the International Osteoporosis Foundation recommends that to reach a serum vitamin D level of 75 nmol/L older adults require 20-25 mcg per day. A vitamin D intake lower than 20 mcg/day may be adequate for people who have regular effective sun

exposure. Those who are obese, have osteoporosis or have very limited sun exposure should have as much as 50 mcg a day. Each 2.5 mcg of extra vitamin D will increase serum levels by about 2.5 nmol/L (Dawson-Hughes 2010).

A US study estimated that people of European ancestry need 32.5mcg vitamin D in the winter assuming they have good sun exposure in the summer (Hall 2010). The study also estimated that people with African ancestry need 52.5 - 77.5 mcg vitamin D all year round, especially if they have low sun exposure. This should achieve a plasma vitamin D level of 75 nmol/litre.

<u>1.5 Main dietary sources of vitamin D:</u>

The major dietary sources of vitamin D in Australia are margarine (50% of dietary intake), canned fish such as herrings and salmon (16%) and eggs (10%) (Shrapnel 2006). Other sources in the diet include butter, cheddar cheese and lean meat. Milk is low in vitamin D unless it is fortified, such as Calcium Plus[™], Vitasoy Calciplus[™] and Anlene[™] with 200 mg calcium and 2 mcg vitamin D per 100mL.

It is estimated that the typical adult dietary vitamin D intake is 2.0-2.2 mcg/day by women and 2.6-3.0 mcg/day by men (Nowson 2002). This paper states: "Adequate Intake of vitamin D is unlikely to be achieved through dietary means, particularly in the groups at greatest risk, although vitamin D-fortified foods may assist in maintaining vitamin D status in the general population".

A review paper says that "The relatively high dietary recommendation for vitamin D for elderly people cannot be met through the existing food supply and supplementation appears to be a desirable option for many" (Shrapnel 2006). The paper makes a strong case for increased vitamin D consumption, mainly through fortification of foods like yogurt and milk.

<u>1.6 Vitamin D supplements:</u>

Because many people do not get enough vitamin D from either their diet or through sun exposure, vitamin D supplements remain the main choice to get adequate vitamin D. Patients who have low plasma vitamin D are either given an injection of vitamin D or recommended to take tablets. Vitamin D supplements are readily available, such as Ostelin® which has 25 mcg cholecalciferol per tablet.

<u>1.7 Vitamin D in mushrooms:</u>

Mushrooms can also contribute significantly to dietary vitamin D as shown in Figure 2 where the contribution of mushrooms is compared with other dietary sources.

Mushrooms have relatively high levels of ergosterol, which, under the action of UV light is converted to ergocalciferol (known as vitamin D_2), in levels of 20 mcg/100g or more. Ergosterol is a component of mushroom cell walls (just as cholesterol is found in animal cell walls). Vitamin D_2 is inactive, but on consumption is converted to 25-hydroxycholecalciferol in the liver, then to 1,25-dihydroxycholecalciferol (Calcitriol) by the kidneys.

Figure 2



Vitamin D in food (mcg)

Currently, Australian adults get only around half their vitamin D needs through food such as oily fish, margarine and eggs. The mushroom has the potential to be the only food that can provide the dietary Adequate Intake of vitamin D in a single serve.

The first paper to measure the vitamin D in edible mushrooms was published in 1935 (Sceunert 1935). In 1994 Finnish researchers confirmed that mushrooms naturally contain vitamin D_2 when growing in the wild (Mattila 1994). The action of sunlight on the surface of the mushroom stimulates the conversion of the natural ergosterol to vitamin D_2 , commonly reaching levels of 10-40 mcg per 100g of fresh mushroom (Mattila 1994; Mattila 2002). There are only small amounts of vitamin D_2 in commercially grown mushrooms because they are grown in darkness and additional lighting tends to affect growing room temperature, running costs and mushroom quality.

The United States Department of Agriculture (USDA) has a database value of 15 International Units (0.375mcg) of vitamin D per 84g serving of conventionally produced mushrooms (www.ars.usda/gov/nutrientdata). Although not included in the USDA database, ergosterol and sterol content of mushrooms were also determined and are anticipated to be included in SR23, September 2010.

Since 2005 there have been a series of experiments that subjected mushrooms to UV light to observe the effect on vitamin D_2 production when using light to mimic what occurs in nature (Jasinghe 2005a; Teichman 2007; Roberts 2008). Vitamin D_2 production in response to UV light was the same whether mushrooms were treated one day or four days after harvest (Roberts 2008). Exposing sliced mushrooms to UV light was more effective in generating vitamin D_2 than with whole mushrooms due to the greater surface area of exposed tissue (Ko 2008).

Mushroom industries around the world did not exploit the vitamin D_2 advantage until 2007 when further research from Pennsylvania State University showed that UV light applied to commercial mushrooms dramatically boosted vitamin D_2 to levels relevant

to public health. Using UV lights, mushrooms growers have produced vitamin D mushrooms in the US since 2009.

The University of Western Sydney has completed a trial using light exposure and found that mushrooms can easily reach the Adequate Intake levels of vitamin D in a single serve. They also showed that the vitamin D is stable and well absorbed from the mushroom (Koyyalamudi 2009a).

Since then, the Australian mushroom industry has collaborated with Warsash Scientific, agents for Xenon pulsed light equipment from the United States, and the University of Western Sydney to test the effect of pulsed light on vitamin D levels in mushrooms post harvest. Punnets of mushrooms of two sizes (35 mm and 50 mm diameter) were placed on a conveyor belt and passed under pulsed light for 1-2 seconds, resulting in vitamin D_2 levels at least 10 mcg (400 IU), the amount recommended each day for adults 51-70 years.

1.8 Vitamin D bioavailability from mushrooms:

The vitamin D from both wild mushrooms and cultivated mushrooms subjected to UV light is bioavailable and easy to absorb (Koyyalamudi 2009a; Outila 1999; Jasinghe 2005b). Jasinghe concluded that after being absorbed the vitamin D_2 in mushrooms is converted to 25-hydroxycholecalciferol and actively assists in bone mineralisation in rats. A further rat study by the same research group (Jasinghe 2006) confirmed that the bone mineral density of the femur was increased after being fed mushrooms high in vitamin D_2 . The amount of vitamin D in plasma reflected the amount of vitamin D_2 in the mushrooms fed to rats, increasing as the vitamin D_2 in the diet increased (Koyyalamudi 2009a).

In a human trial Outila found that vitamin D_2 in mushrooms (as a broth) raised plasma vitamin D_2 as effectively as taking an equivalent vitamin D_2 supplement over three weeks. The study was not on fresh mushrooms. The first human clinical trial on vitamin D_2 bioavailability in fresh mushrooms is currently being conducted in the US by the USDA.

There is a recorded case of a patient who significantly raised his plasma vitamin D levels by consuming 200g of stir-fried mushrooms daily for three months. He bought the mushrooms from a retail outlet and placed them under a UV-B light at a distance of 15 cm. The length of light exposure was not specified. After three months his plasma vitamin D was raised by 129% (Ozzard 2008).

1.9 Effect of cooking and storage of mushrooms on Vitamin D content:

A paper by Mattila (1999) tested the effect of cooking on the ergocalciferol (vitamin D_2) content of wild mushrooms (ie. it didn't include *Agaricus*). After the mushrooms were fried for five minutes there was at least an 85% retention of vitamin D_2 . This is likely to be similar in *Agaricus* mushrooms. Furthermore, there is very little loss of vitamin D_2 when mushrooms are refrigerated for eight days (Koyyalamudi 2009a) or frozen for three or nine months (Mattila 1999).

1.10 Vitamin D toxicity:

The Upper Level of Intake for any nutrient is defined as "The highest average daily nutrient level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases" (NHMRC 2005). The UL for vitamin D is 80 mcg/day for anyone over one year of age.

A chronic intake of vitamin D is indicated as being in excess of 50,000 International Units (1250 mcg) per day for weeks or months. Acute intake is constituted by a single dose of 4,000,000 International Units (100,000 mcg). (Dunn 2006)

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SECTION 2: COMMERCIAL PRODUCTION OF VITAMIN D MUSHROOMS

Recent research in the United States and Australia has investigated the use of ultraviolet (UV) light to convert erogosterol to vitamin D_2 in mushrooms. This follows early work in Finland (Mattila et al 1994 and 2002) which confirmed that mushrooms growing naturally in the wild contain vitamin D_2 .

2.1 United States research:

A proposal by the United States Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition led to Pilot Studies in 2005-2006 to investigate the optimisation of vitamin D_2 and ergosterol content of white button and portabella mushrooms.

The American Mushroom Council facilitated studies to determine the duration of UV light exposure and harvesting/processing techniques to maximise vitamin D_2 content.

Pennsylvania State University studied the influence of UV-B light (290-315nm) on vitamin D_2 content when mushrooms were exposed during the growing and harvesting periods. Mushrooms were harvested after 0, 1, 2, 3, 6, and 24 hours exposure with the distance from light source varying between 7 and 30cm.

Monterey Mushrooms studied the influence of UV-C light (290-320nm) on vitamin D_2 content when mushrooms were exposed postharvest. White button and portabella mushrooms were used with exposure times of 0, 1, 2, 5, 10 and 15 minutes.

Sylvan Research analysed several samples for vitamin D_2 using previously reported HPLC methods (Perera et al 2003). Depending on exposure type and time vitamin D_2 values ranged from 5.2mcg/g dry weight to 8.2mcg/g dry weight suggesting the UV treated mushrooms could be a significant source of vitamin D_2 .

Substantiation of the preliminary results from Sylvan Research was undertaken by Dr Mattila of MTT Agrifood Research, Finland using widely referenced analytical methods for vitamin D_2 (Mattila et al 1994). Analyses of mushroom samples from both Pennsylvania State University and Monterey Mushrooms showed that an 84g serving of mushrooms exposed to UV light could provide vitamin D_2 in amounts greater than 100% of the US Adequate Intake (AI). Storage of treated mushrooms at +12°C for three days resulted in some loss of vitamin D_2 but the amount remaining was still greater than 100% of the Adequate Intake (AI).

Discolouration due to UV exposure has been reported (Mau et al 1998) and is thought to influence acceptability of fresh mushrooms. Monterey Mushrooms tested mushrooms for whiteness after 0, 1, 5, 10 and 15 minutes exposure to UV light. Discolouration and reduction in brightness increased with longer exposures. They were only slight after one minute's exposure but more severe after 15 minutes.

These results led to studies by Professor Robert B Beelman, Pennsylvania State University, using pulsed UV light, which delivers energy at a high peak power level in a very short time. When compared to UV-B floodlighting systems, the time is considerably less for the same amount of exposure.

Beelman's studies (2008) using fresh, sliced *Agaricus* mushrooms employed pulsed UV light exposure of 4, 10 and 20 seconds. Vitamin D_2 levels reached respectively 6, 12 and 16 times the daily need for each 84g serving.

Note: A serve of mushrooms in the US is 3.0 US ounces (84g), while the Australian mushroom industry promotes a mushroom serve size of 100g, equivalent to 3 medium sized button mushrooms.

Following Dr. Beelman's studies, Xenon Corporation conducted tests with fresh, whole, white and Portabella mushrooms and confirmed that, using a 1 second exposure to pulsed UV light, vitamin D_2 levels reached to 5 - 10 times daily need with whole, white mushrooms and 1 - 2 times AI with Portabellas.

Subsequent work by Beelman and Kalaras (2008) extensively studied pulsed light treatment of white button, brown button, Shiitake and Oyster mushrooms. For all mushrooms types, only 1 pulse of UV light applied at a 3 pulse per second rate, increased vitamin D_2 to well over 100% of daily need in a single serving.

2.2 Australian research:

Professor Beyer of Pennsylvania State University presented results from United States research at the 2007 Australian Mushroom Industry conference at Leura, NSW. (Beyer 2008)

Immediately after the conference, trials were undertaken on Graham Price's Dubbo farm utilising UV-B flood light treatments on growing crops. Treated mushrooms were analysed for vitamin D_2 at the University of Western Sydney along with mushrooms which were treated postharvest at the University with UV light from a UV-C germicidal lamp.

While results from the growing room treatments were disappointing due to low intensity of UV at the growing bed surface, postharvest treatment with UV-C light achieved significant vitamin D_2 levels. Levels increased with exposure time and intensity of UV light (proximity of mushrooms to the lamp). Vitamin D_2 levels of 4, 10 and 14 times Australian Adequate Intake were obtained following 2.5, 5 and 10 minutes exposure respectively with mushrooms placed 30cm beneath the lamp. (Koyyalamudi et al 2009)

More recently, the Australian industry in conjunction with the University of Western Sydney and Warsash Scientific – the Australian agents for Xenon pulsed light equipment – treated punnets of whole and sliced fresh *Agaricus* mushrooms with a range of pulses.

Punnets of whole mushrooms of two sizes (35mm and 50mm in diameter) were placed on a conveyor belt and passed under the UV lamp which was set to deliver a range of pulses. Two punnet sizes were used -200g (single layer of mushrooms) and 500g (double layer of mushrooms).



Picture 1: Xenon pulsed UV light equipment set up for trial treatments of punnetised mushrooms.

Note: lamp housing positioned at an angle over the line of the conveyor belt in order to deliver required intensity and frequency of UV light.



Picture 2: Punnet of mushrooms (200g.) fed on conveyor belt to pass under pulsed UV light. Top of the mushrooms at an optimum distance of 31.7mm (1.25 inches) below lamp housing window.



Picture 3: Punnets of large button mushrooms for UV pulsed light treatment. Note: single layer of mushrooms; caps upwards; average – 9 mushrooms per punnet approx 200g/punnet.

Three pulses, each of $\frac{1}{3}$ of a second, produced at least Adequate Intake (AI) levels of vitamin D₂ in all treatments. Mushrooms in the 200g punnets all contained well over AI levels of vitamin D₂ as did the upper mushrooms in the 500g punnets. While the lower level mushrooms in the 500g punnets had lower levels of vitamin D₂, the amounts were still in excess of AI levels. (Figure 3)



Figure 3

 Times Daily Adequate Intake are based on 10mcg vitamin D₂/100g fresh mushrooms.

indicates variance for each treatment

Vitamin D_2 content of whole button mushrooms (*Agaricus bisporus*) after treatment with pulsed UV light.

Random samples were taken and analysed as follows:

- Group A: single layer in 200g punnets.
- Group B1: double layer in 500g punnets.
- Group UL: upper layer in 500g punnets
- Group LL: lower layer in 500g punnets

Mushrooms were freeze dried/analysed immediately after UV treatments.

Vitamin D_2 levels in all mushrooms remained in excess of AI amounts after 8 days cool storage which simulated post-treatment retail supply chain conditions. (Figure 4)



Figure 4

• Times Daily Adequate Intake are based on 10mcg vitamin D₂/100g fresh mushrooms.

indicates variance for each treatment

Vitamin D_2 content of whole button mushrooms (*Agaricus bisporus*) after treatment with pulsed UV light and subsequent storage.

Random samples were taken and analysed as follows:

- Group A: single layer in 200g punnets.
- Group B1: double layer in 500g punnets.
- Group UL: upper layer in 500g punnets
- Group LL: lower layer in 500g punnets

Mushrooms were cool stored for 8 days after treatment prior to freeze drying/analysis.

Further work at the University of Western Sydney subjected sliced mushrooms to a range of pulsed UV light treatments. United States research had already shown that higher levels of vitamin D_2 were produced from treating sliced mushrooms compared to whole buttons. This is presumably due to exposure of gills and a greater surface area of mushroom tissue being exposed to the UV light. Results with sliced mushrooms treated at the University of Western Sydney confirmed those from the United States. Three pulse UV treatments of multiple layers of sliced mushrooms in 200g punnets produced vitamin D_2 levels well in excess of AI amounts. Light treatments of a single layer of slices produced even higher vitamin D_2 levels. (Figure 5)



Picture 4: 200g punnets of sliced mushroom. Multiple layers of slices.



Picture 5: Single layer of sliced mushrooms being fed under pulsed UV light lamp.



Figure 5.

* Times Daily Adequate Intake are based on 10mcg vitamin $D_2/100g$ fresh mushrooms.

indicates variance for each treatment

Vitamin D_2 content of sliced button mushrooms (*Agaricus bisporus*) after treatment with pulsed UV light.

Random samples were taken and analysed as follows:

- Group C1: multiple layers in 200g punnets.
- Group C2: single layer on trays.

Mushrooms were freeze dried/analysed immediately after treatment.

Again, the vitamin D_2 levels remained in excess of AI after 8 days of cool storage. (Figure 6)



Figure 6

• Times Daily Adequate Intake are based on 10mcg vitamin D₂/100g fresh mushrooms.

indicates variance for each treatment

Vitamin D_2 content of sliced button mushrooms (*Agaricus bisporus*) after treatment with pulsed UV light and subsequent cool storage.

Random samples were taken and analysed as follows:

- Group C1: multiple layers in 200g punnets.
- Group C2: single layer on trays.

Mushrooms were cool stored for 8 days after treatment prior to freeze drying/analysis.

Pulsed UV light treatment is likely to be utilised by organisations treating relatively large volumes of mushrooms using pre-packed punnets at a relatively rapid throughput. However, some growers may wish to treat small volumes of mushrooms to supply local or specialised outlets. These growers are likely to use flood UV lights to produce vitamin D_2 mushrooms, rather than the pulsed light system.

A further trial at the University of Western Sydney utilised flood lighting from UV-C germicidal lamps to treat 200g (single layer) and 500g (double layer) punnets of fresh button mushrooms.

Two UV-C germicidal lamps were suspended above a bench measuring 1.10 metres x 0.56 metres on which punnets of mushrooms were placed with the edges abutting.



Picture 6: Punnets of white button mushrooms $-12 \ge 200$ g single layer (left) and $9 \ge 500$ g. double layer (right) – on a 1.10m. ≥ 0.56 m bench beneath two UV-C germicidal lamps. Lamps suspended 30cm or 40cm above mushrooms.

Lamp heights of 30cm and 40cm above the mushrooms were compared along with exposure times of 5, 10 and 20 minutes.

All exposure times at both lamp heights produced vitamin D_2 levels well in excess of AI levels with 20 minutes exposure at the 30cm height giving the highest levels (Figure 7). The very highest levels of vitamin D_2 were in mushrooms from punnets which received the highest intensity of UV light.



Figure 7

 Times Daily Adequate Intake are based on 10mcg vitamin D₂/100g fresh mushrooms.

indicates variance for each treatment

Vitamin D_2 content of whole button mushrooms (*Agaricus bisporus*) after treatment with flood UV-C light for 3 exposure periods

Random samples were taken and analysed as follow:

- Group A: lamps 30cm above mushrooms; freeze dried/analysed immediately
- Group B: lamps 40cm above mushrooms; freeze dried/analysed immediately
- Group C: lamps 30cm above mushrooms; freeze dried/analysed after 8 days cool storage.
- Group D: lamps 40cm above mushrooms; freeze dried/analysed after 8 days cool storage.

Mushrooms sampled from the 200g single layer punnets developed higher vitamin D_2 levels than those sampled from the double layer 500g punnets. Some mushrooms sampled from the 500g punnets were from the bottom layer and received less UV light than mushrooms on the top layer. However, overall level of vitamin D_2 in the whole punnet was still well in excess of AI.

Vitamin D_2 levels in mushrooms from all treatments remained in excess of AI after 8 days cool storage which simulated post-treatment retail supply chain conditions. (Figure 7)

2.3 Pulsed versus flood lighting:

Ultra-violet light treatment of mushrooms converts cell wall ergosterol into vitamin D_2 (ergocalciferol).

Flood lighting, utilising UV-B or UV-C lamps, produces relatively low intensity UV light which takes several minutes to produce the necessary conversion to vitamin D_2 to achieve Adequate Intake levels. It is anticipated that flood UV lighting will be used on a relatively low volume batch treatment basis with lamps suspended over a bench surface on which punnets of mushrooms are arranged.

University of Western Sydney research utilised a 1.10 metre x 0.56 metre bench to hold 21 punnets of mushrooms. The use of a larger bench would probably require more lamps but suspended at the same height and using the same exposure period as indicated in 2.5.1.

Extended periods of exposure to flood lighting increases the likelihood of mushrooms developing a light brown colouration and losing their bright, white appearance. However, there was no evidence of discolouration in mushrooms treated with UV-C flood lighting for periods of up to 20 minutes at the University of Western Sydney.

The Xenon pulsed system delivers UV-B light at high peak power in a matter of seconds and eliminates the risk of discolouration. This system is particularly suited for incorporation into high volume, continuous throughput mushroom packing lines.

Pulsed light achieves in seconds what takes minutes with flood lighting.

2.4 Pulsed UV light treatment of fresh mushrooms:

In a typical installation of the Xenon pulsed light system, the lamp housing will be mounted over a user-designed tunnel that protects personnel from the flashing light as the punnets of mushrooms are moved by conveyor belt under the lamp. (Williams 2009) The specific level of vitamin D_2 achieved from using a pulsed light system will depend on:

- TYPE OF MUSHROOM
- DISTANCE OF MUSHROOM BELOW LAMP HOUSING WINDOW
- SIZE OF PUNNET
- CONVEYOR BELT SPEED
- TOTAL NUMBER OF PULSES RECEIVED BY EACH PUNNET

It is critical that all the above are optimised in order to achieve the required levels of vitamin D_2 .

Positioning and arrangement of the Xenon pulsed light equipment will need to be designed specifically for each commercial operation.

Technical consultants from Warsash Scientific (Australian and new Zealand agents for Xenon Corporation) should be contacted to provide the specifically designed equipment details.

Warsash Scientific, Unit 7, 1 Marian Street, Redfern, NSW 2016. Tel (02) 9319 0122, Fax (02) 9318 2192. Contact Richard Vincent, email <r.vincent@warsash.com.au>

The diagram below (kindly supplied by the Xenon Corporation, 37 Upton Drive, Wilmington, MA 01887, United States of America) illustrates how the lamp housing is typically positioned over a light blocking tunnel.



Figure 8: Typical placement of pulsed UV light lamp housing over a conveyor belt.

Please note that the measurements are in inches (1 inch = 25.4mm).

When several types of mushrooms are to be treated on a single conveyor belt, provision is made to adjust the height of the lamp housing mounting in order to maintain an optimum height of 32mm (1.25 inches) from the lamp housing window to the top of the mushrooms in the punnets.

The Xenon UV lamp generates high energy light pulses with a spectrum that approximates to that of sunlight.



A key factor in the manner by which the Xenon pulsed UV lamp is able to quickly produce vitamin D_2 in mushrooms is how closely its spectrum compares to that of sunlight.

As shown, sunlight (red curve) has a continuous spectrum from about 350 nanometres to just beyond 800 nanometres. The blue curve illustrated the spectrum from a Xenon pulsed UV lamp showing energy extending below 350 nanometres as well as above 800 nanometres.

Ultraviolet light is mainly in the range of 200 – 400 nanometres.

The flash lamp is mounted in a lamp housing unit with a clear fused quartz window.

Xenon pulsed UV lamps produce infrared energy which causes the housing, lamp and reflector surface temperatures to exceed 50°C during operation. To ensure long life for the enclosed pulsed light lamp, an external cooling blower is required to vent hot air from within the lamp housing. In addition, the cooling blower should remain on for a minimum of 5 minutes when the lamp is shut off.

A controller unit provides all power for the pulsed light lamp as well as controlling the light pulse shape, pulse energy and pulse rate.

Protective shielding must be installed around the pulsed lamp housing window and conveyor tunnel to prevent light spillage into areas where workers would be exposed to the flashing UV light.

The pulse rate of the Xenon lamp is factory-set at 3 pulses per second.

Lamp intensity will gradually diminish over time, based on frequency of use, i.e. number of pulses, and maintenance of lamp housing. Replacement of lamp is best performed based on monthly UV light intensity measurements (Appendix D), correlated with measured vitamin D_2 level changes. Records for the initial year of operation will establish the best lamp replacement protocol.

Warsash Scientific technical staff will design an appropriate configuration of lamp housing position (angle over the conveyor belt), conveyor speed and pulse count for each specific commercial installation.

They will also advise on the design of the light blocking tunnel to accommodate the appropriately angled lamp housing.

2.4.1 Probable installation:

BASED ON AUSTRALIAN RESEARCH RESULTS, IT IS LIKELY THAT PRODUCERS WILL UTILISE A PULSED LIGHT SYSTEM WHICH DELIVERS 3 PULSES OF UV LIGHT IN ONE SECOND TO PUNNETS OF SINGLE LAYER (200g) OR DOUBLE LAYER (500g) BUTTONS OR 200g PUNNETS OF SLICED MUSHROOMS

2.4.2 Standardising the operation of a pulsed light system:

Prior to commercial operation of the pulsed UV light system, trial punnets of mushrooms should be treated and samples sent to a National Association of Testing Authorities (NATA) recognised laboratory that is experienced in vitamin D_2 analysis using the method described by Koyyalamudi et al (2009).

Results will confirm that positioning of pulsed light equipment, conveyor speed and pulse number are correctly coordinated to produce the required level of vitamin D_2 . Details for suitable commercial analytical laboratories are given in Section 4 of this manual.

2.5 Flood UV light treatment of fresh mushrooms:

A typical installation for 'flood' lighting punnets of mushrooms with ultraviolet light from germicidal UV-C lamps will have the lamps suspended horizontally above a bench on which punnets of mushrooms are arranged.

The installation used for experimentation at the University of Western Sydney (UWS) used two germicidal UV-C lamps suspended horizontally and centrally above a bench measuring $1.10m \times 0.56m$ in which $12 \times 200g$ and $9 \times 500g$ punnets of mushrooms

were arranged (200g punnets measured 18cm x 14cm; 500g punnets measured 17.5 x 17.5cm)



Picture 7: Punnets of white button mushrooms $-12 \ge 200$ g single layer (left) and $9 \ge 500$ g double layer (right) – on a 1.10m. x 0.56m bench beneath two UV-C germicidal lamps. Lamps suspended 30cm or 40cm above mushrooms.

Suspending the lamps 30cm or 40cm above the top of the punnets enabled sufficient intensities of UV light to reach the mushrooms in all punnets and generate vitamin D_2 levels well in excess of AI following exposure of 5, 10 or 20 minutes.

Using this information as guide, it is likely that growers will suspend lamps 30 - 40cm above the punnets and use an exposure time of 10 - 15 minutes. Bench size will determine the number of lamps required, based on UWS experience, and the number of punnets treated in each batch.

Solid protective screens must be erected vertically along the outer edges of the bench to prevent spillage of UV light into the work areas around the treatment facility. The screens must be sufficiently tall to prevent any UV light reaching workers in the area. At the same time reflectors should be fitted above to lamps will deflect UV light downwards towards the mushrooms.

Extraction fan(s) should be positioned over the bench above the lamps in order to extract any ozone/ hot air produced. The extracted air should be exhausted well away from work areas and preferably outside.

Technical consultants from Sylvania Lighting Australasia Pty Ltd should be contacted to provide specific details for installing a flood light system.

Sylvania Lighting Australasia Pty Ltd – Customer Service 1300 728 988; email: <sylvania@sla.net.au>.

2.5.1 Probable installation:

BASED ON AUSTRALIAN RESEARCH RESULTS, IT IS LIKELY THAT PRODUCERS WILL UTILISE A FLOOD LIGHT SYSTEM WHICH HAS AN EQUIVALENT ARRANGEMENT TO TWO GERMICIDAL UV-C LAMPS SUSPENDED 30 - 40CM ABOVE A 1.0 METRE X 0.5 METRE BENCH HOLDING 20 - 24 200g OR 500g PUNNETS. EACH BATCH OF PUNNETISED MUSHROOMS WILL BE TREATED WITH UV-C FOR 10 MINUTES.

2.5.2 Standardising the operation of a flood light system:

Prior to commercial operation of the flood light system, trial punnets of mushrooms should be treated and samples sent to a NATA recognised laboratory that is experienced in vitamin D_2 analysis using the method described by Koyyalamudi (2009)

Results will confirm that the number of lamps, lamp height, size of the treatment and bench and treatment time are correctly co-ordinated to produce the required level of vitamin D_2 .

Details for suitable commercial analytical laboratories are given in Section 4 of this manual.

2.6 Safety issues for use of UV lamps:

Ultraviolet light wavelengths (200 - 400 nanometres) can cause damage to organic materials such as human tissues. Damage includes fading or darkening and structural damage from the breakdown of molecular bands. Xenon pulsed light UV-B lamps produce ultraviolet and visible light (300 - 800 nanometres) with a similar spectrum to sunlight. In addition, germicidal UV-C lamps produce far UV light (200 - 300 nanometres) and ozone.

Ultraviolet light alone is many times more damaging than visible light. Thus it is important to shield and /or filter UV light to protect personnel.

Continuous exposure without adequate protection can cause eye damage and skin cancer.

PRECAUTIONS FOR PERSONNEL WHO HAVE ACCESS/EXPOSURE TO ULTRAVIOLET LIGHT SOURCES.

• use of safety viewing windows when visible access to UV light sources is required. [Acrylite GP, Acrylite OP-2 and Acrylite OP-3 are recommended materials for use in safety viewing windows.]

• avoid direct exposure to UV light, prolonged exposure to reflected light and UV leakage through cracks, etc.

• **ozone:** pulsed light UV-B lamps do not produce ozone and the cooling air exhausted from the lamp housing does not have to be removed from the workplace. Germicidal UV-C lamps can produce ozone, and the equipment should not be used in a tightly enclosed area. Extraction fans should be used to move air away from the treatment area and operators.

• never look directly at lamps and always wear UV light-blocking safety glasses when operating or in close proximity to the UV equipment.

• keep temperature sensitive materials away from the lamp equipment. [UV lamps also produce infrared energy causing the lamp equipment to heat up to more than $50^{\circ}C$ during operation.]

• allow hot surfaces to cool for at least 5 minutes before handling.

• keep water well away from UV equipment.

• do not open any cover, operate controls, make adjustments, or perform other procedures to a UV light source except those specified in the users manual supplied with the system.

• allow only trained, authorised service personnel to repair the equipment.

2.7 References:

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SECTION 3: QUALITY ASSURANCE AND FOOD SAFETY PROTOCOL.

3.1 Introduction:

The process involved in producing vitamin D_2 in mushrooms requires an evaluation and determination of both quality and food safety aspects. Mushroom businesses that instigate the process of generating vitamin D_2 will already have in place considerable QA and Food safety documentation that covers mushroom farming growing/ harvesting practices.

These may be ISO 9001, Quality Assurance, HACCP, Fresh-care or other commercial programs e.g. Woolworths WQA, Coles specified program e.g. SQF: 1000 / SQF: 2000. These programs are routinely audited by external third party auditors to validate the implementation and compliance against known standards.

This QA Food Safety section can be used as a stand-alone document or may be used as a guide to allow review and inclusion into existing documentation.

3.1.1 Scope:

The Quality Assurance and Food safety information contained in this section relates to practices that are likely to be employed for producing vitamin D_2 packaged mushrooms for the fresh market. This information covers:

- UV pulsed light on a continual flow belt
- batch treatment using fixed UV lamp flood lighting
- whole button mushrooms
- sliced button mushrooms

3.1.2 Policy:

The policy is to ensure that mushroom businesses producing vitamin D_2 mushrooms do so with a full understanding of Quality and Food Safety implications. Commercial ramifications associated with Quality or Food Safety non-conformance will have significant, industry-wide impact.

Quality aspects pertain to:

- Consistency of achieving the vitamin D levels as claimed on the packaging.
- Freshness and appearance of the mushrooms
- Retention of typical mushroom flavor, odour and texture

Food Safety pertains to:

A safe food product for human consumption, free of harmful bacteria and chemical residues, foreign matter and compliance to stated claims that appear on packaging...

3.1.3 Responsibilities:

It will be the responsibility of each mushroom enterprise that produces vitamin D mushrooms to review their existing Quality Assurance and Food Safety programs and

to endorse the basic additional requirements as outlined in this document. This will ensure that Quality Assurance and Food Safety certification continues to be achieved with a clear extension to the scope of certification to include the practices in production associated with the production of vitamin D mushrooms.

To ensure that the process is understood and effectively implemented it will be the responsibility of each business owner to ensure that adequate procedural training is provided to key operational personnel. In-house or external training needs to ensure that the principles of the UV light treatment are understood as well as the monitoring of critical operational aspects.

This includes:

- calibration and routine checking of equipment.
- work safe requirements for the equipment and immediate area.
- label requirements, e.g. Best Before date requirements.
- other label information and bar coding to identify the product.
- in-process record preparation, e.g. Good Manufacturing Practice (GMP) check lists.
- corrective actions requirements where system failures occur.

3.2 Procedures:

3.2.1 Mushroom quality aspects:

Only freshly harvested and good quality (visually presentable) mushrooms should be selected and packed in trays for vitamin D treatment.

Similar sized mushrooms should be selected and other features should be in accordance with the typical AMGA specification shown below.

Table of AMGA typical specifications:

Vitamin D white button mushrooms (Agaricus bisporus).

General Appearance		
Colour	White cap and stalk.	
Visual appearance	Refer customer specifications.	
Sensory	Fresh firm texture, typical mushroom flavor free from	
	off odours or taste.	
Shape and size	Uniform size with well rounded, dome shape cap and	
	straight, cylindrical stem.	
Maturity	Firm, but gills not exposed.	
Major Defects		
■Refer typical or		
customer specification		
Discolouration	Evidence of discolouration due to too much UV light	
	intensity or exposure.	
Temperature injury and or	Evidence of surface dryness.	
dryness in appearance		
Insect /disease/pest damage	Refer customer specifications.	

Marks/ blemishes/	Refer customer specifications.		
physiological disorder			
Minor Defects			
Refer typical or			
customer specification			
Consignment			
Refer typical or			
customer specification			
Packing : Trays	Food grade approved tray material		
	Food grade over wrap		
Cartons / Retail packs	Refer customer specification (may be generic		
	pre-labelled carton)		
Labelling	Description, nutritional claims, to be in compliance		
	with food labeling laws and requirements.		
	All other labelling requirements, e.g. identification,		
	grade/ class/size, date harvest, best before date,		
	minimum net weight to be in compliance with		
	customer specifications and applicable legal		
	requirements.		
Best Before	To be in compliance with specifications and /or		
	industry standard practices.		
Receival condition	• Refer typical or customer specification		
Contaminants	• Refer typical or customer specification		

It is also important that the vitamin D process minimizes the amount of mushroom handling.

3.2.2 Tray packing:

Depending on tray/pack size, mushrooms should be placed with the cap facing upwards towards the light source.

The tray over-wrap may be applied either before or after UV light exposure.

Key points: Fresh good quality mushrooms selected Similar size mushrooms selected Minimize handling

• Caps upwards towards light source

The procedure for producing vitamin D mushrooms should be regularly checked to ensure consistency of operation. Consistency of the operation is important in ensuring that the required vitamin D levels are achieved. It is worth noting that over-exposure to UV light is just as big a problem as under-exposure since the consequences may reflect on quality as well as truth of labelling and statements related to vitamin D levels.

3.2.3 Pulsed UV light method:

The following procedures should be read in conjunction with the instructions provided by the supplier of the light system and the manufacturer of the conveyor belt system. The pulsed light equipment is pre-set to delivers 3 pulses of UV light per second and, hence, conveyor belt speed will determine the amount of light received by each tray of mushrooms. As the flow of trays through and under the light source is continuous, calibration of the belt speed in relation to the dimensions of the mushroom tray is extremely important to ensure the desired vitamin D levels are achieved. The supplier of the pulsed light equipment will advise on calibration.

It is possible that fabric conveyor belts, as opposed to chain link conveyers are more prone to slippage that would influence the amount of pulsed UV light received by each tray of mushrooms.

3.2.3.1 Calibration:

The pulsed light equipment will be calibrated for individual business at the time of installation. This will include belt speed, pulse light frequency, tray pack size, distance from light source to mushrooms, etc.

The various parameters should then be maintained with belt speed being checked regularly.

Any changes to the original settings, such as belt speed, tray pack size, etc. should only be made in consultation with equipment supplier.

3.2.3.2 Test run:

- Place empty test trays at the intended rate on the conveyor belt.
- Check that light pulses are being delivered at regular $\frac{1}{3}$ second intervals as trays pass beneath the light.

3.2.4 Flood UV light, batch method:

This method uses UV light to treat batches of trays of mushrooms arranged on a bench beneath the UV lamps. Results from trials to determine the lighting and bench arrangement are reported in Section 2 of this manual.

Individual mushroom growers need to consult with the lighting supplier to design the specific configuration for their own operation. The next critical part of the process is in managing the time for which the trays of mushrooms are exposed to UV light.

Key points for flood UV light process:

• Ensure capability of the light unit or units to spread UV light across the bench.

• Ensure fixed positioning of the light unit or units at correct height above the bench surface.

- Check daily that the light(s) are working.
- Ensure correct placement of the trays of mushrooms beneath the light source.
- Monitor and record time for which each batch of mushrooms is treated.
- Regular check safety issues with UV light screening..

REMEMBER UV light is harmful to people

3.2.5 Records:

A process record needs to be developed for both types of vitamin D practices.

3.2.5.1 Records required for pulsed UV light method:

- Belt speed check:
- Light pulsing regularly as pre-set
- Label information e.g. harvest date...... Best Before date......
- No of trays cartonspacked
- Room identification: Picker identification:
- Check weights, (trays): hourly.....
- Cold room temperature:
- Safety checks about equipment:

A GMP Checklist is included as Appendix A.

NOTE!! These are the minimum QA records that will be required.

3.2.5.2 Records required for flood UV light, batch method:

- Light source working:
- Height of light above bench top correct:
- Treatment commencement time each batch:
- Treatment completion time each batch:
- Label information e.g. harvest date...... Best Before date......
- No of trays cartonspacked
- Room identification: Picker identification:
- Check weights, (trays): per batch:
- Cold room temperature:
- Safety checks about equipment:

A GMP Checklist is included as Appendix B.

NOTE!! These are minimum QA records that will be required.

3.2.6 Mushroom tray labelling requirements:

The mushroom tray shall be in compliance with customer specification requirements and applicable legal requirements.

There will be a labelling difference between convention tray-packed mushrooms and vitamin D mushrooms with the provision of identification detail of vitamin D treatment and any nutritional value claims. Bar code will be different.

3.2.7 Retention samples:

- When supplies are made directly or indirectly to major supermarkets there will be a requirement for holding retention samples for a defined period, (not less than the Best Before date). Sampled UV D mushrooms should be retained for the same period.
- Retention samples should be held at 1 4°C.

- Prior to disposal of retention samples, quality should be checked and recorded.
- •

3.3. Support Procedures:

3.3.1 Training:

Refer to existing training procedures and extend training requirements to include practices associated with producing vitamin D mushrooms.

The key points are:

- Checking operation of light / conveyor system (especially speed),
- GMP check list compliance,
- Safety checks, (may be part of the GMP checklist requirements),
- Labelling requirements,
- Corrective action requirements,
- Retention sample requirements.

3.3.2 Materials acquisitions (purchasing):

Refer to existing purchasing procedures and extend requirements to include materials used in the practices associated with producing vitamin D mushrooms.

The key points are:

- Approved status of tray suppliers.
- Letter which states of food safety status of tray material.
- UV light specifications.
- UV light suppliers.
- UV pulse light technical support suppliers.

3.3.3 Worker safety:

Refer to existing safety protocols and extend requirements to include requirements for use of UV light equipment for producing vitamin D mushrooms.

The key points are:

- Safety aspects of the conveyor belt system.
- Safety aspects of preventing light emission to outer areas, and to personnel.
- Safety wear provisions for operators.

NOTE!! This is not an exhaustive safety check list.

3.3.4 Equipment maintenance:

Refer to suppliers manuals on equipment maintenance and on light emission guidelines.

3.4. Hazard Analysis (HACCP):

3.4.1 Scope:

The scope of this HACCP assessment, audit and validation is limited to the steps involved as demonstrated on the accompanying flow chart.

- Selection of mushrooms
- Tray packing and preparation for UV light treatment
- UV light treatment
- Removal from light source
- Labelling
- Storage

The following process as well as activities associated with storage and transport of packed mushrooms activities are deemed to be adequately covered through existing QA and HACCP documentation.

3.4.2 Flow chart:

The following flow chart provides a typical description of the steps involved in producing vitamin D mushrooms. Risk assessment, food safety and quality remain almost identical irrespective of the processes used, i.e. pulsed UV light process or flood UV light batch process.



Suggested Risk Assessment table formats are shown in Appendix C.

HACCP Verification and Validation requirement are shown in Appendix D.

Note ! This HACCP analysis should be read in conjunction with the grower's existing Food Safety / HACCP program as this commences from the point of harvesting fresh mushrooms.

3.4.3 Responsibilities:

Existing QA, Food Safety and HACCP personnel, that administer and review programs that are in place for all other aspects of the mushroom business, should include activities involved in producing vitamin D mushrooms within their responsibilities.

Responsible personnel:

SECTION 4. VITAMIN D2 ANALYTICAL LABORATORIES

There is a strict requirement that all vitamin D_2 analyses be undertaken by laboratories that are NATA CERTIFIED for the specific test required.

These laboratories are chosen based on their ability to undertake the analytical procedures used during the vitamin D_2 trials.

Charges for vitamin D_2 analysis are considerably higher than tests that are routinely performed, e.g. chemical residues and or microbiological testing.

Consequently, there may be financial advantages in a planned testing protocol for all businesses that are involved in vitamin D_2 mushroom analysis.

Please note!!! When analysing for low levels of vitamins, results from more than one test sample will statistically provide greater test result confidence than results from a single test sample. Consequently, the preferred testing protocol may be based on a 3 sample testing program where an average vitamin D_2 content would provide greater confidence in the results.

NOTE: Further review of the sampling and testing protocols may be required and may be influenced by testing laboratory advice and external third party auditors as well as customers.

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Laboratories:
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Symbio Alliance 44 Brandl Street, Eight Miles Plains, Qld 4113 Phone 61 07 3340 5700 61 07 3219 0333 Fax **Contact: David Phillips** Costs: Valid until June 30th, 2011 1 - 4 samples \$255.00 (excl. GST) 5 - 9 samples \$229.00 per test (excl. GST) > 10 samples \$204.00 per test (excl. GST) National Measurement Institute The Loading Bay, Unit.1, 153 Bertie Street, Port Melbourne, Vic 3207 Phone: 61 03 9644 4861 61 03 9644 4999 Fax: Contact: Paul Adorno (61 03 9644 4861) Costs: valid until: November 17, 2010 1-3 samples \$270.00 per batch (excl. GST) 4 - 6 samples \$211.00 per batch (excl. GST) \$189.00 per batch (excl. GST) 7 + samplesNote: 1. A standard handling fee of \$33.00 applies inclusive of GST. 2. A minimum invoice of \$275.00 inclusive of GST applies, (includes sample handling fee).

(Limit of reporting: 5mcg/100g)

Appendix A: GMP Checklist - Pulsed UV light method

Date:..... Assessor:.....

CHECK	TIME	COMPLIANCE		Sign
Operational aspects		Acceptable	Rectify	
Belt speed				
check				
Light	1.			
operational	2.			
check	3.			
(2 hourly)	4.			
	5.			
End of	•••••			
production				
Protective				
light shields				
in place				
Label check				
Harvest date				
check				
Best before				
date check				
Room No. s				
No. trays				
packed				
No. cartons				
packed				
Retention	Day 10	Appearance comment:		
samples taken	evaluation			
and held			1	
Cold room	AM°C			
temperature	PM.			
	°C			

Appendix B: GMP Checklist - Flood UV light UV method

Date:..... Assessor:.....

CHECK	TIME	COMPLIANCE		Sign
Operational				
aspects		Acceptable	Rectify	
Table set up				
check				
Light	Batch 1.	Time	Time	
operational	Batch 2.	on	out	
check	Batch 3.	Time	Time	
	Batch 4.	on	out	
	Batch 5.	Time	Time	
		on	out	
		Time	Time	
		on	out	
		Time	Time	
		on	out	
Light check				
(End of				
production)				
Protective				
light shields				
in place				
Label check				
Harvest date				
check				
Best before				
date check				
Room No. s				
No. trays				
packed				
No. cartons				
packed				
Retention	Day 10	Appearance comment:		
samples taken	evaluation			
and held			1	
Cold room	AM°C			
temperature	PM .			
	∣°C			