DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date · AUG 18 1999

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredients:	*Ganoderma lucidum* Spore Powder
Firm:	GloboAsia LLC
Date Received by FDA:	June 28, 1999
90-day Date:	September 25, 1999

99 AUG 19 P2:57

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 953-03 16 after September 25, 1999.

Robert J. Moore, Ph.D.

955-0316

RPT 52

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

AUG 1 8 1999

Nancy Kan, Ph.D.
Senior Regulatory Scientist
GloboAsia, LLC
7250 Parkway Drive
Suite 340
Hanover, Maryland 21076

Dear Dr. Kan:

This is to notify you that your submission pursuant to section 4 13(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) dated May 20, 1999, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., *Ganoderma Lucidum* Spore Powder) was received by the Food and Drug Administration (FDA) on June 28, 1999. Your submission will be kept confidential for 90 days from the date of receipt, and after September 25, 1999, your submission will be placed on public display at Dockets Management Branch (Docket No. 953-03 16). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

Sincerely,

Robert J. Moore, Ph.D.
Senior Regulatory Scientist
Division of Programs and Enforcement Policy
Office of Special Nutritionals

GloboAsia LLC
美國環亞藥業有限公司
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72 Hanover Parkway Drive Suite 210 340
Maryland
Phone: 410-712-0600 Fax: 410-712-9547

June 23, 1999

Robert J. Moore, Ph.D.
Senior Regulatory Scientist
Division of Programs and Enforcement Policy
Office of Special Nutritionals (HFS-456)
Center for Food safety and Applied Nutrition
U.S. Food and Drug Administration
200 C Street, S.W.
Washington, D.C. 20204

RE: 75-day Premarket Notification for New Dietary Ingredient

Dear Dr. Moore,

As the authorized U.S. agent and on behalf of our client Green Power Health Products International Company, Ltd. ("Green Power"), notice is hereby given pursuant to the requirements of Section 413 (a)(2) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 350B) of the intent of Green Power to market a new dietary ingredient, Ganoderma Lucidum Spore Powder, in the U.S. The brand name for the product is Enhanvol Ganoderma Sporo-Pollon (100%). Accordingly, an original and two copies of this notification and related product information are submitted for your reference.

The new dietary ingredient will be sold in 300 mg capsules. The recommended dose is taking orally l-2 capsules each time, up to 3 times a day.

Five toxicity tests using this new dietary ingredient were included in this submission to support that this ingredient is safe to use. In the acute toxicity test, 40 mice were giving an oral dose of Spore Powder between 2.15 and 21.5 g/kg body weight. All animal survived and no toxic reactions were observed over one week. The estimated LD_{50} is larger than 21.5 g/kg and this compound is not considered toxic according to the acute toxicology classifications. This high dose of 21.5 g/kg is more than 800 times the recommended maximum daily oral dose (1.8 g) for a person of 70 kg body weight.

The accumulative toxicity test in 60 mice given daily dose for over 21 days showed that no significant difference in the changes of body weight between the control and the treatment groups. Furthermore, no abnormalities of the internal organs were observed for both groups. It is concluded that this dietary ingredient did not have any accumulative toxicity in animals.

Other toxicity studies indicated that this dietary ingredient did not induce mutations in the mouse micronucleus test and in the Ames test. It did not cause abnormalities of mouse sperms.

Based on the information submitted, we have concluded that this dietary ingredient, Ganoderma Lucidum Spore Powder, will reasonably be expected to be safe under the recommended Directions For Use.

Please direct all correspondence to me and fell free to call me at (410) 712-0609 if you have any question regarding this matter.

Sincerely yours,

Nancy Kan, Ph.D.
Senior Regulatory Scientist
GloboAsia LLC

Enclosures

cc. Green Power Health Products International Company, Ltd.

Ganoderma Lucidum Spore Powder

Table of Content

Introduction

Ganoderma Lucidum Spore Powder

1. Introduction

1.1. *Ganoderma lucidum* is a traditional Chinese medicine

Ganodema lucidum is a Basidiomycete. The family of Basidiomycetes consists of over 25,000 different species of fungi including mushrooms. *Ganoderma Zucidum* is also referred as "Ling Zhi" (Chinese name) or "Reishi" (Japanese name). It is considered a medicinal mushroom and a precious herb in traditional Chinese medicine. The entire mushroom is used. *Ganoderma lucidum* is recorded in the Chinese medical classics. It is described in details in Compendium of Material Medica, authored by Li Shi Zhen, a famous medical scholar in Ming Dynasty (1368 – 1644). This compendium was later translated into several languages, such as Japanese, English, French, German, Russian and Latin in the 20^{th} century. As "Ling Zhi" was also prominently mentioned in the famous 14^{th} century Chinese folklore "The Story of a White Snake", it has been widely known in the Orient and considered a very valuable herb.

The uses of *Ganodema Zucidum* described in ancient Chinese medical books are to promote longevity and maintain vitality of the human body. Many beneficial effects of *Ganodema lucidum* have been claimed. Most of these claims have not been studied in controlled clinical trials, although there has been an abundance of clinical use, *in vitro* and animal data. The major benefit of the herb appears to be its immuno-modulating action, resulting in enhancement of immunity, improvement of liver functions, improvement and restoration of the normal functions of the respiratory system, and prevention of certain viral infections. The anti-hypertensive action of the herb demonstrated in the animal may be important in regulating the cardiovascular system and lowering blood pressure in humans. The anti-tumor and anti-HIV protease activities found *in* vitro and in the animal may help explain the claims that G. *Zucidum* could prevent cancer, hepatitis B, hepatitis C and hepatoma, although clinical data to support the claims is not available.

1.2. *Ganoderma lucidum Spore*

A mature *Ganodenna Zucidum* mushroom produces millions of air-born spores. The spores, when mature, are ejected into the air often forming a light cloud near the mother mushroom. Therefore, the spores of *Ganoderma Zucidum* are also called spore powder or sporo-pollen. Under an electron microscope, mature *Ganodenna Zucidum* spores are oval shaped, about 8-12 x 6-7 mm.

Each spore is covered with two layers of very tough wall called the sporoderm. Natural germination process is very slow with a low germination rate. Even under optimal condition, budding occurs in 24 to 48 hours, and mycelia formed after 72 hours with a germination rate of 3 – 15%.

1.3. New Dietary Ingredient: Ganoderma Lucidum Spore Powder

Each *Ganoderma Zucidum* spore contains a complete copy of the hereditary blueprint for making of the plant. New substances, collectively called bio-ingredients, are synthesized during the vigorous process of germination. The *Ganoderma Zucidum* spores are also used as herb. However, as the spores are covered with two layers of very tough wall (sporoderm), it is questionable whether the nutrients inside the spores can be efficiently absorbed when ingested intact.

The new dietary ingredient, Ganoderma Lucidum Spore Powder, is made from processed spores. Proprietary procedures are utilized to select the spores, promote germination, and micro-break the sporoderm. The purpose of this process is to promote the synthesis of bio-ingredients, disrupt the sporoderm to enhance body absorption, and retain the activities of the bio-ingredients throughout the processing procedures. The manufacturing process is described in Section 4. After further drying and refinement, the resultant powder form is the dietary ingredient, Ganoderma Lucidum Spore Powder.

This ingredient is then encapsulated to produce the brand name product, Enhanvol Ganoderma Sporo-Pollen (100%).

The human uses of the entire mushroom of *Ganoderma Zucidum* have been documented for a long period of time. In comparison, the use of pure *Ganoderma Zucidum* spores is of recent decades. The new dietary ingredient, made from processed spores using a recently invented proprietary technique, had been subjected to toxicity studies to ensure its safety for human use. Results of the toxicity studies of this product are summarized in Section 5 and described in Section 6.

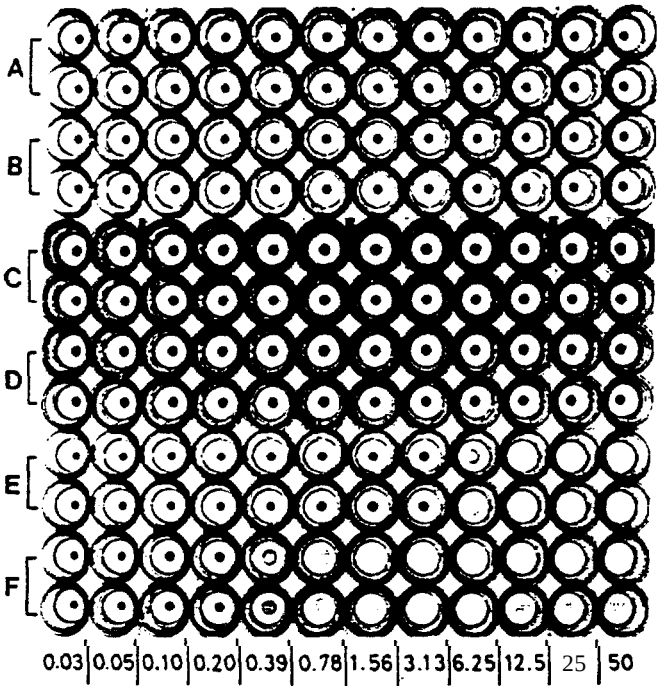


FIG. 3. Hemagglutination activity of LZ-8. Hemaggiutination activity toward human red blood cells of types A *(A),* B *(B),* AB *(C),* and 0 *(D)* and sheep red blood cells *(E)* were determined as described under 'Experimental Procedures." Duplicate assays are shown and as a control, serially diluted Con A (30 ng/ml to 50 μg/ml) was added instead of LZ-8 to sheep red biood cells *(F).*

TABLE III

Effects *of* LZ-8 *on* the *production of active systemic anaphylaxis in mice*

CFW mice were sensitized subcutaneously (s.c.) or intraperitoneally (i.p.) and then shocked with BSA intravenously (i.v.), and the response was noted as described under "Experimental Procedures." Mice receiving LZ-8 were given 6.9 mg/kg body weight twice per week, altogether six times in Experiment 1. and 7.1 mg/kg body weight twice per week, altogether seven times in Experiment 2. As a negative control, 1 mg ovalbumin (OA) was administered instead of BSA as the shocking injection in Experiment 1. The effect of LZ-8 treatment simultaneous with the shocking injection was also studied *in* Experiment 2.

Experiment no.	Sensitizing injection	Shocking injection	LZ-8 treatment	Result S/T[a]	Result D/T[b]
1.	BSA (s.c.)	BSA (i.v.)		10/10	4/10
	BSA (s.c.)	OA (i.v.)	−	0/10	0/10
	BSX (s.c.)	BSA (i.v.)	+	0/10	0/10
2.	BSA (i.p.)	BSA (i.v.)		10/10	4/10
	BSA (i.p.)	BSA-LZ-8 (i.v.)	−	9/10	2/10
	BSA (i.p.)	BSA (i.v.)	+	0/10	0/10

[a] Number with anaphylactic symptoms/total number of mice.
[b] Number with anaphyiactic deaths/total number of mice.

was not caused by cell-mediated delayed type reaction. After 2 and 3 days, no difference in footpad thickness (swelling reduced) was noted within the control or LZ-8-treated groups, although 1 of 10 mice in the control group remained positive 1 day after footpad injection.

We compared some mitogens, especially Con A which is able to stimulate production from spleen cells of a suppressive factor against antibody production (16), with LZ-8 for effect on blast-formation stimulatory activity using mouse spleen cells. From a dose-response study of either LZ-8 or Con A alone, maximum uptake of [^{3}H]thymidine was observed at concentrations of 3.13 and 6.25 μg/ml, respectively (Fig. 5*A*).

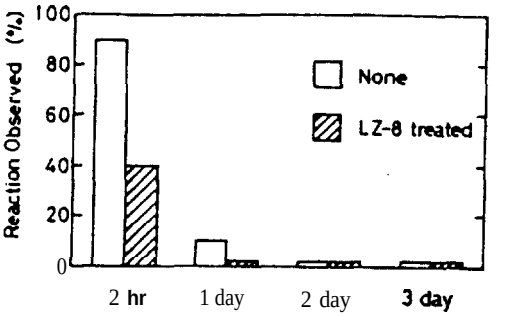


FIG. 4. Effect of LZ-8 on Arthus reaction in *mice.* CFW mice, untreated or treated with LZ-8, were sensitized to BSA and then tested for ability to produce an Arthus reaction as &scribed under 'Experimental Procedures."

When LZ-8 and Con A were added in combination, maximum [^{3}H]thymidine uptake was shifted to 3.13 μg/ml total mitogen, or 1.56 μg/ml of each, and the amount was intermediate between the maximum values for LZ-8 or Con A alone. On the other hand, a dose-response study of LPS revealed a saturable effect on [^{3}H]thymidine uptake (Fig. 5*B*). This effect of LPS has been noted previously (17). Combined addition of LZ-8 and LPS produced maximum [^{3}H]thymidine uptake at 3.13 μg/ml total mitogen, or 1.56 μg/ml of each, and in this case the combined maximum response was greater than that of the individual mitogens alone. At concentrations below those required for maximum [^{3}H]thymidine uptake (0.10-3.13 μg/ml total or 0.05-1.56 μg/ml individual mitogen), LZ-8 and LPS appeared to have an additive effect on uptake, as the value for the mitogens added in combination closely approximated the sum of the values for the individual mitogens alone. In the comparison of LZ-8 with Con A (Fig. 5*A*), below concentrations required for maximum [^{3}H]thymidine uptake (same as above), the uptake produced by combined addition of mitogens exceeded the sum of the values for the individual mitogens alone.

DISCUSSION

Estimation of the purity and yield of LZ-8 obtained following our isolation procedure differed greatly depending on the assay method for determining LZ-8 activity (Table I). The hemagglutination assay gave a final purification of 20.4-fold with a yield of 13%, whereas the more sensitive blast formation assay gave 88.8-fold purification with a 57% yield A reason for the unexpected increase in LZ-8 activity determined by blast formation assay after the gel filtration step may be removal of cytotoxic material present in the crude extract which could be affecting the bioassay (thy&dine uptake).

Examination of the hemaggiutination activity of LZ-8 with sheep and human red blood cells revealed ability to aggregate only sheep red blood cells and not human red blood cells of any types (Fig. 3). Binding sites for LZ-8 selectively expressed on the surface of sheep red blood cells may be postulated, but the exact mechanism of hemagglutination by LZ-8 remains unknown. Failure of seven kinds of mono- and disaccharides to inhibit the reaction suggests that sugar is not involved in the interaction. Goldstein et *al.* (18) have defmed a lectin as a sugar-binding (glyco)protein which agglutinates cells; then it should be possible to define specificity of the lectin in terms of mono- or disaccharides that inhibit the reaction. Thus, whereas as LZ-8 has hemagglutination activity and some lectins are potent mitogens, LZ-8 is not a lectin *per se.* Nevertheless, LZ-8 possesses mitogenic activity toward mouse

Product
Specifications

Ganoderma Lucidum Spore Powder

2. Product Specifications

Product Name:	Enhanvol Ganoderma Sporo-Pollen (100%)	
Content:	Each gelatin capsule contains 300 mg of Ganoderma Lucidum Spore Powder (100%)	
Manufacturer:	Green Power Health Products International Company, Ltd. 710A Argyle Centre, 688 Nathan Road Mongkok, Kowloon, Hong Kong	
Heavy Metals:	Arsenic	< l.Oppm
	Cadmium	< 1.0 ppm
	Mercury	< 0.5 ppm
	Lead	< 1.0 ppm
	Copper	< 20 ppm
Microorganisms:	Total plate count	Less than 10 CFU/g
	E. coli	Less than 30 CFU/100g
	Pathogenic bacteria	Not detected
Packaging:	300 mg/capsule 60 capsules/bottle Net weight 18 g/bottle	

Directions for Use:

Take 1-2 capsules each time, up to 3 times a day.

Nutritioal
Analysis

3. Nutritional Analysis

The analysis report is attached in Appendix A.

Test Facility:	Analytical Chemical Services of Columbia, Inc. 9151 Rumsey Road, Suite 190 Columbia, Maryland 21045, U.S.A.
Tests conducted by:	Dr. Reyaz A. Kango
Test procedures:	Standard procedures as described in "AOAC Official Manuals of Analysis" 15th edition (1990), AOAC International, Arlington, Virginia, U.S.A. (Copies of the methods, except the pathogen screen, are attached in Appendix A.)
sugar:	AOAC 977.20, page 1030
Protein:	AOAC 976.05, page 72
Sodium:	AOAC 985.35, page 1110
Arsenic, Cadmium, Lead:	AOAC 986.15, page 237
Mercury:	AOAC 977.15, page 263
Pathogen screen:	Standard microbiologic method
Product Name:	Enhanvol Ganoderma Sporo-Pollen (100%)
Lot tested:	02/99
Lot expiration date:	02/01
Test date:	06/01/99

Ganoderma Lucidum Spore Powder

Test report date: 06/08/99

Test result:

Test	Concentration	Nutritional Value*
sugars		
Fructose	0.66%	0
Glucose	1.78%	0
Total sugars	2.44%	0
Protein	33.3%	0
Sodium	17180 ppm	0
Heavy Metals		
Arsenic	0.80 ppm	
Cadmium	0.18 ppm	
Lead	Not detected	
Mercury	Not detected	
Pathogen Screen		
Coliform	Not detected	
Salmonella	Not detected	
Staphylococcus	Not detected	
Yeast & Mold	< l/g	
Total Plate Count	< 10/g	

*. Based on 500 mg serving size.

Manufacturing
Process

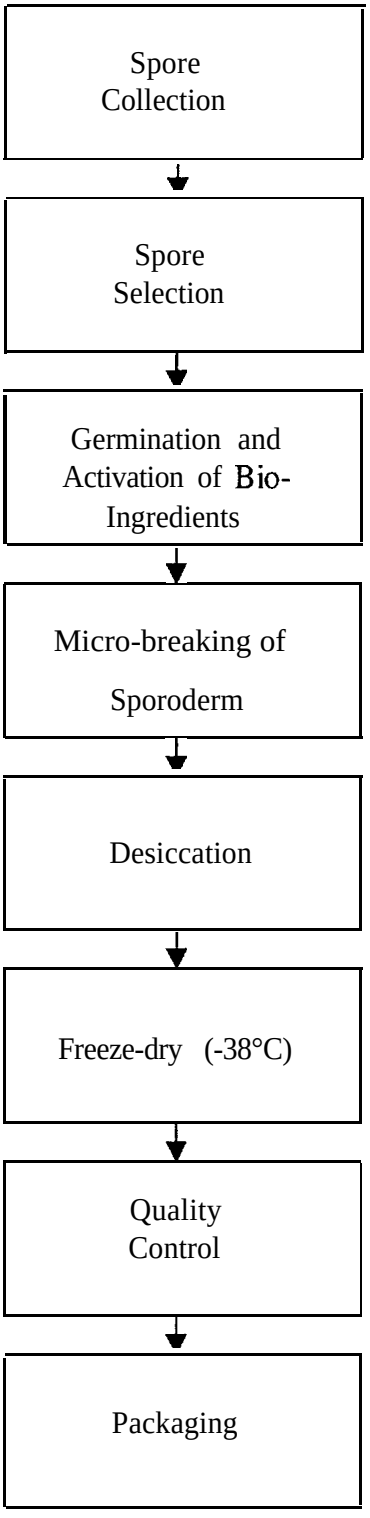
4. Manufacturing Process

A flow chat of the manufacturing process is presented in the following page.

The new dietary ingredient, Ganoderma Lucidum Spore Powder, is manufactured using a proprietary technique, which can be described as sporo-germination activation and micro-breaking of sporoderm.

The process is briefly described as following. The spores are collected from the fungi, *Ganoderma lucidum*. Then, matured well-formed spores are selected. The selected spores then undergo sporo-germination activation, that is, cultivation in liquid media under controlled temperature for a certain period of time followed with incubation under controlled temperature and humidity. Successful germination rate is 95% or higher. The spores are further subjected to enzyme treatment at low temperature to micro-break the sporoderm while preserves the bio-ingredients in their active state. The processed spores are then dried via desiccation and freeze-drying, and refined into powder form to yield the final ingredient, Ganoderma Lucidum Spore Powder. Quality of the powder ingredient is inspected. The pure ingredient is then encapsulated in gelatin capsules and packaged to produce the final brand name product, Enhanvol Ganoderma Sporo-Pollen (100%).

Manufacturing Process Flow Chart



Summary of
Safety Considera

5. Summary of Safety Considerations for Ganoderma Lucidum Spore Powder

Five toxicity studies of the dietary ingredient, Ganoderma Lucidum Spore Powder [as in the brand name product, Enhanvol Ganoderma Sporo-Pollen (lOO%)] were performed during 1996 at the Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China. An official report was issued by the government regulatory agency in August 1996. A copy of this toxicity report, "Report of the Public Health Inspection Results" for Ganoderma Lucidum Spore Powder, in the Chinese language is presented in Appendix B together with its English translation. The results from these studies are briefly summarized in the following paragraphs.

- The acute toxicity test in mice demonstrated that the oral LD_{50} of Ganoderma Lucidum Spore Powder in mice was more than 21.5 g/kg body weight. The highest dose used in the study, 21.5 g/kg, is more than 800 times higher than the recommended maximum daily dose of 1.8 g for an average 70 kg human.

- The accumulative toxicity study in mice, receiving an accumulative dose of 129 g/kg over a 25-day period, demonstrated no significant effects on the body weight of the test animals. No abnormalities were observed in the internal organs examined compared to control groups.

- Ganoderma Lucidum Spore Powder did not induce mutations of the femur bone marrow cells in the mouse bone marrow micronucleus test.

- Study on the malformation of mouse sperms showed that at the high dose of 10g/kg, Ganoderma Lucidum Spore Powder did not induce abnormalities in mouse sperms.

- The Ames test indicated that Ganoderma Lucidum Spore Powder did not induce, directly or indirectly, mutations in the bacterial strains tested.

- It is therefore concluded that Ganoderma Lucidum Spore Powder was not toxic to the test animals at the doses used. The results of the animal toxicity tests are presented in the following section.

Based upon the toxicity study results, we conclude that Ganoderma Lucidum Spore Powder is considered safe to use at the recommended maximum oral daily dose of 1.8 g (300 **mg/capsule,** 2 capsules each time, three times a day).

Toxicity Studies

6. Toxicity Tests in Animals

This section presents the results of the five toxicity studies reported in the Chinese document, "Report of the Public Health Inspection Results" for Ganoderma Lucidum Spore Powder, which is summarized in Section 5. A copy of the Chinese document and its English translation are attached in Appendix B.

6.1. Test Facility

Food Safety and Inspection Laboratory, Institute of Health, Guang Dong Province, China.

6.2. Test Date

March 18, 1996

6.3. Report Date

August 2, 1996

6.4. Material

6.4.1. Test Compound

The test compound, Ganoderma Lucidum Spore Powder, abbreviated as Spore Powder below, does not dissolve in water, and has a brown color with a fragrance unique to this family of fungi. The test compound was made into desired concentrations for the following experiments with 3% starch solution.

6.4.2. Animals

Healthy, white NIH mice were supplied by the Guang Dong Province Medical Animal Farm, China. The mice weighed between 16 to 23 grams.

6.5. Methods and Results:

6.5.1. Acute Toxicity Test in Mice

Method:

Forty (40) NIH mice (20 females and 20 males), weighing between 18 to 22 grams, were randomized into four dose groups according to the Horn's methodology. One dose of the test product was given to the mice by gavage on empty stomach. The mice were observed for one week. The results are shown in Table 1.

Table 1. Acute Toxicity Study Results

Dose (g/kg)	No. of Animals		No. of Dead Animals	
	Male	Female	Male	Female
21.50	5	5	0	0
10.00	5	5	0	0
4.64	5	5	0	0
2.15	5	5	0	0

Conclusion:

No adverse reactions to the test compound were observed in the mice. The LD_{50} value was therefore larger than 21.5g/kg body weight. The test compound was determined to be non-toxic.

6.5.2. Accumulative Toxicity in Mice

Method:

Sixty NIH mice, weighing between 18 to 20 grams, were divided into treatment groups (20 females and 20 males) and the control group (10 females and 10

males). The test was performed according to an accumulative index methodology. The animals were given a daily dose for consecutive 2 1 days. The initial dose was equivalent to one tenth of the LD_{50}. After 21 days, the mice in the treatment group were given an extra dose equivalent to LD_{50} (2 1.5 g/kg, the highest dose in the Acute Toxicity Test in Mice described in Section 6.5.1). After observation for three more days, the mice were sacrificed and the experiment was terminated. The liver, kidneys and testes were taken from the sacrificed mice for macroscopic observation.

Results:

(1) The accumulative dose: Initial dose was 2.15 g/kg. At the end of the experiment (Day 25), the accumulative dose was 129 g/kg body weight.

(2) Body weights: There were no marked differences in the net weight gain between the treatment and control groups. The female mice in the treatment and control groups had an average net weight gain of 4.6g and 4.1 g, respectively, The male mice in the treatment and control groups had an average net weight gain of 6.3g and 6.9g, respectively.

(3) Macroscopic examination of internal organs: No abnormalities were observed during the macroscopic examination of the internal organs in both the control and treatment groups.

Conclusion:

The Spore Powder did not have any accumulative toxicity in mice.

6.5.3. Mouse Bone Marrow Micronucleus Test

Method:

Seventy (70) NIH mice, weighing between 20 to 23 grams, were placed into six dose groups of 0, 0.63, 1.25, 2.50, 5.00 and 10.00 g/kg. Each group consisted of 10 mice, 5 females and 5 males. The seventh group was the positive control

group (cyclophosphamide). The experiment was performed according to the "Procedure of Toxicity Evaluation for Food Safety". The mice were dosed in two gavage administrations. The mice were sacrificed six hours after the second administration. The bone marrow from both femurs was removed and made into slides, which were subsequently stained and examined under microscope. Ten thousand polychromatic erythrocytes (PCE) were examined. The numbers of micronucleated polychromatic erythrocytes (MN-PCE) were determined. The Micronucleated Ratios were then determined as the number of MN-PCE per 1000 PCE. The results are shown in Table 2.

Table 2. Mouse Micronucleus Test Using Sporoderm Powder

Dose (g/kg)	No. of Animals Male	Female	No. of PCE Examined	No. of MN-PCE	Micronucleated Ratio (0/00)
0	5	5	10.000	14	1.6
10.00	5	5	10,000	13	1.3
5.00	5	5	10,000	10	1.0
2.50	5	5	10,000	10	1.0
1.25	5	5	10,000	12	1.2
0.63	5	5	10,000	13	1.3
Cyclophosphamide (0.06)	5	5	10,000	249	24.9"

*. The placebo control group and the treatment groups were compared to the cyclophosphamide positive control group, $p < 0.001$, using two-tail T test.

Conclusion:

No significant differences among the Micronucleated Ratios of the placebo control and the dose groups were observed. In comparison, the cyclophosphamide positive control group was significantly different from the

placebo control group ($p < 0.001$). The experimental results indicated that the Spore Powder did not induce mutations in the mouse micronucleus test.

6.5.4. Study on the Malformation of Mouse Sperms

Method:

Twenty-five NIH male mice, weighing 16 – 17 grams, were randomized into five groups. They were given the test compound once a day by gavage for five consecutive days, at the dose levels of 0, 2.50, 5.00, and 10.00 g/kg. The positive control compound, cyclophosphamide, was administered intraperitoneally (i.p.) at the dose level of 0.04 g/kg. After 35 days, the mice were sacrificed. Both testes were removed, made into slides, and stained following the standard procedure. Under microscope through oil lens, 5000 sperms were examined. The number of abnormal sperms was obtained. The abnormality rate was expressed as abnormal sperms per 1000 sperms examined. The results are shown in Table 3.

Table 3. Results of the Mouse Abnormal Sperms

Dose (g/kg)	No. of Animals	No. of Sperms Examined	No. of Abnormal Sperms	Abnormality Rate (0/00)
0	5	5,000	62	12.40
10.00	5	5,000	56	11.20
5.00	5	5,000	43	8.60
2.50	5	5,000	73	14.60
Cyclophosphamide (0.04)	5	5,000	364	72.30*

* Following the Wicoxon Serial Examination method, the placebo control group and the dose groups were compared to the cyclophosphamide control group, $p < 0.01$.

Conclusion:

There were no significant differences between the treatment groups and the placebo control group. The most common abnormality of the sperms in the treatment and placebo control groups was unfixed-shape. The next most common abnormality was a swelling head. No other abnormalities were observed. In the cyclophosphamide positive control group, the most common abnormality of the sperms was unfixed-shape. Occasionally, no-hook-shape, banana-shape and curly-tail-shape sperms were observed.

The Spore Powder at the highest dose of 10 g/kg did not induce abnormalities in mouse sperms.

6.5.5. Ames Test

Method:

The bacterial strains (TA97, TA98, TA100, and TA102) were provided by the Beijing Food Inspection Office, Department of Health, China. The bacterial strains were examined for certain characteristics and S9 reactivity and were found suitable for the Ames test. The Ames test in the presence and absence of S9 extract was performed according to standard procedures. Duplicate experiments were performed independently. Three plates were used for each dose level. The results are shown in Table 4.

Table 4. Results of the Ames Test with Spore Powder

	Dose (μg/plate)	TA97		TA98		TA100		TA102	
		- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
Natural mutation	0	140 ± 7	147 ± 8	33 ± 3	36 ± 4	142 ± 7	144 ± 7	250 ± 17	275 ± 19
Sample	5	132 ± 6	150 ± 7	35 ± 3	38 ± 4	142 ± 6	144 ± 9	260 ± 19	275 ± 20
	50	138 ± 7	159 ± 8	34 ± 4	36 ± 5	162 ± 7	161 ± 11	248 ± 21	255 ± 18
	500	155 ± 6	148 ± 7	29 ± 3	35 ± 3	142 ± 8	167 ± 10	242 ± 21	262 ± 19
	5000	145 ± 7	154 ± 7	32 ± 3	34 ± 4	156 ± 9	160 ± 10	258 ± 20	274 ± 18
2-AF			1635 ± 38	-	4850 ± 95	-	2876 ± 50	-	530 ± 9
Dexon		1342 ± 41	-	1088 ± 27	-				
NaN_3	45					2640 ± 76	-		
MMC								2210 ± 62	-

MMC = mitomycin C

Conclusion:

The numbers of mutated colonies in the treatment groups were comparable to those of the naturally occurred mutations for the four bacterial strains tested, regardless of the doses and the presence or absence of the S9 mixture. In contrast, the positive control groups (2-AF, Dexon, NaN_3 and MMC) showed markedly higher numbers of mutated colonies than the naturally occurred mutations. It is concluded that Spore Powder did not induce mutations in the Ames Test.

Pcol & Clinica
Studies in Lit.

7. Pharmacology and Clinical Studies of *Ganoderma lucidum* Reported in the Literature

Over one hundred oxygenated triterpenens have been isolated from *G. lucidum*. Of these, ganoderic acid β, lucidumol B, ganodermanondiol, ganodermanontriol and ganolucidic acid A showed activity to inhibit human immunodeficiency virus (HIV) protease in a recent report (Min BS, Nakamura N, Miyashiro H, et *al., Chem. Pharm. Bull.(Tokyo),* 1998, 46: 1607- 16 12). A new protein, LZ-8, has been isolated from *Ganoderma lucidum* and shown to be a member of the immunoglobulin superfamily (Kino K, Sone T, Watanabe J, et al. *Int. J. Immunopharmacol.*, 199 1, 13: 1109- 1115; van der Hem LG, van der Vliet JA, Bocken CFM, et al., *Transplantation,* 1995, 60:438-443). Treatment with LZ-8 prevented BSA-induced systemic anaphylaxis in CFW mice (Kino K, Yamashita A, Yamaoka K, et al., *J. Biol. Chem.*, 1989, 264:472-478) and the development of autoimmune type I diabetes in NOD mice (Kino K, Mizumoto K, Sone T, et al., *Diabetologia,* 1990, 33:7 13-7 18). These results demonstrated an immunosuppressive action of *G. lucidum* in vivo. *G. lucidum* is very rich in polysaccharides. It has been found that crude or partially purified polysaccharides of *G. lucidum* significantly inhibited the growth of locally implanted S 180 sarcoma and reduced tumor metastasis in mice (Miyazaki T & Nishijima M, *Chem. Pharm. Bull.(Tokyo),* 1981, 29:361 l-36 16; Maruyama H, Yamazaki K, Murofushi S, et *al., J. Pharmacobio-Dyn.*, 1,989, 12: 118-123). Wang et al (Wang SY, Hsu ML, Hsu HC, et al., *Int. J. Cancer,* 1997, 70:699-705) discovered that the anti-tumor effect *of G. lucidum* polysaccharides is mediated by cytokines released from activated macrophages and T lymphocytes treated with G. *lucidum*. Another study (Lieu CW, *Lee SS* & Wang SY, *Anticancer Res.,* 1992, 12:1211-12 16) was consistent with this conclusion, demonstrating that the polysaccharide fraction of *G. lucidum* (PS-G) induced differentiation of human leukemic cell line U937. However, the differentiation was induced by the conditioned medium from PS-G stimulated human blood mononuclear cells. PS-G itself did not have any effect on the target cells. Yet another pharmacological effect of G. *lucidum* is its anti-hypertensive

action and an inhibitory effect on angiotensin converting enzyme (ACE) (Lee SY & Rhee HM, *Chem. Pharm. Bull.(Tokyo)*, 1990, 38:1359-1364; Morigiwa A, Kitabatake K, Fujimoto Y & Ikekawa *N, Chem. Pharm. Bull.(Tokyo)*, 1986, 34:3025-3028). Extract of *G. Zucidum* was shown to decrease systolic and diastolic blood pressure in rabbits, probably due to its central inhibitory effect on the sympathetic nerves. Furthermore, the water-soluble fraction of *G. Zucidum* was shown to suppress platelet aggregation of bovine blood in vitro (Shimizu A, Yano T, Saito Y & Inada Y, *Chem. Pharm. Bull.(Tokyo)*, 1985, 33:3012-3015).

In the literature, mice were able to tolerate intraperitoneal injection of water- or alcohol-soluble extractions of *Ganoderma Zucidum* spores up to 40 g/kg body weight (Liu G, Bao, T, Niu X, et al., *Chin. Med. J.*, 1979, 92:496-500).

In a clinical study, ten patients (8 males and 2 females) with atrophic myotonia were given a water-soluble preparation of *Ganodenna Zucidum* spores by intramuscular injection (Fu HD & Wang ZY, *J. Tradit. Chin. Med.*, 1982, 2:63-65). The patients received an initial dose of 400-800 mg/day for a period of time for a total dosage of 38.4-180 g. The patients were followed up from 8 months to 6.8 years. No short-term and long-term adverse reactions were observed.

A clinical evaluation of the anti-hypertensive effect of lyophilized *G. lucidum* extract was reported by Kanmatsuse et al (Kanmatsuse K, Kajiwara N, Hayashi K, et *al., Yakugaku Zasshi,* 1985, 105:942-947). Fifty-three patients with either essential hypertension, mild hypertension, or normal blood pressure were given *Ganodenna Zucidum* extract as oral tablets. Each patient took 6 tablets containing 240 mg of the extract every day for a period of 6 months. The result showed that *G. Zucidum* had blood-pressure lowering effect on patients with essential hypertension, and did not have any side effects on patients with essential or border line hypertension during 6 months of oral intake.

Conclusion

8. Conclusion

Based upon the toxicity study results, we conclude that Ganoderma Lucidum Spore Powder is considered safe to use at the recommended maximum oral daily dose of 1.8 g (300 **mg/capsule,** 2 capsules each time, three times a day).

References

9. References

A copy of each article is attached in Appendix C.

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Appendix A.

Appendix A. Nutritional Analysis Report and Standard Test Procedures

Appendix A.1 Nutritional Analysis Report

ANALYTICAL CHEMICAL SERVICES
OF COLUMBIA, INC.

410-730-7782
800-842-ACSC (2272)
Fax: 410-730-8340

8 June, 1999

Dr. Nancy Kan
7250 Parkway Drive, Suite 340
Hanover, MD 21076

Dear Dr. Kan:

Enclosed please find the results on "Enhanvol" (Ganoderma Sporo-pollen) capsules. Our studies have shown that there are not any significant amounts of protein, sugars or nutrients in them. Mercury and lead are below detection limits, however 0.8 ppm of arsenic and 0.18 ppm of cadmium are present in these samples.

The pathogen screen results did not show any coliform, salmonella or staphylococcus in them. Total plate count is less than 10/g and yeast and molds are also less than l/g in them.

Sincerely,

Reyaz A. Kango, Ph.D.
Senior Chemist

9151 Rumsey Road . Suite 190 . Columbia, MD 21045

Table I

Parameter	Concentration	Nutritional Value*
Sugars		
Fructose	0.66%	0
Glucose	1.78%	0
Total Sugars	2.44%	0
Sodium	17180 ppm	0
Protein	33.3%	0
Heavy Metals		
As	0.80 ppm	
Ed	0.18 ppm	
Hg	Not Detected	
Pg	Not Detected	
Pathogen Screen		
Coliform	Not Detected	
Salmonella	Not Detected	
Staphylococcus	Not Detected	
Yeast & Mold	< 1/g	
Total Plate Count	< 10/g	

* Based on 500mg serving size.

Appendix A.2 Fructose and Glucose Tests