



Memorandum

~~SECRET~~ DOCKETS TRANSMITTAL MEMO

0432 '03 JAN 27 P2:23

Date: *JAN 23 2003*
From: Consumer Safety Officer, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-821
Subject: 75-Day Premarket Notification of New Dietary Ingredients
To: Dockets Management Branch, HFA-305

Subject of the Notification: *Pueraria mirifica*

Firm: Triarco Industries

Date Received by FDA: 8/09/02

90-Day Date: 11/07/02

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

Gloria Chang
Gloria Chang, R.Ph./Interdisciplinary Scientist

Attachments

95S-0316

RPT 149



400 Hamburg Turnpike
Wayne, NJ 07470
973-942-3100 973-942-8873 fax

November 5, 2002

Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Attention: Felicia B. Satchell, Director
Division of Standards and Labeling Regulations

Re: Premarket Notification for *Pueraria Mirifica*

Dear Ms. Satchell:

Please allow this letter to serve as our response to your correspondence of October 22, 2002 regarding the above product. With regard to the issue of confidentiality, we consider our entire submission to be confidential, however, the following information is considered to be the most highly confidential and proprietary aspect of the submission and we would appreciate it not being placed on public display: *Anything pertaining to toxicity studies, long term human studies and any referenced correspondence and/or information from any Thai government employee or agency.*

Should you have any questions concerning the above, please feel free to contact the undersigned.

Very truly yours,

A handwritten signature in black ink that reads "Mark Anderson, Ph.D." with a stylized flourish at the end.

Mark Anderson, Ph.D.
Director, Research & Development

/gk



400 Hamburg Turnpike
Wayne, NJ 07470
(973) 942-5100 ext.30 (Phone)
(973) 942-9100 (Fax)

Fax

To:	Felicia B. Satchell, Director	From:	Dr. Mark Anderson
Fax:	301-436-2639	Pages:	2, including cover sheet
Phone:		Date:	11/05/02
Re:	Premarket Notification for <i>Pueraria Mirifica</i>	CC:	

• **Comments:**

Please see attached correspondence regarding the above.



OCT 22 2002

Rodger R. Rhode, Jr., President
Triarco Industries
400 Hamburg Turnpike
Wayne, New Jersey 07470

Dear Mr. Rhode:

This is in response to your notification dated May 17, 2002, you submitted pursuant to 21 U.S.C. 350b(a)(2) and 21 Code of Federal Regulations (CFR) Part 190.6 and initially received by the Food and Drug Administration (FDA) on May 20, 2002. On July 15 2002, we requested additional information in accordance with 21 CFR 190.6. We received a facsimile from you with this information on July 19, 2002. Subsequently, on July 25, 2002, we requested additional information. FDA received the additional information on August 9, 2002, which is the new effective filing date. Your notification concerns the substance, *Pueraria mirifica*, that you assert is a new dietary ingredient. Your notification did not specifically identify the author of the Latin binomial name of *Pueraria mirifica*. For purposes of this letter, we will assume that the complete Latin binomial name of your ingredient is *Pueraria mirifica* Airy Shaw & Suvatbandhu and will use this name when referring to the ingredient in this letter.

Your notification states that, under the recommended conditions of use, the maximum daily intake of *Pueraria mirifica* Airy Shaw & Suvatbandhu root extract powder will be 100 mg and that the dietary supplement containing this ingredient would not be recommended for use by pregnant or lactating women.

Under 21 U.S.C. 350b(a)(2), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient is required to submit certain information to FDA at least 75 days before a new dietary ingredient or a dietary supplement containing it is introduced or delivered for introduction into interstate commerce. This information must include the basis on which the manufacturer or distributor has concluded that the new dietary ingredient or a dietary supplement containing the new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under 21 U.S.C. 350b(a)(2), there must be a history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions

recommended or suggested in the product's labeling, will reasonably be expected to be safe. If this requirement is not met, the new dietary ingredient or dietary supplement containing it is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B), because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your notification and has significant concerns about the evidence on which you rely to support your conclusion that the ingredient, *Pueraria mirifica* Airy Shaw & Suvatabandhu, will be reasonably expected to be safe for the suggested or intended uses.

You indicate in your notification that the ingredient is an extract of the root of *Pueraria mirifica* Airy Shaw & Suvatabandhu that is prepared by soaking dried roots of the plant in ethanol for 48 hours. The extract liquid is then concentrated and supplied in a powder form at a concentration ratio of 20:1. The concentrated extract is mixed with di-calcium phosphate and dried before packing. You state that the extract contains phytosterols, isoflavonoids, and coumestans and that phytosterolstoestrogens; miroestrol, isomiroestrol and deoxymiroesterol, appear to be unique constituents of the ingredient.

In both the human and animal studies, it was unclear as to whether the test substances used in the studies are the same as that of the ingredient in your notification. Moreover, your submission provides no information that the test substances used in the referenced studies are qualitatively or quantitatively similar to your ingredient or how these studies are relevant to evaluating the safe use of your ingredient under the recommended conditions of use.

Furthermore, the other information in your notification is inadequate to provide a basis to conclude that your ingredient is reasonably expected to be safe. This information consists of personal testimonials, general information on the effects of specific isoflavones and phytoestrogens from other food sources, and general descriptive information on herbal raw materials, that does not qualitatively and/or quantitatively provide any relevant evidence to support the safe use of your ingredient, the ethanol extract of *Pueraria mirifica* Airy Shaw & Suvatabandhu root. Further, the evidence of history of use you submitted referenced the use of the dried root or rhizome rather the ethanol extract of *Pueraria mirifica* Airy Shaw & Suvatabandhu root. Therefore, the evidence of history of use of your ingredient is inadequate.

In conclusion, the information in your notification does not provide an adequate basis to conclude that *Pueraria mirifica* Airy Shaw & Suvatabandhu is reasonably expected to be safe when used under the recommended or suggested conditions of use. Therefore your product may be adulterated under 21 U.S.C. 342 (f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that it does not present a significant or unreasonable risk of illness or injury. Introduction of such products into interstate commerce is prohibited under 21 U.S.C. 331 (a) and (v).

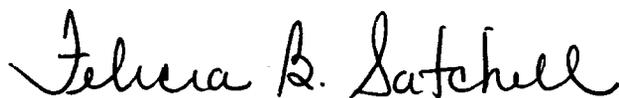
Page 3 – Mr. Rodger R. Rhode

Your notifications will be kept confidential for 90 days after the filing date of August 9, 2002. After November 6, 2002, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. However, any trade secret or otherwise confidential commercial information in the notifications will not be disclosed to the public.

Prior to November 6, 2002, you may wish to identify in writing specifically what information in your notifications you believe is proprietary for FDA's consideration. Nevertheless, our Center's Freedom of Information Officer has the authority to make the final decision about what information in the notifications should be redacted before they are posted at Dockets.

If you have any questions concerning this matter, please contact us at (301) 436-2371.

Sincerely yours,



Felicia B. Satchell
Director
Division of Standards
and Labeling Regulations
Office of Nutritional Products, Labeling
and Dietary Supplements
Center of Food Safety
and Applied Nutrition

Premarket Notification for

***Pueraria candollei* var. *mirifica* root extract**

as a New Dietary Ingredient

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I. Introduction

Pueraria candollei var. *mirifica* Airy Shaw et Suvat (hereinafter referred to as *P. mirifica*) is a Thai plant noted for very large bulbous rhizomes that grow along its roots. The dried powder of the root has traditionally been used as a folklore remedy for menopause-related vasomotor symptoms for centuries. The root of *P. mirifica* has been sold as a food supplement or non-prescription herbal medicine to the general public in Thailand for over fifty years.

Characterization studies carried out since the mid-twentieth century have determined that the rhizomes of *P. mirifica* contain various phenol estrogenic compounds, many of which are found in soy. In recent years, the properties of dried powder obtained from this root have been studied. Standardization and fingerprinting of this material have been ongoing for several years; marker compounds include: daidzin, puerarin, genistin, and daidzein. This document is submitted in support of a notification of intent to market a standardized extract of the dried powder of the root of *Pueraria candollei* var. *mirifica* Airy Shaw et Suvat (*P. mirifica*) as a new dietary ingredient.

II. Origin and Description of *P. mirifica*

P. mirifica is a member of the family Leguminosae, sub-family Papilionoideae, belonging to the soybean and pea sub-family of plants. The *Pueraria* species are strong climbers, creeping in and over low vegetation or climbing high in tall trees. At least 76 different sub-species of *Pueraria* have been taxonomically identified world-wide, many of which are found in Asia, Australia, Africa and North, Central and South America.¹ *P. mirifica* is found within the boundaries of Thailand in mixed forest areas located in the north, west, and northeast parts of the country, at elevations between 300 and 800 meters.^{2,6}

P. mirifica is a perennial woody climber, with multiple large tubers along its root system that can weight 10 to 70 kilos per tuber. A voucher sample of *P. mirifica* is kept at the School of Agriculture, University of Chiang Rai, Thailand. A taxonomist's certified voucher sample of the plant parts of *P. mirifica* is on file at Flora Research Laboratory, Inc., San Juan Capistrano, California, dated February 12, 2002, that was certified by Thawatchai Wongprasert, Taxonomist, Thai Herbarium Center, Department of Forestry, Ministry of Agriculture.³ *P. mirifica* is also grown for study purposes in experimental plots at Thai agricultural universities.

III. History and Traditional Use of *Pueraria candollei* var. *mirifica*

According to historians, the root of *Pueraria mirifica* was first described over 900 years ago in Buddhist scriptures discovered in the ruins of the ancient city of Pookham City (Pukam; now located in Burma).⁴ Traditionally, *Pueraria mirifica* root has been used in Thailand for the relief of vasomotor symptoms (hot flashes and night sweats) associated with menopause.^{2,4} As part of

the current practice of botanical medicine in Thailand, menopausal women are advised to consume powders obtained from the roots of *Pueraria mirifica* once a day before bedtime to alleviate hot flashes and night sweating.⁵

IV. Chemistry of *P. mirifica* Root Extract

Extracts of *P. mirifica* root have been characterized extensively and contain three main classes of compounds: phytosterols, isoflavonoids (isoflavones and isoflavone glycosides), and coumestans.⁶⁻

¹⁸ A number of individual constituents have been identified:

a. Phytosterols:

- beta-sitosterol
- deoxymiroestrol
- isomiroestrol
- isomiroestrol-7-methyl ester
- miroestrol
- miroestrol-3-methyl-ester

b. Isoflavonoids:

i. Isoflavones

- daidzein (7,4 -dihydroxyisoflavone)
- genistein (5,7,4 -trihydroxyisoflavone)
- kwakhurin (3-[2-(3,3-dimethylallyl)-4,6-dihydroxy-3-methoxyphenyl]-7-hydroxyisoflavone)
- kwakhurin hydrate
- formononetin (7-hydroxy-4 -methoxyisoflavone)

ii. Isoflavone glycosides

- daidzin (daidzein-7-O-glucoside)
- genistin (genistein-7-O-glucoside)
- puerarin (6 - O-beta-apiofuranoside)
- puerarin-6 -monoacetate
- mirificin (puerarin-6 -O-beta-apiofuranoside)

c. Coumestans

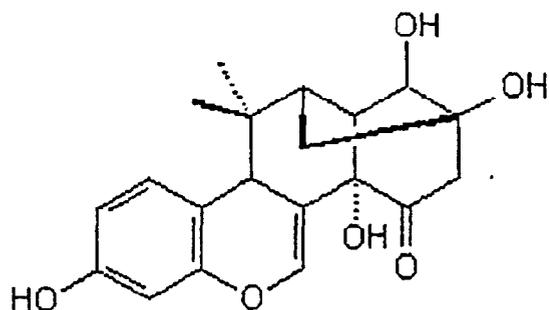
- coumestrol (3,9-dihydroxycoumestan)
- mirificoumestan (3,9-dihydroxy-8-methoxy-7-(3,3-dimethylallyl)-coumestan)
- mirificoumestan glycol (3,9-dihydroxy-8-methoxy-7-(2,3-dihydroxy-3-methylbutyl)-coumestan)
- mirificoumestan hydrate

d. Others

(+)-tuberosin
pterocarpene
puemircarpene (3,9-dihydroxy-8-methoxy-7-prenylpterocarpene)

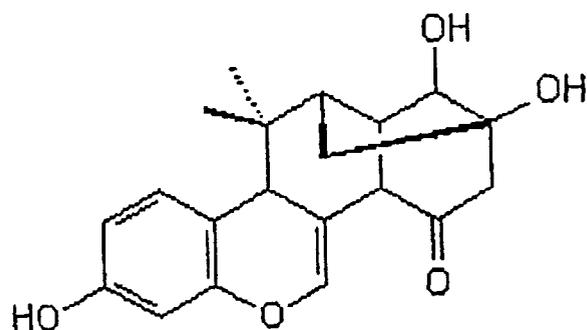
Phytoestrogens in *P. mirifica* root extract that are unique to this species include miroestrol [3,14,17,18-beta-tetrahydroxymiroestrol-1,3,5(10),7-tetraen-15-one] and its related phytosterols, isomiroestrol and deoxymiroestrol. Isomiroestrol and deoxymiroestrol are structurally similar to genistein and daidzein, respectively (see Figures 1 and 2, below).

Figure 1.



miroestrol

Figure 2.



deoxymiroestrol

It has been claimed that the HPLC peak characterized as miroestrol may actually represent the presence of deoxymiroestrol in the extract.¹⁹ These investigators suggested that this peak (and therefore the miroestrol content of the sample of extract being analyzed) may be artifactual and may result from a combination of deoxymiroestrol and isomiroestrol during the sample isolation procedure. However, independent confirmation has not been reported.

The HPLC fingerprint for *P. mirifica* root liquid and powder extract is attached as an exhibit and marked as Figure 3 (Lot # DPM-KK/LB-39) and Figure 4 (Lot # CPM-SA-31). HPLC analysis of *P. mirifica* root extract is characterized by two tall peaks on the HPLC, shown as peak 1 and peak 2, which represent miroestrol and puerarin, respectively. Smaller peaks 3 through 5 represent daidzin, genistin, and daidzein, respectively (see Figures 5 and Figures 6).

V. Standardization and Quality Control of *P. mirifica* Root Extract

Recent unpublished studies carried out in Thailand have identified correct species identification, source, location, atmospheric conditions during growth, age of plant, harvesting period, drying process, storage conditions, and production processes as the most important factors determining the composition of extracts of *P. mirifica* root.²⁰ The following conclusions have been drawn concerning the variability of the composition of *P. mirifica* root extracts:²¹

- *P. mirifica* root extracts obtained from the same sub-species and location (province, district, village or mountain) and during the same harvesting period have the same fingerprint, but exhibit different peak heights on HPLC analysis. This suggests that they contain the same chemical constituents but in different quantities.
- *P. mirifica* root extracts obtained from the same sub-species and location but during different harvesting periods exhibit increased variability in the quantities of the phytoestrogen compounds that they contain. The differences can be as great as three-fold.

- *P. mirifica* root extracts obtained from the same sub-species and during the same harvesting period but from different locations are slightly different in both HPLC fingerprint and peak heights. This suggests that there are small differences in chemical composition and that the quantities of phytoestrogen compounds present is different.
- *P. mirifica* of the same sub-species but harvested from different locations and during different harvesting periods may have as much as 10-fold differences in the quantities of phytoestrogen compounds present.

Because the composition of dried *P. mirifica* root can vary considerably, efforts to develop a standardized extract began in the 1980 s. Laboratory standards are available for the isoflavone glycosides puerarin, daidzin, and genistin and the isoflavone, daidzein, and they can be used as marker compounds in the standardization of extracts of *P. mirifica* root. Miroestrol cannot be used for quantitative standardization of *P. mirifica* root extract because no laboratory standard for it is available.

Companies offering standardized *P. mirifica* root extract have developed quality control/quality assurance (QC/QA) procedures and good manufacturing practices for the harvesting, storage, and manufacture of the extract. These procedures and practices were developed under guidance from the Thai Ministry of Public Health and the Food and Drug Administration (TFDA) and National Institutes for Health (TNIH) of the Thai Ministry of Agriculture, with assistance of the Department of Pharmaceutical Chemistry, Mahidol University, Thailand.²² In addition, the harvesters of *P. mirifica* in Thailand that supply the root to companies engaged in the manufacturing of the standardized extract receive extensive training and must be approved as wild-crafters by the Forestry Department of the Thai Ministry of Agriculture, Bangkok.

HPLC fingerprint-validated standardized *P. mirifica* root extract is available from Smith Naturals Co. Ltd, Bangkok, Thailand.

The complex structure of miroestrol has thwarted attempts to manufacture the compound synthetically.²³ No attempt to synthesize deoxymiroestrol or isomiroestrol has been reported.

VI. Estrogenic and Estrogen Antagonistic Phytoestrogen Isoflavones of *P. mirifica* Root Extract

Structural similarities between miroestrol and estradiol suggested that miroestrol-containing *P. mirifica* root extract might possess estrogenic properties. Subsequently, it was reported that *P. mirifica* root extract exhibited weak estrogenic activity, comparable to that exhibited by some soy products, when fed to ovariectomized rats.^{24,25}

P. mirifica root extract contains inactive glucosides of the plant phytoestrogen isoflavones, genistein and daidzein. Ingestion of the glucosides is followed by complex enzymatic conversions in the human gastrointestinal tract that produce the biologically active heterocyclic phenols, genistein and daidzein.²⁶ These phenols exhibit weak estrogenic activity when present in low concentrations (less than 1% of the estrogenic activity of an equimolar concentration of estradiol²⁷). For example, the ingestion of foods containing substantial amounts of the

phytoestrogen glucosides has significantly reduced the incidence of hot flashes in perimenopausal women.^{26,28}

In contrast, these compounds act as estrogen antagonists when they are present in higher concentrations.^{29,30} The results of animal studies suggest that phytoestrogen isoflavones may inhibit chemically-induced mammary gland tumorigenesis^{26,31,32,33,34,35,36,37} and tumor metastasis^{38,39,40,41} when ingested in sufficient amounts. It is likely that the estrogen antagonist activity of plant phytoestrogen isoflavones is not mediated by the estrogen receptor and therefore is capable of inhibiting the expression of the weak estrogenic effects of low concentrations of these compounds.^{26,33,42,43,44}

A recent series of studies conducted by Emory University School of Medicine in Atlanta, Georgia, with the cooperation of the Department of Obstetrics and Gynecology, Phramongkutklo College of Medicine, Bangkok, Thailand, have shown that *P. mirifica* root extract (obtained from Smith Naturals Co Ltd., Bangkok) exhibits potent anti-estrogenic activity against aggressive breast cancer cell lines *in vitro*, especially the proliferative estrogen receptor-positive (ER+) breast cancer lines, T47-D, MCF-7, and ZR-75-1 (obtained from the MD Anderson Cancer Institute and the National Cancer Institute).⁴⁵

VII. Safety of *P. mirifica* root extract

P. mirifica root extract is not mutagenic and is without toxic effect in laboratory animals during long-term ingestion of up to 6 times the recommended daily human dose. Acute exposure to the equivalent of 14,000 human doses over 14 days, or to the equivalent of 45,000 human doses over 90 days, have not produced evidence of toxicity. Daily intake of 6 times the recommended daily human dose has not produced adverse effects or signs of toxicity in healthy menstruating women aged 20 to 49 years during 6 months of continuous daily supplementation. Testimonial letters from experts have attested to the safety of *P. mirifica* root extract in humans. *P. mirifica* root extract is very safe for human use as recommended for at least 6 months.⁴⁶

Bacterial Mutagenicity (AMES) Assay

P. mirifica root extract is not mutagenic. Two Ames tests were conducted in June of 2001, using the incubation method where the results were the mean standard deviation of two plates from two independent experiments. The mutagenicity assay was performed according to standard methodology.^{47,48,49} Control solvents included distilled water and dimethyl sulfoxide (DMSO). Salmonella typhimurium strains TA 98 and TA 100 were obtained from Dr. Taijiro Matsushima (Japan Bioassay Research Center, Japan Industrial Safety and Health Association, Kanagawa, Japan). Two-fold criteria were used for data evaluation.⁴⁷ The tested materials were to be considered to be mutagenic when a dose-related increase in relevant colony count was observed, the number of colonies per plate with the test substance is more than twice that of the negative control, and when a reproducibility of test results is observed. *P. mirifica* root extract test results were consistently negative.^{50,51}

b. Animal Studies

Several animal toxicology studies have been completed on *P. mirifica* root powder using both crude powders and standardized extracts.

P. mirifica root extract produces no toxic effects or changes in liver function or histology when given to rats in daily doses of up to 2000 mg/kg for up to 14 days.^{52,53,54} This level of intake (2000 mg/kg for 14 days) is equivalent to a total exposure of up to 28,000 mg/kg and is comparable to 1,000 recommended daily doses (2 mg/kg) taken daily or 14,000 recommended daily doses total over 14 days. In an attempt to determine the LD₅₀ for *P. mirifica* root extract, a single dose of 40 g/kg (equivalent to 20,000 recommended daily doses taken at once) failed to induce signs of acute or subacute toxicity in mice.⁵⁵

In long-term feeding experiments, daily doses of 10 mg/kg (equivalent to 5 times the recommended daily dose) or 100 mg/kg (50 times the recommended daily dose) did not produce toxic effects when given to rats for 90 days (total cumulative exposure: 270 mg or 2700 mg).⁵⁵ In addition, daily intakes of 0.4 mg or 4.0 mg (about 6 times the recommended daily dose for a 50 kg human) added to a mixture of other herbal extracts produced no toxic effects when given to rats for 36 weeks.⁵⁶

However, in one study, a daily dose of 1000 mg/kg for 90 days (equivalent to about 45,000 recommended daily doses) induced reversible anemia and pathologic changes in the kidneys and testicles.⁵⁷ In another study, 12 weeks of ingestion of 40 mg/day (about 60 times the recommended daily dose for a 50 kg human) resulted in significant hepatomegaly and hypertrophy of the kidneys, heart and brain (although these effects were not apparent after only 4 weeks).⁵⁶

These findings indicate that *P. mirifica* root extract is without toxic effect in laboratory animals during long-term ingestion of up to 6 times the recommended daily human dose.

More detailed descriptions of the studies cited above follows.

Acute Toxicology Studies

P. mirifica root extract was given to ICR species mice (10 males and 10 females) by gavage in two doses of 20 g/kg, 6 hours apart. The mice were then observed for 14 days. No mortality, signs or symptoms of toxicity, or gross pathological changes were found.⁵⁵ The *P. mirifica* root extract tested was Lot No. SPM-SA-29C/29E (Smith Naturals Co Ltd., Bangkok, Thailand) and a confirmatory HPLC fingerprint was done on June 12, 2001, by the Department of Chemistry, Rangsit University, Bangkok, Thailand (attached). (Marker compounds are identified as 1 through 5 with an index provided to each compound to the right of the chromatogram.)

This study was repeated three times, with identical results, by the Department of Medical Sciences, Ministry of Public Health, on May 24, 2001 (Lot No. SPM-SA-25-PE); July 12, 2001 (Lot No.

SPM-SA-29C); and July 12, 2001 (Lot No. SPM-SA-29E). The Department's Acute Toxicity Test Method is attached. At the end of each of these three studies, the conclusion of the Ministry's toxicologists was:

Observation results show that *Pueraria mirifica* powder given as 40 g/kg produced no signs or symptoms of acute toxicity in mice and did not cause animal deaths. Therefore, the LD-50 value is greater than 40g/kg.⁵⁸

An acute toxicity study was performed in male and female rats using a standardized *P. mirifica* root extract (Lot # SMP-SA-05, Smith Naturals, Co., Ltd., Bangkok, Thailand) given orally. A confirmatory HPLC fingerprint was done by the Department of Chemistry, Rangsit, University, Bangkok, Thailand (Lot SMP-SA-05 chromatogram attached). The dose of extract given was based on the recommended daily dose of 100 mg of standardized *P. mirifica* root extract for a woman weighing 50 kg. Male and female rats were given daily doses of standardized *P. mirifica* root extract containing 0.126 or 0.63 micrograms of miroestrol (10 and 50 times the recommended daily dose, respectively) via intragastric tube. No significant evidence of toxicity was found in either sex at the lower dose (0.126 micrograms). However, at the higher dose (0.63 micrograms), significantly higher organ weights and increased blood chemistries were noted, but these were not considered to be of a pathological nature.⁵⁴

Subacute Toxicology Studies

Liver enzymes and function were studied in 20 male albino rats given either 0, 10, 100 or 200 mg/kg of *P. mirifica* root extract for 14 days via intragastric tube. On day 15, blood was collected via the infraorbital sinus and the serum examined for GOT and GPT activity. A histopathologic examination of the liver was performed. No significant histopathological differences were found between the treated and control groups; however, the size of liver cells in the *P. mirifica*-treated rats at the dosage of 10 mg/kg was found to be smaller than in the rats given placebo. This finding was not repeated in the liver tissues of the rats receiving the higher doses.⁵³

In an oral subacute toxicology study, Wister rats were given *P. mirifica* root extract at a dose of 2,000 mg/kg body weight for 14 days. At the end of the study, no mortality, signs or symptoms of toxicity, or gross pathological changes were found.⁵²

Chronic Toxicology Studies

A chronic toxicology study in rats treated orally with *P. mirifica* root extract at daily doses of 10, 100 and 1,000 mg/kg for 90 consecutive days revealed that the growth rate and food consumption of rats receiving *P. mirifica* root extract at the daily doses of 100 and 1,000 mg/kg were significantly lower than those of the control groups. Hematological results indicated that *P. mirifica* root extract at the daily dose of 1,000 mg/kg caused anemia with significant decreases in hematocrit, the number of erythrocytes, and plasma hemoglobin in both sexes. Two weeks after withdrawal of supplementation with *P. mirifica* root extract, the hematologic changes in male rats reversed, whereas in only two out of four females, the hematocrit returned to normal. The numbers

of white blood cells and platelets in male rats receiving the highest dose were significantly lower than those of the control group but these changes were not observed in female rats of the same dose group. Serum biochemical examination showed that total cholesterol concentrations in male rats receiving *P. mirifica* root extract at each dose were significantly lower than that of the control group; these changes were observed in females only at the daily doses of 100 and 1,000 mg/kg. At post-mortem examination, the weights of both testes from male rats receiving the highest dose were significantly lower than those of the control group. The uterus of females receiving 100 and 1,000 mg/kg appeared swollen and the actual uterine weights and percent relative uterine weights of these two groups were significantly higher than those of the control group. Histopathological examinations indicated that male rats receiving the highest daily dose of *P. mirifica* root extract had a significantly higher incidence of testicular hyperemia than the control group. Female rats receiving the highest daily dose of *P. mirifica* root extract had significantly higher incidence of kidney tubular casts than did the control group. Taken together, it was found that *Pueraria mirifica* at the daily doses of 10 and 100 mg/kg given orally in rats did not cause any significant pathologic changes.⁵⁷

Toxicology Study of Thai Traditional Formulation Containing *P. mirifica* Root Extract

In some areas of Thailand, women consume a traditional Thai formulation to relieve menopausal symptoms that includes *P. mirifica* root extract and three other botanical preparations: *Terminalia bellerica*, *Terminalis chebula* and *Phyllanthus emblica* (known together as the Tripala).

Toxicology studies have been performed in rats fed this traditional formulation. The dosage administered to rats was based on the dosage recommended in Thai traditional medicine for a 50 kg adult human: 150 mg/day of the Tripala (as 50 mg of *Terminalia chebula*, 50 mg of *Terminalia bellerica*, and 50 mg of *Phyllanthus emblica*) plus 100 mg of *P. mirifica* root powder. The combination of the Tripala and *P. mirifica* is referred to hereafter as *P. mirifica*/Tripala. The 150 mg/day dose of the Tripala is equivalent to 0.6 mg/day in rats. An equivalent human dose of *P. mirifica* root extract of 100 mg/day in rats is 0.4 mg/day. A 10-fold human equivalent dose of *P. mirifica* root extract in rats is 4 mg/day, while a 100-fold human equivalent dose is 40 mg/day.

The *P. mirifica*/Tripala toxicology study compared *P. mirifica*/Tripala to distilled water. *P. mirifica*/Tripala was fed at three different daily doses: a dose containing the recommended daily human dose of *P. mirifica* root extract of 100 mg of , a 10-fold higher dose, and a 100-fold higher dose. In each study, female rats were given *P. mirifica*/Tripala via intragastric tube for 1-day, 4-weeks (one month), 12-weeks (3 months), 24-weeks (6 months), or 36-weeks (9 months). No evidence of increased toxicity was found in any animal, compared to control animals, after either 1 day or 4 weeks.⁵⁶

After 12 weeks, the rats exhibited no significant differences in body weights or organ weights or pathology. However, the hematocrit was significantly lower and the plasma SGOT activity significantly higher in the rats receiving 0.4 mg/day of *P. mirifica*/Tripala than in those receiving Tripala alone. The rats receiving 4 mg/day of *P. mirifica*/Tripala exhibited significantly lower hematocrit and plasma hemoglobin concentration and significantly higher plasma glucose concentration and SGOT activity than did the rats receiving Tripala alone.

When Tripala was administered chronically for 24 weeks, with the exception of a significantly lower liver weights, no significant differences were seen in body weight or weights of any organs. Histological examination found no pathologies in any of the organs. Blood analysis revealed that the hematocrit, plasma hemoglobin and urea concentrations and plasma SGOT and SGPT activities in those receiving Tripala alone did not differ significantly from the control group. However, plasma glucose concentrations were significantly lower in those receiving Tripala ($p < 0.05$). The group receiving *P. mirifica*/Tripala for 24 weeks exhibited no significant differences compared to the rats receiving Tripala alone, except for significantly lower plasma glucose concentrations among the rats receiving either 0.4 mg/day and 4 mg/day of *P. mirifica* root extract. Rats receiving Tripala combined with 40 mg/day of *P. mirifica* root extract had significantly lower hematocrit and plasma SGPT activity than did the rats receiving Tripala alone ($p < 0.05$; $p < 0.01$, respectively).

After 36 weeks, the rats receiving Tripala alone exhibited no significant differences in body weight or the weights of any organ compared to the control group. In contrast, the rats receiving Tripala combined with 40 mg/day of *P. mirifica* root extract had significantly lower body weights and significantly higher weights for the liver, uterus, adrenal glands and brain than did the control group. The rats receiving *P. mirifica*/Tripala (containing 40 mg/day of *P. mirifica* root extract) had significantly higher plasma SGPT activity compared to the Tripala only group. Moreover, the group receiving *P. mirifica*/Tripala (with 40 mg/day of *P. mirifica*) had significantly lower hematocrit than the group receiving Tripala only ($p < 0.05$).

Human studies

A safety and efficacy study was conducted at a university hospital in Japan by a Japanese and Thai research team on the safety of *P. mirifica* root in healthy menstruating women (see letter attached from the principle co-investigator, Assoc. Prof. Yuthana Smitasiri, dated January 17, 2002, regarding this study).⁵⁸

The Japanese-Thai study was conducted at the School of Medicine, Saint Mariane University, Tokyo, Japan.⁵⁹ (Kuramoshi, T. and Smitasiri, Y. Preliminary study of *Pueraria mirifica* in Japanese females. English, unpublished, 2000.) 50 healthy menstruating volunteer females, ages 20 to 49, were given between 100 to 600 mg orally of *Pueraria mirifica* root powder daily as capsules for 7 days, two weeks after menstruation. The crude root powder was obtained from a certified harvester in Kanjanaburi Province, Thailand, and confirmed taxonomically and by HPLC fingerprint as *P. mirifica* root. Compared to prestudy measurements, there were no significant changes in female hormones (serum estrogen, urine estrogen, urine pregnanediol concentrations), kidney function (total urine volume, specific gravity, creatinine clearance), blood chemistries (plasma total protein, triglycerides, sodium, potassium, chloride, calcium, or total phosphate concentrations; serum total cholesterol concentrations; plasma GOT or GTP activities), white blood cell counts (neutrophil [segmented and non-segmented], eosinophil, basophil, lymphocyte, and monocyte), hematocrit, plasma hemoglobin concentrations, blood platelet counts, white blood cell counts (WBC), or red blood cell counts (RBC) 14 days after oral intake ended. Six out of 50 subjects (12%) reported that they menstruated earlier or later than expected. There were no reports of abnormally heavy, severe, or missed menstruation.

In the letter received from Dr. Smitasiri, Associate Professor of Reproductive Physiology at the University of Mae Fah Luang, Thailand, dated January 17, 2002,⁶⁰ he states that the data from this study shows a very low order of side effects and no significant changes in any clinical markers during the 4 week period of this study.

An inquiry to the Thai Ministry of Public Health, which regulates food supplements, and the country's expert on *P. mirifica*, resulted in two letters^{61,62} attesting to the safety record of this food supplement.

In Thailand, *P. mirifica* is regulated as an over-the-counter food supplement by the Thai FDA. The first letter⁶¹ is authored by Dr. Pakdee Pothisiri, Director-General of the Department of Health, Ministry of Public Health. He is the former Director of the Thai FDA and Director-General of the Department of Medical Sciences. In addition, he is a former Senior Researcher at the U.S. NIH, and Chairman of the Codex Alimentarius Commission of the World Health Organization/United Nations in Rome. Thailand's FDA is within the Ministry of Public Health.

The second letter⁶² is from Prof. Yuthana Smitasiri, Dean, School of Agricultural Technology, Mae Fah Luang University, Chian Rai, Thailand. He has studied *P. mirifica* for over 20 years as an animal toxicologist and specialist in reproductive physiology at three major Thai universities. The Ministry of Public Health regards Prof. Smitasiri as a leading expert on *P. mirifica*. Both letters state that, in Thailand, there is no record of any significant adverse events in the Thai population related to the use of *P. mirifica* root or *P. mirifica* root extract.

VIII. Summary

Given the widespread and historic use of *P. mirifica* root as a botanical medicine and food supplement for the relief of vasomotor symptoms associated with menopause, combined with the evidence from animal toxicological studies, the Japan-Thai safety/efficacy study in healthy women, and written verification of the lack of adverse reports associated with its consumption in the country of origin, the risk associated with human consumption of standardized *P. mirifica* root extract in a dietary supplement is extremely low.

Supporting this opinion is a letter from Dr. Yuthana Smitasiri, Associate Professor in Reproductive Physiology at the University of Mae Fah Luang in Thailand, who states that he has studied this botanical for over 30 years. In his letter, dated January 23, 2002 (attached), he states:⁶³

P. mirifica has been available as a traditional medicine in Thailand for over fifty years, and a regulated herbal medicine for the last 10 years. It is a popular herbal medicine today throughout Thailand and even neighboring countries such as Myanmar. Although health claims are not permitted for this botanical, it is offered by more than 25 different Thai manufacturers and can be found readily available in public markets, pharmacies, and health clinics. No prescription is required and it is not registered as a drug but as a food supplement. Toxicology studies in various animals have been conducted at Thai universities on several animal species and been found to be non-toxic at levels of intake

well above those recommended for use by Thai manufacturers. Considerable work has been done by Thai university chemists and foreign chemists characterizing the principle compounds found in *P. mirifica*, with particular interest shown in the isoflavones, many of which are found in soybeans, which is not surprising as *P. mirifica* is a member of the same botanical family. Since the contents of *P. mirifica*'s isoflavones varies depending on the time of harvest and location, there is increasing demand among health practitioners for the extract of the product to insure consistent levels of the major isoflavones found in the finished product.

Manufacturers generally recommend a daily intake of between 50 to 100 milligrams of the extract or crude powder. No contraindications are known. No significant non-transient adverse events have been reported to date.

In Dr. Smitasiri's opinion, *P. mirifica* root powder probably has been used by Americans of Thai descent for many years, primarily as a benefit for their aging parents or relatives, to continue their family's traditional use of this botanical. He also points out that, One of the principle reasons for Thai people consuming this root in crude or semi-crude form, or as an extract, is to relieve symptoms associated with the end of menstruation in women, which is often referred to as post-menopausal symptoms.

Finally, Health Canada has not objected to the importation of *P. mirifica* into Canada. A signed letter from Health Canada's Health Products and Food Branch, dated December 4, 2000, to that effect is attached.⁶⁴

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