DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

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Memorandum

Date: November 6, 2003

From: Consumer Safety Officer, Division of Dietary Supplement Programs , Office of Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305

Subject of the Notification: Luteolin

Firm: Synorx, Inc

Date Received by FDA: January 17, 2003

90-Day Date: April 4, 2003

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.

Victoria Wellwood
CSO.

95S-0316

RPT166

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

APR - 2 2003

Mr. Thomas Lahey
Synorx, Inc.
1031 Calle Trepadora, Suite D
San Clemente, California 92673

Dear Mr. Lahey:

This is in response to your letter to the Food and Drug Administration (FDA) dated January 6, 2003, making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413 of the Federal Food, Drug, and Cosmetic Act and 21 CFR 190.6). Your letter notified FDA of your intent to market luteolin, a flavonoid, as a new dietary ingredient. Each pill will contain a concentration of 25mg per tablet or capsule. The recommended use is to take one to three tablets/capsules per day.

Under 21 U.S.C. 350b(a)(2), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such 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 section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement may be 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 submission, and the agency has significant concerns about the evidence on which you rely to support your conclusion that luteolin will reasonably be expected to be safe. You state in your submission that "luteolin is a naturally-occurring flavone flavonide long-established as a component in many commonly consumed foods...." However, you provide no evidence of safety for the compound luteolin, the subject of the notification under proposed conditions of use. The notification referenced two preclinical oral dosing studies but failed to provide the articles as required by 21 CFR 190.6(b)(4). The same was true for a chronic toxicity study for 50-times the adult dose. It is unknown whether these reference citations provide any support for the safety determination of luteolin. The reference articles in the notification are of general information and do not support the safety evaluation of luteolin.

Your submission contains no information to support that historical use of this product, isolated luteolin, when used under the conditions recommended or suggested in the notification is reasonably expected to be safe.

For the reasons discussed above the information in your submission does not provide an adequate basis to conclude that luteolin, when used under the conditions recommended or suggested in your submission, will reasonably be expected to be safe. 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 such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of January 17, 2003. After April 17, 2003, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to April 17, 2003, you may wish to identify in writing specifically what information in your notifications you believe is proprietary, trade secret or otherwise confidential information, which should not be disclosed to the public.

We note that your notification does not include either a phone or facsimile (fax) number or an electronic mail address as a means to contact you. We note that Karen A. Weaver, J.D., R.Ph. submitted the notification on your behalf, and we will fax a copy of this letter to her.

If you have any questions concerning this matter, please contact Victoria Lutwak at (301) 436-2375.

Sincerely yours,

Susan Walker, M.D.
Acting Director,
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety
and Applied Nutrition

cc:
HFA-224 (yellow box copy)
HFS-810/Walker
HFS-810/Lutwak 4 copies
HFS-840/Rader
R/D:HFS-810:Vlutwak 301-436-1775
Revised:HFS-810:VL:
Reviewed:HFS-811:RMoore:
HFS-800:SWalker:
F/T:HFS-810/Barr: 3/31/2003
DOC: found via "p" drive:
DSLR/data:NDI/Confidential/Luteolin/resubmission/luteolin 83126. ISO 24

Luteolin
New Dietary Ingredient Submission (Originals)

Submitted by:
Synorx, Inc.
January 6, 2003

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January 6, 2003

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington, DC 20204

Re: New Dietary Ingredient Notification

Dear Sir / Madam:

In accordance with 21 Code of Federal Regulations § 190.6, Weaver & Amin, on behalf of its client Synorx, Inc. hereby notifies the U.S. Food and Drug Administration that the company intends to market a new dietary ingredient known as luteolin and hereby submits the following information in support thereof.

1. The name and complete address of the manufacturer or distributor:
   Synorx, Inc.
   1031 Calle Trepadora, Suite D
   San Clemente, CA 92673
   USA

2. The name of the new dietary ingredient:
   Product Description
   Generic Material: luteolin
   Chemical Name: 3',4',5,7-tetrahydroxyflavone
   CAS No.: 000491-70-3
   Chemical Formula: $C_{10}H_{15}O_6$
   Molecular Weight: 286.24

3. A description of the dietary supplement or dietary supplements that contain the new dietary ingredient including:
   a) the level of the new dietary ingredient in the dietary supplement; and

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b) the conditions of use recommended or suggested in the labeling of the dietary supplement or, if none, the ordinary conditions of use of the supplement.

Description of the Dietary Supplement

Luteolin is a naturally-occurring flavone flavonoid long-established as a component in many commonly consumed foods including broccoli (74.5 mg/kg dry weight)[1] sweet red peppers, celery, parsley, artichoke leaves, green olives, olive oil, sage, thyme, rosemary and chamomile. Luteolin was first found in foods in 1896 by Perkin, *J. Chem. Soc.* **69**, 800 (1896) and by Fleischer, *Ber.* **32**, 1186 (1899); again by Perkin, Horsfall, *J. Chem. Soc.*, **77**, 1315, (1900), and Hyashi, Inoye, *Acta Phytochim (Japan)* **15**, 53 (1949), as cited in the *Merck Index*, 12th edition, 1006 (1996). Luteolin is the major flavonoid present in the hulls of mature peanuts at up to 6,000 mg/kg. Celery contains 200mg luteolin per 1kg fresh weight, and sweet red peppers contain 11mg luteolin per 1kg fresh weight.

Luteolin is also found in many edible tropical plants such as *Capsicum annum* (green chili) at 33mg/kg dry weight, *Capsicum frutescens* (bird chili) at 1035mg/kg dry weight, *Allium fistulosum* (onion leaves) at 391mg/kg dry weight, *Averrhoa belimbi* (belimbi fruit) at 202mg/kg dry weight, *Daucus carota* (carrot) at 37.5mg/kg dry weight, *Apium graveolens* (local celery) at 80.5mg/kg dry weight, and *Garcinia atroviridus* (asam gelugor) at 107.5mg/kg dry weight.

Table 1. below summarizes a partial list of luteolin-containing foods.

Common Name	Luteolin Content	Reference
Artichoke	Various values depending on extraction and concentration processes.	Brown Evans, Free Radical Research, Vol. 29 pp. 247-255.
Broccoli	74.5 mg/kg	1
Bird Chili	1035 mg/kg	1
Onion leaves	391 mg/kg	1
White radish	9.0 mg/kg	1
French bean	11.0 mg/kg	1
Celery	200 mg/kg	1
White celery stalks	22-36 mg/kg	8
Celery, dried	358 mg/kg	4
Onion leaves	391 mg/kg	1
Red bell pepper	13-31 mg/kg	4
Sweet red peppers	11.0 mg/kg	1
Carrots	37.5 mg/kg	1
Olives	various	Amiot 1986. Rovellini 1997
Olive Oil	10 mg/kg	5
Sage	550 mg/kg	3
Dried sage	11 mg/kg	7
Dried Peppermint	50 mg/kg	7

Dried Thyme	160 mg/kg	7
Perilla seeds	300 mg/kg	10
Lentils	various	D' Arcy and Jay Phytochemsity 17 (4) 826-827 (1978)
Japanese Barnyard Millet	3.8 mg/kg	11
Belimbi fruit	202.5 mg/kg	1
Belimbi leaves	464.5 mg/kg	1
Dried asam gelugur	107.5 mg/kg	1
Limau purut leaves	30.5 mg/kg	1
Compositae	400 mg/kg	Igile, et. al J. Agric. Food Chem 1994 42 2445-2448.
Vitex rotundifolia fruit	100 mg/kg	9

Luteolin exhibits many of the same properties as other bioflavonoids, such as its usefulness as an antioxidant and its role in inflammatory and vasodilatory processes. Luteolin's molecular structure most closely resembles quercetin, a major flavonol flavonoid.

The company expects to market luteolin for its antioxidant properties. No additional bioflavonoid or other dietary supplement ingredients are expected to be added to the product formulation. The formulation also contains binders, fillers, technical manufacturing ingredients, or flavors such as calcium carbonate, microcrystalline cellulose, citric acid, xylitol, fructose, or artificial orange flavor.

References:

1. The Merck Index, Twelfth Edition, 1996, p. 959.

2. Miean KH; et al., *Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants*, J Agr Food Chem 49(6): 3106-3112 June 2001.

3. Hollman, Peter and Arts, Ilja, *Flavonols, flavones and flavanols – nature, occurrence and dietary burden*, J. Sci. Food Agric. 0022-5142 2000.

4. Karakaya, Sibel; Nehir EL, Sedef, *Quercetin, Luteolin, Apigenin and Kaempferol contents of some foods*, Food Chemistry 66(1999) 289-292.

5. Hertog, Michael G.L.; Hollman, Peter C.H.; et al., *Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands*, J. Agric. Food Chem. 1992, 40, 1591-1598 and 2379-2383.

6. Brenes, Manuel; Garcia, Aranzazu, *Phenolic Compounds in Spanish Olive Oils*, J. Agric. Food Chem. 1999, 47, 3535-3540.

7. Duh, Pin-Der; et al., *Extraction and Identification of an antioxidative component from peanut hulls*, JAOCS, Vol. 69, no.8 (August 1992)

8. Kanazawa, K. et al., *Luteolin: A Strong Antimutagen against Dietary Carcinogen, Trp-P-2, in Peppermint, Sage, and Thyme*, J. Agric. Food Chem. 1995, 43, 410-414.

9. Crozier, Alan et al, *Quantitative Analysis of the Flavonoid Content of Commercial Tomatoes, Onions, Lettuce, and Celery*, J. Agric. Food Chem., 1997 45, 590-595.

10. Shin, K. H. et al, *Isolation of an aldose reductase inhibitor from the fruits of Vitex rotundifola*, Phytomedicine Vol. 1, 1994 pp. 145-147.

11. Yamamoto, H. J. *Inhibitors of Arachidonate Lipoxygenase from Defatted Perilla Seed*, Agric. Food Chem 1998 46, 862-865.

12. Watanabe, Mitsuru *Antioxidative Phenolic Compounds from Japanese Barnyard Millet (Echinochloa utilis)* Grains J. Agric Chem., 1999, 47, 4500-4505.

Level of the New Dietary Ingredient

Luteolin will be supplied in a dietary supplement oral tablet or capsule dosage form in a concentration of 25 mg per tablet/capsule. Daily dosage recommendations are one to three tablets/capsules per day. Based on the above food source references and consultation with professionals knowledgeable in the field, the company believes a healthy diet contains 2-125 mg of luteolin per day and up to 6 grams of bioflavonoids.

Conditions of Use

Luteolin, as with other major flavonoids, exhibits potent antioxidant activity. It reduces oxidative damage, inhibits lipid peroxidation and neutralizes free radicals. Luteolin showed a concentration-dependent inhibitory activity in several models of oxidative stress. The antioxidant potential of luteolin, measured by Trolox test, is twice as strong as that of Vitamin E. Luteolin has strong scavenging properties for super oxide radicals and has also been shown to strengthen capillaries and act as a smooth muscle vasodilator. Based on the available scientific literature and the apparent consensus among medical professionals in the field, the company expects to associate claims such as "antioxidant", "free radical scavenger" and "supports the cardiovascular system" with the luteolin product.

References:

13. Shimoi, Kayoko; Saka, Norika; et. al, *Metabolic fate of luteolin and its functional activity at focal site*, Biofactors 12 (2000) 181-186.

14. Brown, Jonathan E.; Rice-Evans, Catherine A., *Luteolin-Rich Artichoke extract protects law density lipoprotein from oxidation In Vitro,* Free Rad. Res., Vol. 29, 247-255, 1998.

15. Cai, Qiuyin; Rahn, Ronald O., *Dietary flavonoids, quercetin, luteolin and genistein, reduce oxidative DNA damage and lipid peroxidation and quench free radicals,* Cancer Letters, 119(1997) 99-107.

16. Duarte, Juan; Vizcaino, Francisco Perez; et al., *Vasodilatory effects of flavonoids in rat aortic smooth muscle, structure-activity relationships,* Gen.Pharmac. Vol. 24, No.4. pp 857-862, 1993.

17. Rainova L, Gakhniyan R, *Changes in the resistance of the capillary walls in rats under the effect of flavonoid compounds isolated from Genista L. (Leguminosae),* Farmatsiya (sofia) 28, 5, 39-42, 1978.

4. The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling, reasonably will be expected to be safe. This should include citations to published articles or other evidence that forms the basis for the conclusion that the ingredient is safe. References to published literature should be accompanied by reprints or copies of the references and should be in English:

Toxicity of Luteolin

Luteolin is considered nontoxic. Toxic death could not be effectuated at oral doses of 2.5 g/kg in mice (Huamin, P., Siyuan, X. and Zhaoqing, H.) or 5 g/kg in rat (Nichols, J., Johnson, G. 2000). The LD_{50} by intraperitoneal injection is 180 mg/kg in mice and 411mg/kg in rat. The LD_{50} by intramuscular injection is 592mg/kg in rat.

LD_{50} of Luteolin

Animal	Route	Dose	Reference
Mouse	Intraperitoneal	180 mg / kg	Peng, H.; Xiang, S.; Bi, Z. (1981) Yao Hsueh T'ung Pao 16(ISS 2), 11-13 Chavant, L.; Combie, H.; Crs, J. (1975) Plant. Med. Phytother. 9(4), 267-272 Dai, L.M., Cheng, H., Li, W.P., Liu, S.Q., Chen, M.X., Xu, S.Y. (1985) Acta Anhui Med. Univ. 20, 1-3.

Rat	Intraperitoneal	411 mg/kg	Annui Cooperation Group, Preliminary experimental study of Aruga decumbens Thunb. against chronic bronchitis, (1973) Chin. Herb. Med. Commun. 2, 18-23
Rat	Intramuscular	592 mg / kg	Dai, L.M., Cheng, H., Li, W.P., Liu, S.Q., Chen, M.Z., Xu, S.Y. (1985) Acta Anhui Med. Uni. 20, 1-3

Reproductive or Carcinogenic Toxicity

Luteolin safety data includes findings of no mutagenic activity via Ames testing and no existence of harmful metabolites via degradation analysis using bacterium found in the human intestinal tract

Pharmacokinetic Data

Shimoi, et. al found that the dietary form of luteolin (luteolin 7-O-beta-glucoside) is metabolized in the gut to free luteolin. Free luteolin is absorbed through the gut and into the bloodstream whether administered initially as luteolin 7-O-beta-glucoside or as free luteolin. Luteolin was detected in human serum 3 hours after a single 50mg oral dose.

Chronic Toxicity

While the company has identified only one published study that tested chronic toxicity in rats (at a dose 50 times the expected adult dose and demonstrating no toxicity upon chronic administration), luteolin-containing foods enjoy a long history of human consumption. The company anticipates supplying luteolin in a dose of 25-75mg/day – well within nutritional norms and established toxicity parameters.

Manufacturing Practices

Synorx, Inc. contracted with Array BioPharma to develop manufacturing methods that will result in a finished product containing a concentration of luteolin greater than 90%. Array BioPharma developed these methods and verified the purity of luteolin via HPLC, 1H NMR and 13C NMR testing (results attached). While the Array BioPharma product resulted in a luteolin content above 95%, the luteolin product subsequently manufactured by Synorx, Inc. for purposes of ramp-up testing using the starting material rutin (Certificate of Analysis attached), supplied by Merck S.A. resulted in a more pure product (13C NMR testing and reverse phase HPLC results attached).

The test results reveal Synorx, Inc. manufactured luteolin at a purity of 99.79%, assuring the absence of contaminants that could negatively affect consumer health and instilling FDA confidence in the product's safety profile. Additional testing of the Synorx, Inc. manufactured luteolin included analysis for

the presence of metals and inorganic sulfate. These data show the residual compounds present in the luteolin manufactured by Synorx, Inc. are in accordance with current Good Manufacturing Practices (cGMPs).

References:

18. Czeczot, H. Tudek, B., et.al, *Isolation and studies of the mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs*, Mutation Research, 240 (1990) 209-216.

19. Braune, Annett; Gutschow, Michael, *Degradation of Quercetin and Luteolin by Eubacterium ramulus*, Applied and Environmental Microbiology, Vol 67, No. 12, 5558-5567, December 2001.

20. Shimoi, Kayoko, et al, *Intestinal absorption of luteolin and luteolin 7-O-β- glucoside in rats and humans*, FEBS Letters 438 (1998) 220-224.

21. MSDS luteolin

22. Certificate of Analysis: rutin (Merck S.A.)

23. Certificate of Analysis: luteolin (Array BioPharma, Inc.)

24. Certificate of Analysis: luteolin (Synorx, Inc.)

25. 13C NMR (Array BioPharma, Inc.)

26. 13C NMR (Synorx, Inc.)

27. Sierra Laboratories Analytical Report.

5. The signature of the person designated by the manufacturer or distributor of the dietary supplement that contains a new dietary ingredient.

Thomas Lahey

Mr. Thomas Lahey
President, Synorx, Inc.

An original and two copies of this notice are submitted pursuant to 21 CFR 190.6(a). Please confirm receipt of this notice and maintain confidentiality of these submitted materials pursuant to 21 CFR 190.6(c) and (e) respectively.

Thank you for your attention to this matter. Please call me if you have any questions.

Sincerely,

Karen A. Weaver, JD, RPh

Enclosures