DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date:

From: Director, Division of Standards and Labeling Regulations, Office of Nutritional Products, Labeling and Dietary Supplements, HFS-820

Subject: 75-Day Premarket Notification for New Dietary Ingredients

To: Dockets Management Branch, HFA-305

New Dietary Ingredient:	Diosmin complex
Firm:	Nutratech, Inc.
Date Received by FDA:	August 21, 2000
90-Day Date:	November 18, 2000

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on pubic display in docket number 95S-0316 after November 18, 2000.

Felicia B. Satchell

Felicia B. Satchell

95S-0316

RPT83

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC

NOV 3 2000

Carl Germano, RD, CNS, LDN
Senior VP Product Development and Research
Nutratech, Incorporated
208 Passaic Avenue
Fairfield, New Jersey 07004

Dear Mr. Germano:

This is to inform you that the notification, dated August 21, 2000, you submitted pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act was received and filed by the Food and Drug Administration (FDA) on August 21, 2000. Your notification concerns a combination of substances called "diosmin complex" that you assert is a new dietary ingredient. This notification is a resubmission of a new dietary ingredient notification for diosmin dated April 3, 2000.

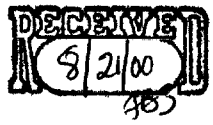
Your notification will be kept confidential for 90 days following the date of its receipt. After November 18, 2000, the notification will be placed on public display at FDA's Dockets Management Branch in docket number 95S-0316. However, any information that is trade secret or otherwise confidential commercial information in the notification will not be disclosed to the public.

Please contact us at (202) 205-4168, if you have any questions concerning this matter.

Sincerely,

Felicia B. Satchell

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



August 17, 2000

Felicia B. Satchell, Division Director
Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Re: Diosmin Complex

Dear Ms. Satchell:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, this submission responds to your letter of June 16, 2000. That letter responded to Nutratech's April 3, 2000 submission regarding Diosmin. In this connection, Nutratech, Inc proposes to market a dietary ingredient, Nutratech's Diosmin Complex, 90% diosmin and 10% hesperidin. Diosmin Complex is intended for use in dietary supplements at a level not to exceed 500 mg per day with meals and not for any longer than 3 months. Enclosed are an original and 2 copies of this submission.

This submission provides additional safety data to that which was previously submitted. As set forth in the submission, Nutratech's Diosmin Complex is intended for use by adults only for no more than 3 months. Various longer term studies support the 3-month length of time recommended by Nutratech for use of Nutratech's Diosmin Complex. The submission also provides a recommended daily dose limit that, along with the length of use limit, will be set forth on the Diosmin Complex raw material label and product specification. Nutratech's submission elaborates on the pharmacology and chemical structure of diosmin and hesperidin. The relationship of hesperidin to diosmin is explained—specifically, the fact that Nutratech's Diosmin Complex is a standardized mixture of diosmin and hesperidin. This mixture is comparable to the products studied in clinical trials that are presented in this submission. The safety of both diosmin and hesperidin are also described, and the following comments from FDA's June 16, 2000 letter are addressed specifically:

- In response to the FDA comment that

  ...studies using diosmin alone were not included in your submission. Instead, the studies in your submission used Daflon, a diosmin hesperidin mixture, micronized to increase absorption, (and) ...the submission contains no explanation or information that provides a valid basis to conclude that studies of Daflon are suitable to establish the safety of a dietary supplement containing a different substance, namely diosmin,

  The relationship of hesperidin to diosmin is explained—specifically, the fact that Nutratech's Diosmin Complex is a micronized purified flavonoid fraction containing 90% diosmin and

EAST COAST OFFICE 208 Passaic Avenue Fairfield, NJ 07004 973.882.7773, 888.466.8872 Fax: 973.882.9666
WEST COAST OFFICE 17258 South Main Street Gardena, CA 90248 310.515.7722 888.515.8833 Fax: 310.515.7766

10% hesperidin. This mixture is comparable (identical) to the products studied in clinical trials and presented in this submission. Nutratech's submission elaborates on the pharmacology and chemical structure of diosmin and hesperidin.

- In response to the FDA comment that

  .....there is inadequate information to provide reasonable assurance that such ingredient (diosmin) does not present a significant or unreasonable risk of illness or injury,

  The results of more than 35 studies, conducted over the past 30 years, enrolling more than 10,000 participants, to demonstrate the safety and efficacy of diosmin and hesperidin are included. The results of these studies substantiate that Nutratech's Diosmin Complex is reasonably expected to be safe under the conditions of use set forth above.[1]

As a dietary ingredient for use in dietary supplements, Nutratech's Diosmin Complex will be marketed as a powder and labeled with a recommended dose of 500 mg per day orally (50% of the dose typically used in studies) for adults only and no longer than 3 months. In addition, Diosmin Complex is not intended for use by pregnant or lactating women or children and will be so labeled.

In a letter dated August 4, 2000 (attached herewith), to Ms. Virginia Wilkening, Nutratech counsel, Anthony L. Young, set forth the rationale for a meeting with FDA to discuss Nutratech's Troxerutin Complex submission. Those reasons apply equally with respect to Nutratech's Diosmin Complex submission.

If you have any questions, please contact us or Nutratech's counsel, Anthony L. Young, Piper Marbury Rudnick & Wolfe LLP, 1200 19th Street, NW, Washington, DC 20036, (202) 861-3882; fax (202) 223-2085; email: anthony.young@piperrudnick.com. We would appreciate the opportunity to discuss this submission and any questions FDA staff may have about the conditions of use recommended for Diosmin Complex.[2]

Yours truly,

Carl Germano, RD, CNS, LDN
Senior VP Product Development & Research
Nutratech, Inc

Janice Roma Kane, DO
Product Development & Research
Nutratech, Inc

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1 To the best of Nutratech's knowledge, all studies bearing on the safety of Diosmin Complex reasonably available to Nutratech have been provided in this submission and in Nutratech's prior submission.

2 In its initial submission, Nutratech noted that Diosmin is presently on the market in the United States as a dietary supplement. Nutratech is investigating whether diosmin was marketed in the United States prior to October 15, 1994.

1200 Nineteenth Street, N.W.
Washington, D.C. 20036-2412
www.piperrudnick.com

PHONE (202) 861-3900
FAX (202) 223-2085

ANTHONY L. YOUNG

anthony.young@piperrudnick.com
PHONE (202) 861-3882
FAX (202) 223-2085

August 4, 2000

Ms. Virginia L. Wilkening
Deputy Director of Programs
Office of Nutritional Products, Labeling and Dietary Supplements
Center for Food Safety and Applied Nutrition
Food and Drug Administration
HFS 165
200 C Street, S.W.
Washington, D.C. 20204

Re: Nutratech, Inc. – Troxerutin Complex

Dear Ms. Wilkening:

We are writing on behalf of Nutratech, Inc. By letter of March 27, 2000, Nutratech submitted a new dietary ingredient notification for troxerutin. The Administration responded by letter of June 9, 2000. By letter dated August 1, 2000, Nutratech has responded to the issues raised in the Administration's letter.

This letter is to request that the Administration promptly review the August 1, 2000 responsive submission by Nutratech and agree to meet and confer with the company prior to making any further written response to the company. The reason for doing so is that Nutratech has diligently sought to meet the requirements of Section 8 of the Dietary Supplement Health and Education Act of 1994 and to address all of the issues raised by the Administration in its June 9, 2000 letter.

The Troxerutin Complex which Nutratech seeks to market as a dietary supplement is presently being offered for sale by others for use in dietary supplements and there are dietary supplements on the market with this

ingredient. Where a company respects the new dietary ingredient provisions of DSHEA, it is important that the Administration be willing to interact with that company. Such interaction is especially important and necessary where the Administration has not yet developed guidelines for new dietary ingredient submissions and has publicly stated that such guidelines are an important goal to be achieved. Moreover, letters back and forth with 75 day FDA review periods are not an efficient way to communicate. Interaction with those filing new dietary ingredient notifications will provide for open discussion and fleshing out of scientific issues and help make the submission and review process more meaningful and expeditious.

Sincerely yours,

Anthony L. Young

ALY/jek

cc: Mr. Carl Germano
Dr. Janice Kane
Nutratech, Inc.

DIOSMIN COMPLEX—90% Diosmin and 10% Hesperidin

Basis for Concluding Diosmin is Reasonably Expected to be Safe

This submission is in response to FDA's letter of June 16, 2000 regarding Nutratech, Inc's plans to market the dietary ingredient Diosmin Complex (90% diosmin and 10% hesperidin). This document provides additional safety data that establish that Diosmin Complex is reasonably expected to be safe under the conditions of use recommended or suggested in its labeling. It addresses the recommended dose and duration of use for diosmin and the fact that diosmin is not intended for chronic consumption in a dietary supplement. The safety of both diosmin and hesperidin are also described, and FDA comments are addressed specifically.

Background: Bioflavonoids

Bioflavonoids, also known as flavonoids, are a class of water-soluble plant pigments. They are one of the most numerous and widespread groups of natural compounds and are found in foods, such as citrus fruits, onions, and soybeans. They are ubiquitous in all major groups of green plants. Hesperidin is the predominant flavonoid in lemons and sweet oranges. Commonly known as "citrus pulp", hesperidin is found in the inner linings of the rind, the inner membranes, and raw pulp of citrus.[1,2] Hesperidin is on the National Nutritional Foods Association list of dietary supplement ingredients in use before October 15, 1994.

Diosmin is a bioflavonoid derived from hesperidin. Diosmin is available under many brand names throughout the world (Table 1). A diosmin and hesperidin formulation was first launched commercially in France in 1971 for the treatment of chronic venous insufficiency (CVI) of the lower limbs; heavy legs, pain, nocturnal cramps, hemorrhoidal disease, and acute hemorrhoidal attacks. As of 1992, a variety of diosmin formulations were marketed in 57 countries, including 8 in Western Europe. Today, its application has been extended to include many other venocapillary disorders, such as varicose veins, venous stasis ulcers, subconjunctival and retinal hemorrhage, and gingival bleeding.[3] In the United States, diosmin is used in dietary supplement formulas. Several dietary supplement manufacturers presently market products containing diosmin in a complex with hesperidin. To date, Nutratech has not been able to establish that this complex was on the market prior to October 15, 1994.

NUTRATECH'S Diosmin Complex

- ***Nutratech's Diosmin Complex is a micronized mixture of 90% diosmin and 10% hesperidin.***

- ***Nutratech's Diosmin Complex is qualitatively and quantitatively identical to the diosmin and hesperidin formulations studied in the clinical trials presented in this submission.***

Nutratech's Diosmin Complex is a mixture of the flavonoids hesperidin and its derivative diosmin, micronized to increase bioavailability. The Nutratech formulation contains 90% diosmin and 10% hesperidin in proportions comparable to other formulations on the market. Accordingly, Nutratech's Diosmin Complex is comparable to products studied in the clinical trials presented in this document. The diosmin formulation submitted by Nutratech, Inc to FDA in its dietary ingredient notification is qualitatively and quantitatively identical to branded diosmin and hesperidin formulations (Table 1), such as Daflon 500 mg®, Capiven®, Venitol®, Detralex®, Arvenum®, and Venotec®, currently on the market outside the United States.

DIOSMIN (CAS 520-27-4)

Systemic name: 4H-1-Benzopyran-4-one, 7-((6-O-(6-deoxy-alpha-L-mannopyranosyl)-beta-D-glucopyranosyl)ox- y)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-
Synonym: 3',5,7-Trihydroxy-4'-methoxyflavone-7-rutinoside
Molecular formula: $C_{28}H_{32}O_{15}$

HESPERIDIN (CAS 520-26-3)
Systematic Name: 4H-1-Benzopyran-4-One, 7-((6-O-(6-Deoxy-Alpha-L-Mannopyranosyl)-Beta-D-Glucopyranosyl)Ox- Y)-2,3-Dihydro-5-Hydroxy-2-(3-Hydroxy-4-Methoxyphenyl)-, (2s)-
Molecular Formula: C_{28}-H_{34}-O_{15}
Synonyms: 4H-1-Benzopyran-4-One, 7-((6-O-(6-Deoxy-Alpha-L-Mannopyranosyl)-Beta-D-Glucopyranosyl)Ox- Y)-2,3-Dihydo-5-Hydroxy-2-(3-Hydroxy-4-Methoxyphenyl)-, (S)-
Note: 7-((6-O-(6-Deoxy-Alpha-L-Mannopyranosyl)-Beta-D- Glucopyranosyl)Oxy)-2,3-Dihydro-5-Hydroxy-2-(3-Hydroxy-4- Methoxyphenyl)-4h-1-Benzopyran-4-One.
National Library of Medicine [2]

NUTRATECH'S DIOSMIN COMPLEX

- ***Nutratech's Diosmin Complex contains 90% diosmin and 10% hesperidin***
- ***Diosmin Complex has shown no mutagenic action or significant effect on reproductive function.***
- ***Diosmin Complex has shown no toxic effect with 35 times the daily dose.***
- ***Diosmin Complex has an excellent safety and tolerability profile in numerous clinical trials.***
- ***Diosmin Complex has been the subject of numerous clinical trials, animal studies, and* in vitro *studies.***

Diosmin Complex has been the subject of numerous clinical trials, animal studies, *and in vitro* studies. Diosmin Complex had an excellent safety and tolerability profile in clinical trials using doses of 1 gram to 6 grams per day orally for up to 1 year. Rarely,

participants experienced mild, transitory adverse events (Table 2), the incidence and nature of which were similar to placebo. Hemodynamic and laboratory parameters were unaffected by long-term treatment and no contraindications to the therapeutic use of Diosmin Complex appeared. Additionally, Diosmin Complex showed no photosensitizing action and caused no drug interactions during trials.[4]

TOXICITY STUDIES IN ANIMALS

Animal toxicology studies and studies evaluating the effect of Diosmin Complex on the digestive tract, reproductive function, and lactation demonstrated an excellent safety profile. In toxicity studies conducted by Heusser and Osswald on rats, diosmin 200 mg/kg per day orally for 50 days produced no toxicity or abnormality in blood count, GOT, GPT, urea, histology, or weight development. In mice, diosmin 620 mg/kg per day orally for 196 days showed no toxicity or abnormality in blood count, GOT, GPT, urea, histology, or weight development. In minipigs, diosmin in doses of 50 and 250 mg/kg per day orally for 180 days produced no systematic abnormalities in clinical, biochemical, or hematological values pointing to a toxic effect.[5]

In a teratogenicity study, Heusser and Osswald discovered no pathology in 126 skeletons of the fetuses of mother mice given sodium salt of diosmin 50 mg/kg per day from the fourth to the 12th postcoital days. Postnatal mortality (20 days) was 19% in the control group and 26% in the diosmin group in another group of mother mice given the same dose of diosmin and allowed to deliver spontaneously. Weight gain, length development, gross behavior, growth of hair and opening of the eyes were identical in both groups. Organ weights, macroscopical and histological findings did not differ significantly in the diosmin and control animals.[5] There were no abnormalities in the skeletons of the fetuses of mother rats given sodium salt of diosmin 100 mg/kg per day from the fourth to the 12th postcoital days. After spontaneous delivery, the average number of animals per litter was 12.9 in the control group and 11.6 in the diosmin group. There were no significant differences between the 2 groups in respect to weight gain, length, gross behavior, hair growth, opening of the eyes, organ weight, macroscopic and histologic examinations.[5]

In an article published in the *International Journal of Gynecology and Obstetrics*, Buckshee cites Bromont's 1985 toxicological dossier, which states that there was no toxicity and no change in fertility or reproductive function in mice at doses 35 times greater than the therapeutic dose during 26 weeks of treatment with Diosmin Complex.[6] Tests on bacteria, human lymphocytes, mouse bone marrow and DNA in HeLa cells revealed no mutagenic effects, and the embryology, peri- and post- natal development of rat offspring bone to treated parents were not affected.[6] Additionally, Meyer observed that there was no genetic toxicity in the bacteria gene mutation test, in an analysis of metaphases in human lymphocytes in culture, in a DNA repair test in a leukaryote system gene mutations test, or in an *in vivo* clastogenic lesions test.[4] Buckshee also cites an unpublished report by Bromet and colleagues discussing a study in which the accumulation of diosmin in the uterus of Wistar rats was 0.02%, the transplacental passage was 0.003%, and passage in breast milk was 1%. Lastly, Buckshee mentions an international marketing survey published in the French Medical Index, based on data obtained in France between August 1995 and August 1996, which reported that 1.4% of all prescriptions of micronized Diosmin Complex were issued to women who, subsequently, had a normal pregnancy.[6]

Hitzenberger cites toxicological studies of Diosmin Complex conducted by Sterz for Les Laboratoires Servier. Sterz reports that in mice and rats an LD50 could not be determined after an oral dose of 3000 mg/kg, and there were no deaths in the 15-day observation period that followed. Substance related changes were also not detected during autopsy. When makaka monkeys were subjected to oral doses of 4500 mg/kg, there were no relevant toxic symptoms.[7] When rats were subjected to doses of 600 mg/kg per day for 13 weeks in subchronic toxicity tests, they produced no toxicological symptoms.[7] Chronic toxicity tests on rats for 26 weeks using doses of 600 mg/kg per day, resulted in no substance related changes. This applies also to the cynomolgus monkeys, who were subjected to similar doses for 26 weeks. Examinations for mutagenicity with various tests showed no effect in that regard. Reproduction-toxicological tests on rats and rabbits as well as peri- and post- natal toxicity studies and further teratogenic studies on rabbits were negative, and fertility was not impaired.[7]

DIOSMIN COMPLEX: RESULTS OF CLINICAL TRIALS

A review by Meyer** published in 1994 in *Angiology: The Journal of Vascular Diseases*, analyzed data on 3075 patients participating in 12 mid- and long- term trials.[a,b,c,d,e,f,g,h,i,j,k,l] Participants were treated with 1 of 2 formulations of Diosmin Complex (90% diosmin and 10% hesperidin), or placebo taken twice daily for 6 weeks to 1 year. The nature and incidence of the side effects, which were found in 10% of all patients treated, were similar in all groups and involved mostly gastrointestinal and autonomic disorders. Of the patients treated with Diosmin Complex, 6.9% experienced abdominal pain, gastric discomfort, epigastric pain, nausea, dyspepsia, vomiting, or diarrhea. Additionally, 1.7% of the patients treated with Diosmin Complex experienced insomnia, drowsiness, vertigo, headache, tiredness, anxiety, cramps, palpitations, or hypotension. Other side effects reported were 1 case of pruritus and 2 cases of menometrorrhagia in the placebo group and 1 case of epistaxis and 1 case of menometrorrhagia in the Diosmin Complex group. There was also 1 case of an eczematiform rash and 1 case of pityriasis rosea not attributable to treatment.[4]

Regardless of the length of the trial, approximately 10% of the patients in the Diosmin Complex (micronized formulation) groups, 13% in the Diosmin Complex (unmicronized formulation) groups, and 13.9% in the placebo groups developed side effects. The percent of patients treated who dropped out of trials because of side effects was 1.1% in the Diosmin Complex (micronized formulation) groups, 4.8% in the Diosmin Complex (unmicronized formulation) groups and 3.2% in the placebo group. Additionally, the 12-study Diosmin Complex review showed:

- No evidence of any change in hemodynamic parameters with Diosmin Complex 1000 mg in a 1-year multicenter trial that enrolled 215 patients.

- Side effects in participants 70 years and older was 16.3% in Diosmin Complex groups and 15.9% in placebo groups, without being significantly different from the total population.

- The incidence of side effects did not differ significantly in Diosmin Complex and placebo groups in individuals with hypertension, atherosclerosis, diabetes, neurologic disorders, psychiatric disorders, or alcoholism.

- When Diosmin Complex was combined with other drugs used to treat concomitant disorders, there was no evidence of any drug incompatibility or interaction in any of the 12 trials.

- Side effects in trials lasting 6 weeks to 2 months were equivalent to those in trials lasting from 6 months to 1 year. **

- No side effects were seen in 18 patients treated at the daily dosage of 3000 mg for 28 days, in 10 patients treated at the daily dosage of diosmin 2000 mg for one month[a] or in 18 patients treated once with 2000 mg of Diosmin Complex.[8]

- There was no change in the laboratory values of 437 patients treated with Diosmin Complex or placebo in 4 trials[b,c,d,e] lasting between 2 and 6 months.

- When photosensitivity was evaluated in 40 high-risk patients (elderly, past history of allergy, or iatrogenic photosensitization), there was no evidence of a photosensitizing effect with Diosmin Complex.[f]

Blood count, hemoglobin, packed-cell volume, prothrombin, creatinine, urea, albumin, fasting blood glucose, total cholesterol, HDL and LDL-cholesterol, HDL/LDL cholesterol ratio, triglycerides, uric acid, calcium, phosphorus, magnesium, transaminases, GGT, and alkaline phosphatase were not modified by treatment with Diosmin Complex in a 1-year multicenter trial conducted by Pointel (cited by Meyer). There was a slight decrease in plasma creatinine in 65.5% of patients that was significant during treatment. There was also a regular but nonsignificant fall in fibrinogen levels in 65.2% of patients. Both of these parameters, however, remained within the normal physiologic range.[4]

In a review of the pharmacoclinical properties of Diosmin Complex, Nicolaides and Geroulakos reported on studies conducted by Tsouderos[9,10], Laurent[11], and Cospite.[12] In total, 183 patients were treated for CVI with Diosmin Complex or placebo. In all studies, Diosmin Complex produced a significant decrease in venous capacitance, venous distensibility, and venous emptying time ($p<0.001$). Clinical side effects were rare and led to treatment withdrawal in only 3 patients. Tsouderos' first study enrolled 20 participants who received Diosmin Complex 1000 mg; his second study enrolled 40 participants who received Diosmin Complex 1000 mg for 2 months.[13,14]

In a double-blind randomized, multicenter trial study to compare micronized Diosmin Complex with a nonmicronized formulation conducted by Cospite and Dominici, 90 patients with CVI of the lower limbs entered the study, and 88 completed treatment. Participants received either micronized Diosmin Complex 1000 mg or nonmicronized Diosmin Complex 900 mg daily for 2 months. In both groups, there were statistically significant changes, but the improvement in the clinical symptoms and the decreases in the venous outflow parameters were more substantial with micronized Diosmin Complex than with nonmicronized. The clinical and laboratory acceptabilities were equal in both groups, and clinical tolerance was satisfactory. Five patients developed epigastric pain in the nonmicronized diosmin group and 7 in the Diosmin Complex group.[12,14]

**Clinical Trials Reviewed by Meyer: [a]Lacombe, [b]Frileux, [c]Delmont, [d]Cope, [e]Pointel, [f]Ortonne, [g]Amiel, [h]Galley, [i]Lagrue, [j]Cospite 1998, [k]Peker, [l]Elbaz, [m]Vicari

Diosmin Complex 1000 mg was given daily for 2 months to 174 women and 26 men with either organic CVI (83) or functional CVI (117) in 2 double-blind, randomized, placebo-controlled trials conducted by Laurent and colleagues. Results showed no allergic reactions or drug interaction, and side effects were of the same type and frequency in both groups. In the Diosmin Complex group, 1 patient experienced hypotension, 4 patients complained of nausea, 1 of headache, 2 of gastric pain, and 1 of insomnia. Only 3 patients dropped out: 1 in the Diosmin Complex group for epigastric pain and 2 in the control group for nausea and hypotension. In the placebo group, 1 patient experienced hypotension, 4 patients complained of nausea, 4 of headache, 2 of gastric pain, 1 of insomnia, 1 of metrorrhagia. Variations in hematological values were within accepted physiological limits.[11,13]

In Guilhou's study of Diosmin Complex for the treatment of venous ulcers, 107 men and women were enrolled in a multicenter, double-blind, randomized, placebo-controlled trial and received a 2-month treatment with Diosmin Complex 1000 mg daily. Ninety-nine individuals completed the protocol, with 6 withdrawals for reasons other than ulcer healing. In the diosmin group, 2 withdrew because of phlebitis and 1 for noncompliance. In the placebo group, 3 individuals withdrew due to mild cutaneous eruptions and 1 for personal reasons. The treatment was well tolerated. Two venous thromboses were diagnosed in the diosmin group, but investigators thought that they were unrelated to treatment. Other adverse events in the placebo group were eczema (2), uritcaria (1), puritis of the scalp (1), and local pain (1). In the Diosmin Complex group, adverse events included skin changes around ulcer (1), asthenia (1), headaches (1), and exacerbation of chronic colopathy (1).[15]

Guillot and colleagues investigated the safety of Diosmin Complex 1000 mg daily for 1 year for the treatment of CVI. Of the 250 participants receiving Diosmin Complex, 170 completed the trial. Laboratory parameters remained constant during the 12 months. Forty-five individuals were excluded from the final analysis because of side effects: 19 dropped out, 10 were excluded for non-compliance, and 12 for causes unrelated to the trial. Side effects were rare and seen in only 20 patients: gastralgia in 7 patients, dizziness in 4, gynecological signs in 7, and cutaneous eruption in 2. Nausea and gastralgia caused 2 participants to withdraw. There was a weight increase in 2 other participants who withdrew, but it was thought not to be related to treatment. Blood pressure measured before and after treatment showed a slight decrease in systolic and diastolic values. Laboratory parameters remained in normal ranges. RBC, WBC, hemoglobin remained unchanged during the trial and SGPT, SGOT, and GGT, alkaline phosphatase, and fibrin showed no modifications. Blood urea varied between 0.32 and 0.34 g/l and creatinine decreased from 87.7 to 84.0 umol/l. Lipids, glucose, magnesium, phosphate, calcium, and urea remained in normal range.[16]

The RELIEF study, a prospective, controlled, multicenter, international study was conducted between March 1997 and December 1998 in 23 countries worldwide with the participation of more than 10,000 people in the Czech and Slovak Republics, Hungary, Poland, Russia, and Spain. The aim of the study was to assess the evolution of quality of life in patients with CVI, with or without venous reflux, treated with micronized Diosmin Complex 1000 mg per day for 6 months. Three thousand one hundred individuals from the European countries were eligible, and 2767 completed the study. Results showed that the evaluation of the overall efficacy of treatment was good or excellent in the

opinion of 77% of the participants and 81% of the investigators. The overall acceptability of treatment was good or excellent by 93% of patients and 94% of physicians.[17]

Manuel y Keenoy and colleagues conducted a double-blind, placebo-controlled study of the effects of Diosmin Complex in 28 individuals with type I diabetes. Results showed a decrease in HbA1c ($p=0.017$), an increase in glutathione peroxidase activity ($p=0.015$), and an increase in the lag time of the copper-induced *in vitro* oxidability of non-HDL lipoproteins ($p=0.005$). The decrease in HbA1c was more pronounced in patients with higher initial HbA1c, but was unrelated to glycemic control. There were no side effects reported with Diosmin Complex.[18]

A single-center double-blind placebo-controlled study was designed by Le Devhat to assess the effects of Diosmin Complex at a daily dose of 1000 mg per day on microcirculatory, hemorheologic parameters, white blood cell counts, and neutrophil activation in patients with CVI. Two parallel groups were treated for 2 months with Diosmin Complex (n=39) or placebo (n=38). Results after 2 months of treatment showed significant reduction of the stasis-induced RBC aggregation index with Diosmin Complex. There was a statistically significant difference ($p<0.001$) between the groups in RBC aggregation, RBC count, microcirculatory BF, amplitude, and frequency of vasomotion. There was no change in the number of total leucocytes, neutrophils or monocytes after 2 months in either Diosmin Complex or placebo.[19]

Serfaty and colleagues conducted an open-label study of premenstrual syndrome (PMS) to evaluate the therapeutic activity of Diosmin Complex. Of 1908 women selected by 887 gynecologists throughout France, 1724 participated in the epidemiological study. Of these 1724 patients, 251 did not finish the study. The reasons for leaving the trial were as follows: 53 patients wished to leave, 44 patients moved or had an unknown motive, 55 patients were non-compliant, and 31 patients thought the treatment was insufficient. Ten patients left because of adverse events: 8 for medical reasons (3 menometrorragia, 2 gastric disturbance, 1 migraine, 1 ovarian cyst, and 1 intercurrent disease requiring antibiotics). There were 2 hospitalizations (appendectomy, hepatitis). Fourteen underwent contraindicated therapy, and 44 had other motives or data were missing. For 35% of the study participants, problems related to PMS were significant and led to time off work in 7.4% of cases. Of the 1724 patients included, 60 (3.5%) had digestive disturbance, 14 (0.8%) menometrorragia, 11 (0.6%) veinocapillary flare-up or peripheral vasodilatation, 6 (0.3%) headache or migraine, and 5 other disturbances. PMS disappeared completely in 37.4% of the 1473 women who completed the trial. The duration of PMS decreased on average by 2.6 days ($p <0.0001$) per cycle. Symptoms of congestion gradually lessened in frequency and severity by about 60%, as did weight gain, which decreased 29% compared with the pre-treatment cycle. The quality of life and the tolerance to the medicine were judged good or excellent by the patients in 87% and 98% of the cases respectively. Therapeutic activity and acceptability of Diosmin Complex was judged good or excellent by the investigators in 87% and 97% of the cases, respectively.[20]

To assess the effects of Diosmin Complex on microcirculatory parameters, a 3-month, double-blind, randomized, parallel-group study was carried out by Belcaro and colleagues. One hundred and four patients with mild CVI were divided into 3 groups according to dose: 500 mg, 1000 mg, and 2000 mg. They were followed for 90 days with visits at 1 and 3 months. Fourteen patients withdrew from the study: 4 in the 500-mg group; 3 in the 1000-mg group; 7 in the 2000-mg group. Nine participants withdrew for

reasons not related to treatment, 3 for adverse events, and 2 were lost to follow-up. Mild adverse events occurred in 1 patient in the 500-mg group (inguinal pain) and 2 patients in the 2000-mg group (gastralgia, cystitis). When the treatment was discontinued, the adverse events disappeared. Hematological and biochemical parameters remained stable throughout the study.[21]

The efficacy and safety of Diosmin Complex was studied by Galley and colleagues in 100 patients with symptomatic capillary fragility in a double-blind, randomized, placebo-controlled trial. Treatment lasted 6 weeks and consisted of 1000 mg daily of either Diosmin Complex or placebo. Patients were examined at weeks 0, 2, 4 and 6. Diosmin Complex was well tolerated, and the rate of side effects spontaneously volunteered by the patients was similar in both groups.[22]

Cesarone assessed capillary filtration by plethysmography in 43 individuals with venous hypertension and in 10 healthy individuals. Half of those with venous hypertension were treated with Diosmin Complex 1500 mg and half with 1000 mg. Healthy participants received 1500 mg. After 4 weeks, there was a dose-related decrease in capillary filtration in those with venous hypertension that were treated with Diosmin Complex. The treatments were well tolerated and patients did not report any adverse effects. [23]

In Menhyei's study (cited by Hitzenberger), 308 patients with CVI received Diosmin Complex 1000 mg for 2 months. The acceptance was excellent and the same in all 3 groups. Hitzenberger also reports on a pilot study conducted by Pecking and Rambert—10 women operated on for breast cancer who received radiation therapy were given Diosmin Complex for 6 months and experienced no adverse effects.[7]

In a 1995 review article in *Drugs of Today*, Godeberge assesses studies conducted by Cospite[24], Copé, Delmont, Godeberge, which enrolled 299 patients to test Diosmin Complex as a treatment for hemorrhoids. In all trials, Diosmin Complex was well tolerated. The side effects, generally transient and mild, were nonspecific, eg, anxiety, shivering, oppressive feeling across the chest, and epigastric pain. The frequency of side effects was similar in both treated and control groups and never required specific treatment. There was no evidence of drug interaction in any of the studies.[25]

Cospite conducted a study of 100 patients undergoing an acute hemorrhoidal attack who were treated with either Diosmin Complex or placebo. Diosmin Complex was given for 7 days at the dosage of 3000 mg for the first 4 days and 2000 mg daily for the following 3 days. One patient in the diosmin group and 5 in the placebo group withdrew from the treatment because of dissatisfaction with the therapeutic results. Four patients in the diosmin group and 3 in the placebo experienced mild digestive side effects; no one stopped because of major side effects. The blood pressure of the participants remained normal and showed no modification attributable to treatment. There was no statistically significant difference between groups. In the Diosmin Complex group, 3 patients experienced gastralgia, 2 diarrhea, 1 abdominal pain, and 1 headache. In the placebo group, 1 patient experienced gastralgia, 1 dyspepsia, and 1 nausea.[24]

A prospective randomized placebo-controlled trial was undertaken by Ho and colleagues to learn the effects of Diosmin Complex on bleeding after hemorrhoidectomy. Two hundred twenty-eight consecutive patients with prolapsed irreducible hemorrhoids underwent an elective hemorrhoidectomy; 114 of those were randomized to receive Diosmin Complex for 1 week after surgery and 114 served as controls. Results showed

that there was no significant difference between the analgesic required, and the hospital stay of each group was the same. The risk of secondary bleeding from hemorrhoidectomy was reduced with postoperative Diosmin Complex, and there were no side effects reported.[26]

In a safety and efficacy study of Diosmin Complex for the treatment of internal hemorrhoids of pregnancy conducted by Buckshee, results showed that treatment was well accepted, and did not affect pregnancy, fetal development, birth weight, or infant growth and feeding. Fifty women with acute hemorrhoids were enrolled in an open study for 8 weeks before delivery and 4 weeks after delivery. Treatment was divided into 3 phases. In the first phase, a loading dose of 3000 mg per day was given for 4 days followed by 2000 mg per day for 3 days. In the second (antenatal) and third (postnatal) phases, a maintenance dose of 1000 mg per day was given for 30 days. Among those recruited, 47 women completed the 7 day loading phase; 44 the antenatal maintenance phase of 8 weeks; and 41 the post natal maintenance phase of 30 days. Four women withdrew: 1 due to nausea and diarrhea in the loading phase and 2 for reasons unrelated to treatment. Five women were lost to follow up. Hemodynamic and biochemical variables showed no significant change with treatment during pregnancy, and were normal at the end of the study. No ultrasonographic fetal abnormalities were detected during the study. One intrauterine death occurred due to a cord around the neck of the fetus. At delivery, gross placental insufficiency was detected in 6 (13.6%) women. The median maturity of the infant at birth was 39 weeks and the weight was 2.9 kg. One infant had a single umbilical artery. At the end of postpartum treatment, 38 infants were breast fed or supplemented artificially and the median weight gain was 1 kg.[6]

A double-blind, comparative, controlled study on the effectiveness of the addition of oral diosmin and placebo to a conservative regimen of bulk laxative in the treatment of acute symptoms of internal hemorrhoids was conducted by Thanapongsathorn and colleagues. One hundred patients who were comparable in age, sex, symptoms, and severity of hemorrhoids were enrolled. During the first 4 days, patients received 6000 mg in 3 divided doses, and then 2000 mg for an additional 10 days. Two individuals in the placebo group left the trial on the fourth day due to clinical deterioration. There were no side effects detected during the study.[3]

Micronized Diosmin Complex Vs Nonmicronized Diosmin Complex

Amato and colleagues conducted a randomized, double-blind, multicenter trial of the pharmacodynamic and clinical effects of Diosmin Complex in comparison with nonmicronized Diosmin Complex. Ninety individuals with chronic venous insufficiency of the lower limbs entered the study. Participants received either 1000 mg of Diosmin Complex or nonmicronized diosmin for 2 months. In addition to data related to adverse effects, tolerance was determined by blood pressure, pulse rate, and blood tests. Results showed that while statistically significant changes were seen in both groups when in comparison with baseline values, improvements in all clinical symptoms were significantly better with micronized Diosmin Complex than with nonmicronized Diosmin Complex. The clinical and laboratory acceptability was similar in both groups. However, the percentage of satisfied patients was 95% in the micronized formulation versus 80% in the nonmicronized diosmin group ($p<0.01$). Among the 90 people included in the study, 2 dropped out: 1 from the micronized Diosmin Complex group for nonmedical reasons and the other from the nonmicronized group for epigastric pain, which

decreased after treatment was stopped. Tolerance was excellent, especially in regard to the gastrointestinal tract.[27]

DOSE CONSIDERATIONS

- ***Nutratech's Diosmin Complex contains 90% diosmin and 10% hesperidin***
- ***Diosmin Complex has been the subject of numerous clinical trials, animal studies, and* in vitro *studies.***
- ***In clinical trials, Diosmin Complex has been given in doses up to 6 grams per day orally for up to 12 months.***
- ***The recommended daily dose for Diosmin Complex is approximately 1/2 of the recommended therapeutic dose for the standard 90% diosmin and 10% hesperidin formulation.***

Diosmin Complex: Dosing in a Therapeutic Setting
Doses for Diosmin Complex used as a dietary supplement have been calculated after an assessment of animal and human clinical trial data. The recommended therapeutic dose for standard Diosmin Complex formulations is 1000 mg to 3000 mg per day. In clinical trials, Daflon 500 mg®, for example, has been given for up to 1 year and in doses up to 6 grams per day.

The usual therapeutic dose for adults with CVI is diosmin 900 mg and hesperidin 100 mg daily. The usual dose for acute hemorrhoidal attacks is diosmin 2700 mg and hesperidin 600 mg per day for the first 4 days, then diosmin 1800 mg and hesperidin 400 mg per day for 3 days and diosmin 900 mg and hesperidin 100 mg daily thereafter. For chronic hemorrhoids, the dose is diosmin 900 mg and hesperidin 100 mg daily. Diosmin has been used in numerous clinical trials lasting from 2 months to 1 year, and loading doses of 3000 mg per day for 4 days have been given without incident (Table 2).

Diosmin complex is not intended for use by children or by pregnant or nursing women and will be so labeled. The safe use of diosmin for the treatment of pregnant or nursing women with hemorrhoidal disease or venous insufficiency has not been established in large-scale clinical trials. However, no deleterious effects have been reported in pregnant women or their offspring after administration during pregnancy.[4]

Diosmin and Hesperidin: Dosing as a Dietary Supplement,
Doses for diosmin, used as a dietary supplement, were calculated after an assessment of animal and human clinical trial data. **As a dietary supplement, DIOSMIN COMPLEX will be marketed as a powder and labeled with a recommended dose of 500 mg per day orally (50% the dose typically used in studies) for adults only for no longer than 3 months.**

- ***The recommended daily dose for DIOSMIN COMPLEX as a dietary supplement is 500 mg per day.***
- ***A 500-mg dose of DIOSMIN COMPLEX is 19% to 50% of the recommended therapeutic dose.***

- ***A 500-mg dose of DIOSMIN COMPLEX is 8% of the maximum therapeutic dose given in a clinical trial.***

- ***The recommended daily dose of DIOSMIN COMPLEX as a supplement is approximately 1/5 to 1/2 the recommended therapeutic dose.***

SUMMARY

Branded formulations of Diosmin Complex have been used safely worldwide for more than 30 years. To date, Diosmin Complex has been the subject of numerous clinical trials (including studies with pregnant women), animal studies, and *in vitro* studies. *In vitro,* diosmin clearly showed an absence of mutagenic activity. In all toxicological experiments, there was an excessively large therapeutic safety margin without any lethal or toxic reactions, good gastrointestinal acceptability, absence of genetic toxicity, and no impairment of reproductive function. There was an extremely low transplacental passage with the amount found in the breast milk less than 1% of the dose administered to the mother.[28] Clinical trials have used doses of 500 mg to 6 grams per day orally for up to 1 year. Throughout these trials, Diosmin Complex has an excellent safety profile and was well tolerated. Adverse events with Diosmin Complex were rare; and when they occurred, they were always mild, and transient. The side effects usually seen were benign cases of digestive intolerance requiring no changes in treatment. In pharmacoclinical studies, the safety of Diosmin Complex has been proved in healthy volunteers, as well as in those with CVI.[23,29] Consistently, hematological and laboratory parameters have been unaffected by treatment of more than 1 year and no evidence has emerged to suggest any contraindication to Diosmin Complex.[28]

Today, diosmin and Diosmin Complex are on the market all over the world, including the United States. Even though diosmin is sold in the United States, Nutratech has not been able to determine that it was on the market prior to October 15, 1994 yet, has decided to provide this full history and bibliography of this ingredient to FDA as a new dietary ingredient notification. Based on the foregoing information, Nutratech firmly believes that Diosmin Complex is reasonably expected to be safe under the condition of use recommended in the labeling that Nutratech will use for this dietary ingredient.

APPENDIX

CLINICAL PHARMACOLOGY

PHARMACOLOGY

In a pharmacokinetic study conducted by Cova and colleagues, the mean plasma concentration of diosmetin, the aglycone form of diosmin, was assessed following the oral administration of diosmin to healthy volunteers (Table 3). Diosmetin was identified by HPLC and LC-MS techniques. No parent compound was present in the plasma at 20 ng/ml, only the aglycone diosmetin with a retention time of 3.4 minutes. The peak plasma level of diosmetin, 417 ng/ml, was reached after 1 hour. Drug levels in the plasma started to decrease slowly after 2 hours, constantly after 24 hours, and were still detectable after 48 hours. The drug was rapidly absorbed, and diosmetin had a plasma elimination half-life ranging from 26 to 43 hours. After oral ingestion of diosmin, there was no urinary elimination of either diosmin or diosmetin. Its minor metabolites were eliminated in the urine, mainly as glucuronic acid conjugates. The presence of degradation products such as alkyl-phenolic acids confirmed a metabolic pattern similar to other flavonoids. The prolonged presence of diosmetin in the blood suggested an enterohepatic circulation, which is known to have the effect of slowing the complete elimination of drugs. Investigators speculated that (1) the high value of the volume of distribution accounted for the low plasma levels compared with the administered dose of diosmin, (2) the apparent volume of distribution of approximately 62.1 liters pointed to an extensive uptake of the compound by the tissues, and (3) the value of the total body clearance accounts for an active metabolism that can occur in the lumen of the gastrointestinal tract or in the liver, before its elimination in the urine, where it cannot be found in unmetabolized forms.[30]

Table 3. Pharmacokinetic parameters (mean ± SD) after a single oral administration of diosmin

Parameters	Mean ± SD
Maximum plasma concentration (ng/ml)	417 ± 94.1
Half life (h)	31.5 ± 8.6
Mean residence time (h)	36.6 h ± 9.9
Area under plasma concentration-time curve (ng/ml h)	5,617.1 ± 1.518.4
Total body clearance (l/h)	1.32 ± 0.42
Volume of distribution (l)	62.1 ± 7.9

Elimination

In a study conducted by Oustrin and colleagues, ^{3}H-labelled diosmin was administered both IV and orally to Wistar rats. Absorption by the GI track was rapid, and the peak plasma concentration was between 1 and 2 hours. Of the organs examined, almost all had 0.1% to 0.2% of the original activity after 48 hours, only the liver had a 1% concentration. Elimination took place in the urine and in the feces. After IV administration, elimination was predominantly in the urine, while after oral administration it was eliminated almost equally in the urine and in the feces, during the first 24-hour period. In the following 24-hour periods, the feces carried the greater portion of diosmin or its metabolites. Binding to the vascular wall was relatively late.[31]

In a study conducted by Servier (cited by Hitzenberger), 12 volunteers received 2-14-10-diosmin 250 mg (25 µCi) one time. The amount of radioactivity was limited to 0,7µCi, which was 50 times less than the dose administered to the monkeys in a similar study. Radioactive levels measured in the urine instead were 13.8 ± 2.9, expressed in percent of the administered dosage. In the feces 80.5 ± 3.5 of the administered radioactivity was found. In this study, neither diosmin nor diosmetin were found in the urine, which points to metabolism either by the intestinal flora and/or to a high first-pass-effect. The metabolites consist of hippuracid, hydroxyhippuracid and cinnamoylglycinacid. Additional metabolites were phenylpropanacid, m-hydroxy-p-methoxy-phenyl-β-hydroxypropan-, and m-phenyl-β-hydroxypropanacid. Substances not metabolized were only found in the feces. Hitzenberger concluded from the results of the Servier study that in regard to metabolism, the first step takes place in the intestinal flora in the form of demethoxylisation, demethylisation or hydroxylisation, and oxidation and conjugation processes take place in the liver.[7]

Mechanism of Action

Diosmin Complex act on the veins, lymphatic system, and microcirculation, increasing venous tone by prolonging the duration of noradrenergic contractions of the venous wall (even in conditions of heat or acidosis).[29,32] Diosmin Complex stimulates lymphagogue activity. It improves the drainage of interstitial tissues by increasing peristaltis and lymphatic flow. This mode of action has been demonstrated in animals [32] and confirmed in man by microlymphography.[32] Diosmin Complex reduces the activation and adhesion of leukocytes and inhibits their migration through the microcapillary endothelium[32,33] and opposes microcirculatory deterioration by inhibiting the expression of certain mediators involved in leukocyte or endothelial adhesion.[32,33] Diosmin Complex increases venous tone by prolonging the activity of parietal noradrenaline. Thus, decreasing venous capacitance, venous distensibility and venous emptying time. Diosmin Complex protects the microcirculation by fighting the microcirculation-damaging process; it combat venous inflammation by decreasing leukocyte activation, and as a consequence, by inhibiting the release of inflammatory mediators, principally free radicals and prostaglandins. Diosmin Complex normalizes capillary permeability and strengthens capillary resistance and acts on the lymphatic system, improving on lymphatic drainage by increasing lymph flow and lymph oncotic pressure.[32]

Diosmin acts by inhibiting the enzyme phosphodiesterase, increasing intracellular cyclic adenosine monophosphate (cAMP) and consequently reducing the level of the main biochemical mediators of inflammation prostaglandin E2 and F2 (PGE2, PGF2), thromboxane B2 (TXB2), and oxygen free radicals. Diosmin reinforces venous tone by prolonging the activity of parietal norepinephrine. In experiments on the saphenous vein strips of dog, conducted by Heusser and Osswald, diosmin blocked the inactivation of exogenous noradrenaline and caused a slow and gradual contractile response of an oil-immersed strip, which was not attributable to the release of noradrenaline.[5] Diosmin exerts a significant potentiation toward NE in both normal and varicose veins under acidotic conditions. Local acidosis depresses reactivity of vascular smooth muscle, especially the response of human isolated saphenous veins to exogenous norepinephrine. In an *in vitro* study, Juteau and colleagues used isolated varicose veins to test the effect of Diosmin Complex and norepinephrine on human rings of veins under acidosis conditions. Results showed that the Diosmin Complex combination induced a shift to the left of the concentration-response curves for norepinephrine. This potentiation was significant in both normal and varicose veins and was increased in proportion with the pathological status of the venous rings.[34]

In an *in vitro* study, the cytotoxic effect of lipopolysaccharide (LPS) on cultivated bovine aortic endothelial cells was attenuated by diosmin. Melzig and Loose speculated that the inhibition of LPS-induced cytotoxicity in bovine aortic endothelial cell cultures by diosmin might be mediated via inhibition of tyrosine kinases. Study data showed that the IC50-value of LPS in the combination with diosmin 8 µmol/l was shifted from 31 to 70 ng/ml in a concentration dependent manner.[35] In another *in vitro* study conducted by Korthuis, diosmin prevented ischemia and reperfusion-induced leukocyte adhesion in skeletal muscle. This anti-adhesive effect appeared to be mediated, in part, by inhibition of induced expression of ICAM-1.[33]

When the lymphatic activity Diosmin Complex was tested in dogs and rats, diosmin induced a lymphatic flow increase that was correlated with the administered doses. The maximal 10 minute period flow after IV injection of D (12.500 mg/kg-1) was 191% higher than the corresponding one in the control group. A correlation between lymphatic flow increase and pulsatility was demonstrated. Infusion of 14C-labelled-D evidenced a clear blood-lymph transfer of the drug: an active transport into the lymph was suggested during a 15-minute to100-minute period from the concentration curves.[36]

Diosmin Complex is a strong inhibitor of Cu(2+)-induced arachidonic acid peroxidation, as revealed by the inhibition of thiobarbituric acid- reactive substance formation in mixed liposomes of phosphatidylcholine and arachidonic acid. Diosmin is a good complexant of Cu2+ ions but not of Fe2+ ions. The Cu2+ complex formation may thus explain part of the antioxidant effect. However, Diosmin Complex is also a good quencher of the singlet oxygen-induced arachidonic acid peroxidation that does not involve metal ions.[37]