



Memorandum

MAR 03 2004

Date: \_\_\_\_\_  
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: Isopropoxy Isoflavone (Ipn-flavone)  
Firm: Perrigo Company  
Date Received by FDA: Oct. 22, 2003  
90-Day Date: January 20, 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.

Jamya A. Jackson Ph.D

95S-0316

RPT 214



Mr. Dave Jespersen  
Director of Regulatory Affairs  
Perrigo Company  
515 Eastern Avenue  
Allegan, Michigan 49010

JAN 5 2004

Dear Mr. Jespersen:

This letter concerns your notification, dated October 9, 2003, which you submitted pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)). Your notification concerning the substance "Isopropoxy Isoflavone" (Ipriflavone) that you intend to market as a new dietary ingredient in the dietary supplement "Ostivone" was filed by the Food and Drug Administration (FDA) on October 22, 2003.

The notification informs FDA that the Perrigo Company intends to market the new dietary ingredient, "Ostivone," in caplet form. The dietary supplement will contain 100 mg Ipriflavone 7-isopropoxy-3-phenyl-4H-1 benzopyran-4-one, 100 IU Vitamin D and 250 mg Calcium per caplet. The notification states that the "suggested use will be for adult women, two tablets per day."

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing 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 is considered 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.

If you have any questions or would like to set up a meeting with FDA concerning this matter, please contact Victoria Lutwak at (301) 436-1775.

Sincerely yours,

A handwritten signature in black ink, appearing to read "Susan Walker". The signature is fluid and cursive, with the first name "Susan" and the last name "Walker" clearly distinguishable.

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



Susan J. Walker, MD  
Division Director  
Division of Dietary Supplement Programs  
Office of Nutritional Products,  
Labeling and Dietary Supplements (HFS-820)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Pkwy  
College Park, MD 20740

Date: January 8, 2004

Dr Susan Walker,

This is in response to your letter dated January 5, 2004 regarding the Perrigo New Dietary Ingredient (NDI) Notification for Isopropoxy Isoflavone (Ipriflavone, trade name Ostivone®) filed by the FDA on October 22, 2003. In your letter you requested additional information to clarify the basis for determining that the subject dietary ingredient is included in the definition of a dietary ingredient under 21 USC 321(ff)(1).

Technical Sourcing International, Inc (TSI) is the supplier of the subject dietary ingredient Isopropoxy Isoflavone. They have independently submitted the requested information to you. As stated in the attached letter from TSI, this ingredient meets the definition of a dietary ingredient for use in a dietary supplement because it falls in category (F), existing as a constituent of (C) an herb or other botanical, in accordance with 21 USC 321(ff)(1).

In addition, this dietary ingredient has been the subject of a previously accepted NDI Notification submitted by TSI to the FDA with a file date of November 19, 1997 (Docket 95S-0316 Item Code RPT No. 22).

This information should adequately clarify your only issue and should not affect the original 75 day time frame, or restart the 75 day cycle.

If you have any further questions, please feel free to contact me.

Sincerely,

David Jespersen  
Director of Technical Services

86947

Enclosure



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**TECHNICAL  
SOURCING**  
International, Inc

January 8, 2004

Susan J. Walker, MD  
Division Director  
Division of Dietary Supplement Programs  
Office of Nutritional Products, Labeling and Dietary Supplements (HFS-820)  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
5100 Paint Branch Pkw  
College Park, MD 20740

Dr. Susan Walker:

On behalf of the Perrigo Company, we are writing in regards to your January 5, 2004 letter concerning the New Dietary Ingredient (a.k.a. "NDI") Notification for Isopropoxy Isoflavone (Ipriflavone, trade name Ostivone<sup>®</sup>) filed by the FDA on October 22, 2003. This ingredient is supplied to Perrigo by our company Technical Sourcing International, Incorporated (a.k.a. "TSI").

Your letter to Perrigo requests additional information to describe the basis that Isopropoxy Isoflavone has been determined to be included in the definition of a dietary ingredient. 21 USC 321(ff)(1) states that a dietary ingredient can be used in a dietary supplement if it is a "...product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract or combination of any ingredient described in clause (A), (B), (C), (D), or (E)."

In response, Isopropoxy Isoflavone (Ipriflavone) can be found in nature in minute quantities in plants of the legume family such as alfalfa and bee propolis. The economics of extracting these minute quantities from plants is not commercially feasible. To produce an economically affordable ingredient, TSI utilizes a synthetic chemical process. Therefore, this ingredient meets the definition of a dietary ingredient for use in a dietary supplement because it falls in category (F), existing as a constituent of (C) an herb or other botanical, in accordance with 21 USC 321(ff)(1).

In addition, this ingredient is the same ingredient that is currently marketed by the acceptance of another NDI Notification submitted by TSI to the FDA. The FDA's file date of that NDI Notification was November 19, 1997 (Docket 95S-0316 Item Code RPT No. 22).

Sincerely,



Kimberly Anderson  
Engineering and Sciences Coordinator  
Technical Sourcing International, Inc.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service  
Food and Drug Administration

Memorandum

Date JAN 16 1998

From Acting Director, Division of Programs and Enforcement Policy, Office of Special Nutritionals, HFS-455

Subject 75-Day Premarket Notification for New Dietary Ingredients

To Dockets Management Branch, HFS-305

017  
APB/FOA  
98  
AP 30  
P 3

New Dietary Ingredient:	Isopropoxy Isoflavone
Firm:	Technical Sourcing International, Inc.
Date Received by FDA:	November 19, 1997
90-Day Date:	February 22, 1998

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after February 22, 1998.

Sincerely yours,

James Tanner, Ph.D  
Acting Director,  
Division of Programs and  
Enforcement Policy  
Office of Special Nutritionals  
Center for Food Safety and  
Applied Nutrition

Attachment

cc:  
HFS-22, CCO  
HFS-450 (r/f, OSN w/control slip:TRAC#55844 & cpy incoming)  
HFS-456 (r/f, Latham, Moore)  
r/d:HFS-456:JELatham;jel:01/07/98:DocName:#55844.mem:Disc4

95S-0316

RPT 22



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Washington, DC 20204

Mr. Steve Lee  
Technical Sourcing International, Inc.  
1742 Misty Meadows  
Sandy, Utah 84093

Dear Mr. Lee:

This is to notify you that your submission pursuant to section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the act) dated November 19, 1997, concerning the marketing of a substance that you assert is a new dietary ingredient (i.e., Isopropoxy Isoflavone) was received by the Food and Drug Administration (FDA) on November 24, 1997. Your submission will be kept confidential for 90 days from the date of receipt, and after February 22, 1998, your submission will be placed on public display at Dockets Management Branch (Docket No. 95S-0316). Commercial and confidential information in the notification will not be made available to the public.

Please contact us if you have questions concerning this matter.

Sincerely yours,

James Tanner, Ph.D.  
Acting Director  
Division of Programs and  
Enforcement Policy  
Office of Special Nutritionals  
Center for Food Safety  
and Applied Nutrition

cc:  
HFA-224 (w/incoming)  
HFS-22 (CCO)  
HFS-456 (r/f, Latham, Moore)  
HFS-450 (r/f, w/ control slip OSN#55844 & cpy incoming)  
f/t:HFS-456:JELatham;jel:01/07/98:DocName:#55844.OSN:Disc4

**tsi** **Technical Sourcing  
International, Inc.**

1742 Misty Meadows  
Sandy, UT 84093  
(801) 523-2666  
(801) 523-3666 FAX

1270 Avenue of the Americas  
Suite 2701  
New York, NY 10020  
(212) 586-7764  
(212) 245-2876 FAX

CERTIFIED MAIL--RETURN RECEIPT REQUIRED

TRADE SECRET, CONFIDENTIAL AND PROPRIETARY INFORMATION (Before  
the FDA's POST) Revised and re-submit NOV. 19, 1997

Director  
Division of Program and Enforcement Policy  
Office of Special Nutritionals  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C Street  
HFS-455  
Washington, D.C. 20204

Dear Sir or Madam:

Notice is hereby given pursuant to the requirements of section 413(a)(2)[(21 U.S.C.350b)] of the Federal Food, Drug, and Cosmetic Act of the intent of Technical Sourcing International, Inc. to introduce a new dietary ingredient, Isopropoxy Isoflavone (the "Ingredient"), into interstate commerce 75 days after this submission. The ingredient occurs in many legume plants such as alfalfa and bee propolis. Based on the information available, we recommend a maximum daily intake of the ingredient of 600 milligrams per day. This is about 1/100 of the non-toxic daily dose performed on many animal model toxicity studies.

The following documents evidencing the ingredient are enclosed with this notice and incorporated herein by reference:

English translation of Imai K, et al , Acute Toxicity Study of Ipriflavone in Mice and Rats, Pharmacology and Therapy, Vol. 13, No 10, 1985,p49-p54

English translation of Tokiwa T, et al., A one-year oral toxicity study of Ipriflavone on beagle dogs, Applied Pharmacology, Vol. 31, No. 1, 1986, p113-p136

Pursuant to 21 CFR section 20.60-61 Technical Sourcing International, Inc. specifically requests that this information be kept confidential and not be disclosed Please contact me if you have any questions on this ingredient.

Thanks.

Sincerely,



Steve Lee  
Technical Sourcing International, Inc.

53844

# A one year (fifty-two week) oral toxicity study of ipriflavone on beagle dogs

## ABSTRACT:

This paper describes a one-year (52 week) oral toxicity study of ipriflavone on beagle dogs.

After 4 weeks of acclimatization, 16 male and 16 female dogs were divided randomly into four equal groups. Ipriflavone was given orally in gelatin capsules at doses of 150, 500, and 1,500 mg/kg per day once daily for 52 weeks. Control animals received an empty gelatin capsule orally.

During the administration period, vomiting and discharge of abnormal feces occurred in animals of all groups, sporadically, or sometimes intermittently. Vomiting was probably due to physical irritation of the stomach caused by administration of large amounts of test compound because the occurrence of this type of disorder is not uncommon in beagle dogs. On the other hand, the feces was mixed with blood rather frequently in 3 animals from the 1,500 mg/kg group. However, like the passage of mucous feces or diarrhea, the passage of bloody feces was probably physiological or sporadic, because the same type of disorder was seen in control animals, and because there were no histopathological changes in the gastrointestinal tract of any animals.

The test compound affected neither the body weight nor the food and water consumption.

There were no changes attributable to the drug in hematology, blood chemistry, urinalysis, organ weight, or histopathology.

The results suggest that the maximum non-toxic dose of ipriflavone for beagle dogs following one oral administration is above 1,500 mg/kg per day.

## INTRODUCTION:

Ipriflavone 7-isopropoxy-3-phenyl-4H-1 benzopyran 4 one

This was introduced and provided by Takeda Pharmaceutical Company and the Hungarian Chinoïn Company. This was tested jointly by these companies for abnormal bone metabolism. This experiment was completed for a 52 week oral administration period.

## EXPERIMENTAL MATERIALS AND METHODS:

### 1. Materials Used:

Ipriflavone, 7-isopropoxy-3-phenyl-4H-1 benzopyran 4 one was acquired from Takeda pharmaceutical Company, Lot No M263-019 (100.0%), M263-020 (99.9%), M263-021 (100.2%). The material consists of a white crystal powder contained in metal containers well protected from sunlight and temperature change.

### 2. Animals used in experiment:

20 (twenty) beagle dogs acquired from White Eagle Labs of the U.S.A. After a four week quarantine period, sixteen dogs were chosen for use. The ages ranged from nine to ten months old. Weight ranged from 8.3 -12.9 kg for females and 5.5-10.3 for males. Room temperatures ranged from 22-28° c and 40-70% humidity. Dogs were exposed to normal room light for 12 hours from 6:00 A.M. to 6:00 P.M. Each occupied a metal cage. Dogs were fed 500 grams of

food produced by Oriental Y East Company.

### 3. Method of administration:

125 mg of ipriflavone per kg of body weight was administered once daily by gelatin capsule. The control group was fed 1% of hydroxymethyl cellulose once daily. The serum concentration of ipriflavone metabolite was detected. Appendix 1 (p. 114) shows the toxicity response for 125, 500, 1000, 1500, and 2000 mg per kg of body weight.

### 4. Observation and examination:

#### 1. General observation.

Dogs were closely observed before administration and after administration in intervals of 1/2, 2, 4, and 6 hours.

#### 2. Body weight

Animals were weighed weekly.

#### 3. Record of food and water intake.

Consumption of water intake was observed and recorded weekly.

#### 4. Hematological examination.

Blood samples were taken once before the experiment and five times during course of this study in intervals of 5, 13, 20, 39, and 52 weeks. RBC, WBC, Microcell Counter CC-108, hematocrit, hemoglobin, Hemoglobin Counter HB-100 were used to check RBC, WBC, platelet, hemoglobin count. Thromboplastin and prothrombin times were also checked. The Brecher method was used to check RET (reticular RBC ration). The Wright-Giemsa method was used to check WBC and bone marrow. EDTA-2K and sodium ascorbate were used to check prothrombin and thromboplastin times.

5. Hemotobiochemical exams were taken to check glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), leucine aminopeptidase (LAP), creatine phosphokinase (CPK), cholinesterase, carbohydrates, bilirubin, urea (BUN), creatinine, total lipids, total cholesterol, neutral lipids, phosphatidyl lipids, fatty acids, inorganic phosphorous, calcium, and total protein. Lactic dehydrogenase and alkaline phosphatase (ALP) were detected in heparin treated serum (Abbott VP Bichromatic analyzer) Serum sodium and serum chloride (Hitachi 775) A/G ratio (A/G albumin globulin ration (Cliniscan, Helena Laboratories)

#### 6. Urine

Urine samples were taken once before the experiment and five times during course of this study in intervals of 5, 13, 20, 39, and 52 weeks. Density sodium content and chloride content of urine were checked using the Hitachi 775 and chloride counter CL-5M. Also, the Abbott VP Bichromatic Analyzer were used to check inorganic phosphorous. Fresh urine pH, protein, glucose, ketone body, urobilinogen, bilirubin were checked by BM test 8-II. The residue of RBC and WBC and epithelial cells were checked.

#### 7. Eye ophthalmofunduscopy

Eye exams were taken once before the experiment and five times during course of this study in intervals of 5, 13, 20, 39, and 52 weeks. The pupils were enlarged by Mydrin and photographed by a Kowa RC-2 camera.

8. Body temperature, EKG, heartbeat, and blood pressure were taken once before the experiment and five times during course of this study in intervals of 5, 13, 20, 39, and 52 weeks. Temperatures were taken through the rectum (BAT-12m, Bailey) EKG (ECG-5403) (Chart speed 50 mm/sec, calibration: 100 mm/mV) Blood pressure (BP-203NP Japan Korin)

#### 9. Pathological examination.

Dogs were administered pentobarbital and blood was drained from the cervical artery. Visceral organs were examined by sight and pathological exams. The visceral organs and cerebrum, and cerebellum, pituitary, thyroid, thymus, heart, lungs, liver, spleen, pancreas, kidney, prostate, ovary, and uterus were all checked by LIBROR EB-2800, EDL-100KM, Shimazu. The visceral organs, duodenum, ileum, rectum, appendix, lymph, tonsils, trachea, esophagus, tongue, gall bladder were prepared in and 10% solution of formalin and 5% formalin. Some organ specimens were prepared in hematoxylin eosin (HE)

10. Statistical methodology. Bartlett 1937, Snedecor and Cochran 1967, Dunnett 1955, Kruskal and Wallis 1952.

### EXPERIMENT RESULTS:

#### 1. General observations

No changes were noticed in the frequency and amount of the feces of the control group versus the experimental group. One dog from each group vomited. Hematuria occurred in one dog of the high dose group. Softened stool and diarrhea occurred in one dog of the middle dose group. At the 37th week, turbid iris occurred in one female dog.

#### 2. During the experiment, no animals died

#### 3. Body weight (see figure 1).

No significant changes were observed.

#### 4. Food intake

There was a slight change in food consumption detected which does not affect the experiment statistics.

#### 5. Water intake

Water intake remained stable throughout the experiment

#### 6. Hematological exam. (See tables 1-3)

#### 7. Hematobiochemical

After fifty-two weeks, two of the female dogs given a medium dose total lipids were

slightly raised. From the high dose group, one female had slightly raised cholesterol and total serum lipids. The slight raise did not present a significant change in statistics.

8. Urine examination (See table 13 and 14)

9. Eye and ophthalmofunduscopy

No abnormalities were noticed in the eye examination.

10. Body temperature and heart temperature. EKG, blood pressure. On the 13th week, one female from the medium dose group was noticed to have a blockage between the ventricular and atrium of the heart. No changes were noticed in the high dose group.

The context of this study was originally written in Japanese. It was translated into English by Steve Lee and Martin Hansen.

Steve Lee 11/18/97

Martin Hansen 11/18/97

*This document contains copyrighted material which maybe  
viewed at:*

***DOCKETS MANAGEMENT BRANCH  
FOOD AND DRUG ADMINISTRATION  
5630 FISHERS LANE, ROOM 1061  
ROCKVILLE, MD 20852***



25-95

October 9, 2003

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

D.B./FDA

Dear Sir or Madam:

The Perrigo Company is notifying the Food and Drug Administration that it will market Ostivone® (Isopropoxy Isoflavone). Following Section 413 (350b) of the Federal Food, Drug and Cosmetic Act and Title 21 of the Code of Federal Regulations 190.6 please find two copies of this notification.

Trade name: Ostivone®  
Labeling: Isopropoxy Isoflavone  
Chemical name: Ipriflavone 7-isopropoxy-3-phenyl-4H-1 benzopyran-4-one  
CAS number: 35212-22-7

The dietary supplement will contain 100 mg Ipriflavone 7-isopropoxy-3-phenyl-4H-1 benzopyran-4-one, 100 IU Vitamin D and 250 mg Calcium per caplet. Suggested use will be for adult women, two tablets per day.

Included in the attached is the basis for safe use. This summary utilized peer reviewed published research establishing the reasonable safe use of this new dietary ingredient under recommended conditions.

Including:

1. Safety Profile
2. Technical Sourcing International, Inc. 75-Day Premarket Notification for New Dietary Ingredients: Isopropoxy Isoflavone, submitted November 19, 1997

Sincerely,

Dave Jespersen Director of Regulatory Affairs

CC:

Kim Anderson Engineering and Sciences Coordinator  
Technical Sourcing International, Inc.



## Safety Profile

### Human Studies

#### **Valente et al., 1994**

In a year long, randomized, double-blind, placebo-controlled study, 20 postmenopausal women (47-65 years of age) each received Ipriflavone (IP) 300 mg twice daily, or a placebo (PL) and a calcium supplement of 1g/day.

Any adverse effects seen during the study were recorded. Six women in the IP group complained of gastrointestinal problems and 3 withdrew from the study but 3 women in the placebo group also had gastrointestinal problem and 2 of these women in the placebo group also withdrew from the study. The gastrointestinal problems were ascribed to the calcium supplement.

Before and after of cessation of the study at the 12<sup>th</sup> month the following parameters were assessed: systolic and diastolic blood pressure, heart rate, respiratory rate, RBCs, hemoglobin, hematocrit, WBCs, platelets, BUN, glucose, creatine, creatinine clearance, bilirubin, SGOT, SGPT, GGTP, LDH, uric acid, total protein, serum sodium, potassium and chloride. These measurements for cardiovascular function, hematological values, liver and kidney biochemical functions revealed no significant differences that could be ascribed to the IP treatment.

Tolerance to IP was good and compliance to the protocol was excellent.

#### **Ushiroyama et al., 1995**

In a randomized, 1.5-year study 98 postmenopausal women were divided into 4 groups. 28 women were given 600 mg IP/day orally, 15 women were given 1 $\mu$ g vitamin D/day orally, 20 women were given IP and Vitamin D in combination and 35 women received no treatment at all.

Patients in any of the four groups did not report serious side effects. Biochemical parameters for kidney, liver, and hematological parameters were unchanged in any of the four groups.

#### **Adami et al., 1997 and Gennari et al., 1997**

In three collaborative studies performed at several Italian medical centers specializing in osteoporosis care the efficacy and safety profiles of IP were determined in about 650 postmenopausal women for 2 years. The subjects were almost equally divided into a treatment and non-treatment group. The studies were randomized, placebo-controlled,



and double-blinded. They received IP at 200 mg per meal (600 mg/day) or a matching placebo. Both groups received 1g of calcium daily.

The safety profile of IP was good. In one study, 16 women treated with ipriflavone and 15 receiving the placebo withdrew from the study. In the second study 26 subjects treated with IP and 28 subjects given the placebo withdrew from the study. In the third study only five patients treated with IP and 3 receiving the placebo withdrew from the study.

Side effects were mainly GI, and reported to spontaneously recover after about 6 months and may be associated with the administration of the calcium supplement. Blood chemistry, body weight, liver and kidney function did not show significant changes. Occasional transient changes were seen; increases in aspartate aminotransferase, alanine aminotransferase, and  $\gamma$ -glutamyltransferase and decreases in white blood cells and lymphocytes, but recovery to normal were observed after discontinuation.

No significant variations in cardiac and pulmonary functions and in body weight were observed.

Good compliance was seen with 85% of the patients taking more than 80% of the study medications during the two-year period.

#### **Maugeri et al., 1994**

Ipriflavone was tested for 2-years along with the use of a calcium supplement in a double blind, randomized, placebo-controlled study. The study initially started with 100 female subjects selected on the basis of being over 65 years, having at least one vertebral fracture in the past and a bone mineral density at the distal tenth of the radius of  $-2 \times$  S.D. of the mean. The IP group received 200 mg of IP, t.i.d. along with 1g of calcium as a supplement.

The total dropouts were reasonably low (16%) and 41 patients in the IP group and 43 patients in the placebo group were evaluated for the efficacy of the treatment. Of the 16 dropouts, 6 patients were dropped due to protocol violations or non-compliance; another 7 patients were excluded because they did not attend the first control examination at 6 months. Only 2 cases dropped out from the IP group and only 1 case from the placebo group because of side-effects such as pyrosis, diarrhea or mild persisting gastralgia, and the total number of patients affected by any side-effect was 6 in the IP group and 3 in the placebo group. Finally, 6 patients (4 and 2 in the IP and placebo groups, respectively) presented mild gastralgia attributable to IP or Ca during the first 6 months of treatment. These complaints disappeared spontaneously and did not require the suspension of treatment or a reduction of the dose.

The following laboratory blood parameters were performed at 0, 6, 12 and 24 months in all patients, and no significant alterations of them were observed: glucose, urea nitrogen,

uric acid, bilirubin, creatinine, SGOT, SGPT, LDH,  $\gamma$ -glucuronyl-transpeptidase, protein distribution, hemochrome, hematocrit, prothrombin time, serum Na, K, Cl, Ca and phosphorus contents, serum lipids; as well as urine analysis and creatinine clearance.

#### **Agnusdei, Crepaldi et al., 1997**

In another 2-year study, 198 postmenopausal women (50-65 years of age) with vertebral bone density 1 SD below the mean value for normal, age-matched, postmenopausal subjects were enrolled in six Italian medical centers and 134 completed 2 years of treatment. All subjects were randomly allocated to a 2-year treatment with oral IP (200 mg t.i.d.) or a matching placebo, according to a double blind, parallel group design. Ninety-eight patients were allocated to the IP-treated group. All patients also received an oral daily supplement of 1g of calcium carbonate.

A complete routine analysis of liver and kidney functions along with hematological parameters were measured before and at the end of treatment period.

Adverse reactions, mainly gastrointestinal, occurred to a similar extent in the two treatment groups. The tolerability profile of IP was very good. The total number of adverse events was similar in both groups. The reasons for withdrawal from the study were mainly due to gastrointestinal disturbances. There were no significant differences in the distribution of the patients in the two groups. No unexpected adverse reactions or deaths occurred during the study.

#### **Agnusdei, Bufalino et al., 1997**

In another Italian multi-medical center, double-blinded, controlled, 2-year study, using IP and PL. Women over 65 years of age, not taking drugs that effect bone metabolism, without severe kidney or liver problems or heart impairment or with out psychosis were enrolled. The subjects (111) received 200 mg at meals (600 mg/day) of IP or PL.

The safety assessment revealed mainly gastrointestinal adverse effects, which were less than placebo (14.5% IP and 16.1% placebo). These adverse events cleared up after temporarily stopping treatment. Few patients presented reversible modification of laboratory parameters.

#### **Alexandersen et al., 2001**

Menopausal women (45-75 years of age) in a 3-year, randomized, double blind, placebo-controlled study took 200 mg t.i.d. of IP or PL. In this multi-center study 234 women were treated with IP.

This study contradicts the preponderance of efficacy data from clinical studies conducted over the past 15 years. The report states that IP does not prevent bone loss but the study found that Bone Mass Densities for the IP population were stable over the duration of the study. The study had high and unexplained dropout rate of 40%, which significantly reduced the statistical power of the study. Additionally, unexpectedly the placebo group's bone densities remained stable and only 4.5% of the placebo group experienced new vertebral fractures, compared to initial hypothesis of 21% that had been seen with a similar population.

IP safety concerns were raised in this study because the mean lymphocyte percentage as it relates to the total white blood cell count cells decreased from 33% to 27% for the total IP group during the first six months and remained at this level throughout the rest of the treatment period. However, these changes are within normally acceptable ranges. While these decreases in lymphocyte count may be statistically significant, the fact that they were subclinical means that their clinical relevance is unclear. In some IP patients the lymphocyte concentrations decreased significantly ( $500/\mu\text{l}$ ). Thirty-one women in the IP group developed subclinical lymphocytopenia, of whom 29 developed it during IP treatment. Of these, 15 (52%) of the 29 had recovered spontaneously by 1 year and 22 (81%) of the 29 after 2 years of discontinuance.

This paper states that there is an additional report of lymphocytopenia in women taking IP, which have generated some concerns regarding safety of IP. The referenced paper does not support this statement.

### **Takeda Corp., 1998**

Summary data from over 60 IP studies performed in Italy, Japan, and Hungary indicate out-of-range lymphocyte counts were less than 3%, sharply contrasting a value of 13% reported in this study. Takeda Pharmaceuticals of Japan, which first introduced IP, indicated only 4 cases (0.06%) of lymphocytopenia in their post-marketing reports.

No other hematological, biochemical, urinary, or physical examination factors were significantly affected.

### **Agnusdei, Camporeale et al., 1992**

The effectiveness of IP in the treatment of Paget's disease was studied in a short randomized, cross-over designed study. Nine males and 7 females were given 600mg/day and 1200mg/day in the cross-over aspect of the study.

Tolerance and compliance were good. No other safety data was given.

**Melis et al., 1992**

To determine if IP is able to enhance estrogen activity on bone metabolism, 133 postmenopausal women were randomly submitted to the treatment with: (1) placebo; (2) 0.15 mg/day of conjugated estrogens; (3) 0.30 mg/day of conjugated estrogens; (4) 0.15 mg/day of conjugated estrogens plus 600 mg/day of IP; (5) 0.30 mg/day of conjugated estrogens plus 600 mg/day of IP. One g/day of calcium supplementation was given to all women.

An increase in bone mineral density was observed after 12 months of treatment in the women treated with 0.15 (not significant) or 0.30 mg/day (P<0.01) of conjugated estrogens associated with IP. After 12 months of treatment, no significant changes in hematologic, hepatic and renal laboratory tests were observed when compared to baseline values.

**Adverse Events Reported**

The table below lists patient reported results from efficacy IP clinical trials: (Generally mild GI disturbances)

Total # *	Dose IP (mg/day)	Dose Ca (g/day)	IP%	PL%	Duration (mo)	Study
28	600	1	21	18	12	Passeri et al, 1992
133	600	1	6	7	12	Melis et al, 1992
40	600	1	15	S	12	Valente et al, 1994
134	600	1	48	40	24	Agnusdei, Crepaldi et al. 1997
196	600	1	30	43	24	Adami et al., 1997
111	600	NA	14.5	16.1		Agnusdei, Bufalino et al. 1997

\*Does not include those individuals that discontinued the trials

Animal Studies

**Tokiwa et al., 1986**

A controlled, oral chronic toxicological study (52-weeks) of IP on beagle dogs resulted in a no-observed-effect level in excess of 1500mg/kg per day. IP was administered to

groups of two males and two females at levels of 0, 150, 500, and 1,500 mg/kg per day via gelatin capsules.

No deaths were seen.

Hematological tests; biochemical examinations of blood; urine analysis; examinations of the fundus; measurements of the body temperature, ECG, heart rate and blood pressure; autopsy; weighing of the organs; and histopathological examinations did not reveal any changes caused by the administration of IP.

Vomiting, along with feces containing blood or mucus and diarrhea were seen either sporadically or intermittently but there were no other symptoms. Vomiting was observed in all groups but was slightly more frequent in the medium and high dosage groups. However, a similar frequency was observed in a female from the control group as well. It was concluded that the vomiting is the result of physical stimulation caused by the administration of large amounts of the IP since vomiting is common among beagles and because white substances similar to the agent were often seen in the vomit of the medium dosage and high dosage animals.

Stools containing blood were observed in all groups and were slightly more frequent in 3 dogs in the high dosage group. However, histopathological examinations did not reveal changes. The frequency of diarrhea or excretion of stools with mucus does not have any correlation with the dosage and thus such phenomena are believed to be physiological. Stools containing white substances were observed in the IP treated groups during the administration period. Excretion of stools with similar white substances was also seen during an earlier five-week toxicity test and analysis confirmed that the white substance is the specimen. Such excretion of the specimen in the stools is frequently observed in the medium and high dosage groups. It was concluded that substantial amounts of IP was excreted without being absorbed when the dosage is in excess of 500 mg/kg and is administered orally in gelatin capsules for 52 weeks.

Transient decreases in weight, food intake and water intake were seen in all non-control groups and sometimes correlated to vomiting or excretion of bloody stools and other abnormal stools. However, similar changes were observed in the control group as well and thus these changes are seen to be physiologic in nature.

### **Imai et al., 1985**

In an acute toxicity test IP was administered in ICR mice and Sprague-Dawley rats at oral dose levels of 2,500, 5,000 and 10,000 mg/kg, subcutaneous dose levels of 1250, 2500 and 5000 mg/kg and peritoneal dose levels of 1,250 and 2,500 mg/kg.

One male mouse out of 10 male mice died in each oral dosing group, and one female mouse in the 2,500 mg/kg oral group died within 30 hours after administration. However no deaths were noted in any group of rats during the 14-day observation period after oral

dosing. One female rat at 1,250 mg/kg and one male rat at 2,500 mg/kg died 9 and 10 days after peritoneal administration respectively. No other deaths occurred in any group.

In mice, digestion was inhibited, which seemed to cause diarrhea to occur within an hour of oral administration. After 3 days the diarrhea abated.

Observation and pathology revealed no other toxicity.

Subcutaneous cysts formed in mice after injection and motor activity decreased but recovered after 2-3 days. At autopsy only the cyst was a noticeable change.

Peritoneal administration decreased motor activity within 10 minutes and within two hours diarrhea occurred. Upon autopsy some white crystalline powder was found in the peritoneal cavity. In rats, within ten minutes after oral administration motor activity was reduced and after 2 hours severe diarrhea was present. Diarrhea was also noticed in the control and may have been caused by the olive oil vehicle.

Some slight nasal bleeding was recorded. All symptoms recovered to normal in three days. Subcutaneous cysts occurred, similar to the mice, in rats and did not resolve for two-weeks. One developed into an ulcer. After autopsy some rats showed a small hemorrhage in the lungs. Peritoneal dosing had the same effect on rats as mice, spontaneous decreased motor activity. Increased breathing rates and bristled hairs were observed.

Autopsy revealed a white crystalline oily mixture, which coated some organs and got into the lungs. The diarrhea seen in the mice and rats could be caused by the IP or the olive oil as seen when compared to the control. The peritoneal injections did not cause any remarkable changes.

### **Ghezzi et al., 1996**

Male Sprague Dawley rats received orally 200 or 400 mg/kg per day of IP or PL for 12 weeks. The treatment was noted as well tolerated and no signs of toxicity were observed and that no relevant structural changes were seen. Bone crystal structure was analyzed. No changes in broadening parameter  $\beta^{1/2}(002)$  and  $\beta^{1/2}(310)$  or in spacing values measured  $d(002)$  and  $d(310)$  as compared with normal rat bone formation which reflect crystallinity and lattice parameters of bone apatite.

### **Otaka et al., 1986**

Japanese workers performed a 1-year toxicity study on IP in Sprague Dawley rats. IP was administered gavage once a day for 52 continuous weeks at 0, 3, 1.0 and 3.0 g/kg. Ten rats/sex were studied in each group.



No changes directly related to IP treatment were seen in terms of general status, deaths, water consumption, urinalyses, ophthalmoscopy, hematological tests, autopsy findings, or histopathology.

An inhibition in weight gain was seen among males of the 3.0 g/kg group and females of each IP group, and a slight reduction in food consumption was seen among females of each IP group. These changes in females did not show obvious dose-dependency and were not considered to be a change of toxicological significance.

A slight increase in the triglyceride level of males of the 1.0 and 3.0 g/kg groups as well as the albumin level of females of the same groups was seen in hematological tests. These changes appear to be related to the increased liver weight, but there was not a consistent tendency among females and males and was not considered a change that was of toxicological significance.

An increase in relative weight of the liver was seen among males of the 1.0 and 3.0 g/kg IP groups and among females of each IP group. The increase in relative liver weight could ascribed to the increase in activity of some drug metabolizing enzymes of the liver of rats known to occur when 0.3 g/kg of IP is administered to rats and may represent a response to consumption of a foreign substance over a long period of time. Thus, obvious changes of toxicological significance were not seen with administration of 3.0 g/kg to male or female rats.

## **Pharmacokinetic and Metabolic Modeling**

### **Kim and Lee, 2002**

Pharmacokinetic parameters of IP were evaluated after intravenous administration of spray-dried ipriflavone with polyvinylpyrrolidone (SIP) at levels of 5, 10, 20, and 40 mg/kg as IP and after oral administration of 50, 100, and 200 mg/kg as IP to rats. The hepatic, gastric, and intestinal first-pass effects of IP were measured after intravenous, intraportal, intraduodenal, and oral administration of SIP equivalent to 20 or 50 mg/kg of IP to rats.

After intravenous and oral administration, the pharmacokinetic parameters of IP were dose-independent. The absolute oral bioavailability was also independent of oral doses with a value of approximately 24%. The low bioavailability may be due to the hepatic, gastric, and/or intestinal first-pass effects.

After intravenous administration, the first-pass effect in the heart and lung could be almost negligible, if any, in rats. Following intravenous and intraportal administration of SIP, approximately 30% of IP absorbed into the portal vein was eliminated through the by a hepatic first-pass mechanism. The area under the plasma concentration-time curve from time zero to time infinity values after oral administration and intraduodenal instillation of 50 mg/kg of IP as SIP were not significantly different, but the values were

significantly smaller than that after intraportal administration of 20 mg/kg of IP as SIP, indicating that gastric first-pass effect of IP was negligible, but intestinal first-pass effect was considerable in rats. Therefore, the low bioavailability of IP after oral administration to rats was mainly due to intestinal first-pass effect. The hepatic first-pass effect and incomplete absorption of IP from rat gastrointestinal tract also contributed to the low bioavailability in rats.

### **Rohatagi and Barrett, 1997**

The deposition of IP is very variable due to the formation of at least 5 major metabolites (M1-5). The M1 and M5 metabolites are active metabolites in the human.

An integrated pharmacokinetic /metabolic model has been developed that predicts the plasma concentration of IP and metabolites after a single oral dose. The model is based on the metabolic conversion of IP to M1, M3, and M4, with conversion of M4 to M5 and the conversion of both M1 and M3 to M2 in the rat. Conversion of M5 to M6 and M7 was insignificant and was ignored. The input function in the model was described by a first-order constant and the disposition of IP and its metabolites was into two-compartment model. The elimination/non-metabolic constants for each analyte accounted for urinary elimination. The model's predictability was studied by examining plasma concentration data from 16 healthy male volunteers given 200 mg of an IP/corn-oil suspension.

### **Rohatagi et al., 1997**

A pilot study was conducted in healthy male volunteers that demonstrated 8- to 20-fold increase in the IP bioavailability after administration of Osteofix tablets in the fed state relative to the fasting state. A four-way crossover study was conducted in 16 healthy male human volunteers, receiving Osteofix in fed state and 50, 100, and 200 mg of an IP corn-oil suspension in fasted state.

All IP administrations were safe and well tolerated.

A reduction was observed in the IP plasma level variability after 100-mg corn-oil administration as compared with that of the tablet. There was an increase in relative bioavailability with the suspension, which was such that the 50mg IP corn oil suspension yielded similar levels as the 200-mg tablet.

M5 was the major metabolite of IP. The area-under-the-curve (AUC) to the last measured time point (AUC<sub>t</sub>) levels were lower for M5, M3, and M2; higher for M1 and M2; and similar for IP after the 50mg/ corn oil dosing, as compared with the values for the tablet. The absorption/formation of M2 was delayed.



Metabolite formation, except for M1 and M5, decreased with increasing dosage, which was accompanied by the prolonged half-life of IP and non-proportional increase in the IP AUC<sub>i</sub>. This may be due to auto-inhibition of IP metabolism, because IP is an inhibitor of CYP3A4, and the metabolism of IP may be partly CYP3A4 mediated.

The bioavailability of Osteofix may be improved by formulations that increase solubility, such as a corn oil suspension.



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