

Memorandum

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Date: JUL 10 2006

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: **“Kaneka Glavoinoid Rich Oil™ Brand of Licorice Flavonoid Oil”**

Firm: Carter Ledyard & Milburn LLP on behalf of Kaneka Corporation.

Date Received by FDA: April 12, 2006

90-Day Date: July 11, 2006

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

____Victoria Lutwak____

19955-0316

RPT348



Food and Drug Administration
5100 Paint Branch Parkway
College Park, Maryland 20740

JUN 27 2006

Mr. Michael C. Davis
Carter Ledyard & Milburn LLP
1401 Eye Street, NW, Suite 300
Washington, District of Columbia 20005

Dear Mr. Davis:

This is to inform you that the notification, dated April 10, 2006, that 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)) on behalf of your client, Kaneka Corporation, was filed by the Food and Drug Administration (FDA) on April 12, 2006. Your notification concerned the substance that you called "Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil" to which you also refer by the abbreviated name, "LFO". "LFO" is derived from the root of *Glycyrrhiza glabra* L.

According to your notification, "[t]he dietary supplement containing the LFO will be in capsule form. The LFO capsules will be clearly labeled and promoted as a dietary supplement.... [E]ach serving of the dietary supplement will contain 30 mg licorice ethanol extract (equivalent to approximately 300 mg LFO). Consumption of up to three (3) servings per day will be suggested or recommended in the label directions...."

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 21 U.S.C. 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.

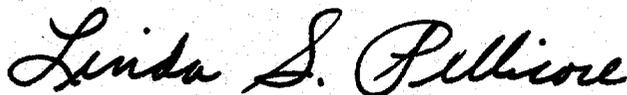
In accordance with 21 CFR 190.6(c), FDA must acknowledge its receipt of a notification for a new dietary ingredient. For 75 days after the filing date, your firm must not introduce or deliver for introduction into interstate commerce any dietary supplement that contains the dietary ingredient that is the subject of this notification.

Please note that acceptance of this notification for filing is a procedural matter, and thus, does not constitute a finding by FDA that the new dietary ingredient or supplement that contains the new dietary ingredient is safe or is not adulterated under 21 U.S.C. 342. FDA is not precluded from taking action in the future against any dietary supplement containing your new dietary ingredient if it is found to be unsafe, adulterated or misbranded.

Your notification will be kept confidential for 90 days after the filing date of April 12, 2006. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

If you have any questions concerning this matter please contact Victoria Lutwak at (301) 436-1775.

Sincerely yours,



Linda S. Pellicore, Ph.D.
Supervisory Team Leader, Senior Toxicologist
Division of Dietary Supplement Programs
Office of Nutritional Products, Labeling
and Dietary Supplements
Center for Food Safety and Applied Nutrition

2006-3231

CARTER LEDYARD & MILBURN LLP
Counselors at Law

Michael C. Davis
Partner

1401 Eye Street, N.W., Suite 300
Washington, DC 20005

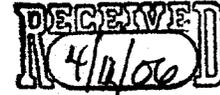
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April 10, 2006



BY HAND

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

Re: New Dietary Ingredient Notification for Kaneka Glavonoid Rich Oil™
brand of Licorice Flavonoid Oil (LFO) at 900 mg/day

Dear Sir or Madam:

On behalf of Kaneka Corporation ("Kaneka"), we respectfully submit the attached information pursuant to section 413(a) of the Federal Food, Drug, and Cosmetic Act, in support of Kaneka's marketing of the new dietary ingredient Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil (LFO) derived from the root of *Glycyrrhiza glabra* L. Three copies of this two volume submission are enclosed.

The dietary supplement ingredient, LFO, is the same as that previously filed (5/05/05) and accepted in RPT 284 (Docket 95S-0316). The conditions of use are presently increased from up to the previous 2 capsules (600 mg) per day to 3 capsules (900 mg) per day, a level that can reasonably be expected to be safe based on the available data.

Based on the availability of new clinical evidence summarized herein, and supported by the data summarized in RPT 284 (Docket 95S-0316), Kaneka affirms that a dietary supplement containing Kaneka's Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil is reasonably expected to be safe when used according to the conditions of use recommended or suggested in the labeling of the dietary supplement.

On behalf of Kaneka, we hereby confirm that this letter and the enclosed Notification contain trade secret or otherwise confidential commercial information that should not be disclosed to the public pursuant to 21 C.F.R. §§20.61 and 190.6(e). More specifically, the Notification contains valuable data and information that Kaneka has held in strict confidence and has not disclosed to any member of the public. A letter identifying such confidential and

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proprietary information and their location(s) within the document will be provided to the Office of Nutritional Products, Labeling, and Dietary Supplements of the Center for Food Safety and Applied Nutrition at a later date.

Any questions related to technical aspects of this notification should be referred to Dr. David H. Bechtel of Cantox U.S. Inc. His contact information is available in Section 1 of the notification.

Best regards,



Michael C. Davis

MCD:lac
Enclosures

cc: Kaku Nakagawa, Kaneka Corporation
Erica Rath, Cantox
Dr. Dave Bechtel, Cantox

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CANTOX
HEALTH SCIENCES INTERNATIONAL

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2006-3231

RECEIVED

APR 12 2006

BY *ATS EID*

**New Dietary Ingredient Notification
for
Kaneka Glavonoid Rich Oil™ brand of Licorice
Flavonoid Oil (LFO) at 900 mg/day**

Submitted by:

CANTOX HEALTH SCIENCES
INTERNATIONAL
1011 U.S. Highway 22 West, Suite 200
Bridgewater, New Jersey
08807

On behalf of:

Kaneka Corporation
Functional Food Ingredients Division
3-2-4, Nakanoshima, Kita-Ku
Osaka 530-8288, Japan

April 6, 2006

CANTOX Offices:

Bridgewater, NJ, USA
908.429.9202

Mississauga, ON, Canada
905.542.2900

Fleet, Hampshire, UK
+44 (0) 870 351 3780

SECTION 1

The name and complete address of the manufacturer or distributor of the dietary supplement that contains a new dietary ingredient, or of the new dietary ingredient.

The manufacturer of the new dietary ingredient is:

Kaneka Corporation
Functional Food Ingredients Division
3-2-4, Nakanoshima, Kita-Ku
Osaka 530-8288, Japan

Direct correspondence to:

David H. Bechtel, Ph.D., DABT
Managing Director and Senior Scientific Consultant
CANTOX U.S. Inc.
1011 U.S. Highway 22, Suite 200
Bridgewater, NJ 08807
Phone: 908-429-9202
Fax: 908-429-9260

SECTION 2

The name of the new dietary ingredient.

Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil (LFO)*.

* The term "LFO" appears throughout this NDI notification and refers to Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil.

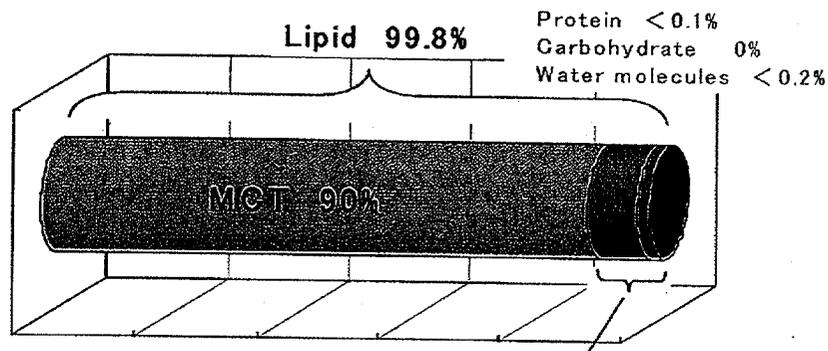
April 6, 2006

SECTION 3

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of the dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.

The dietary supplement ingredient, LFO, is the same as that previously filed (5/05/05) and accepted in RPT 284 (Docket 95S-0316); the conditions of use are herein increased from up to the previous 2 capsules (600 mg) per day to 3 capsules (900 mg) per day.

Figure 1 **Composition of LFO**



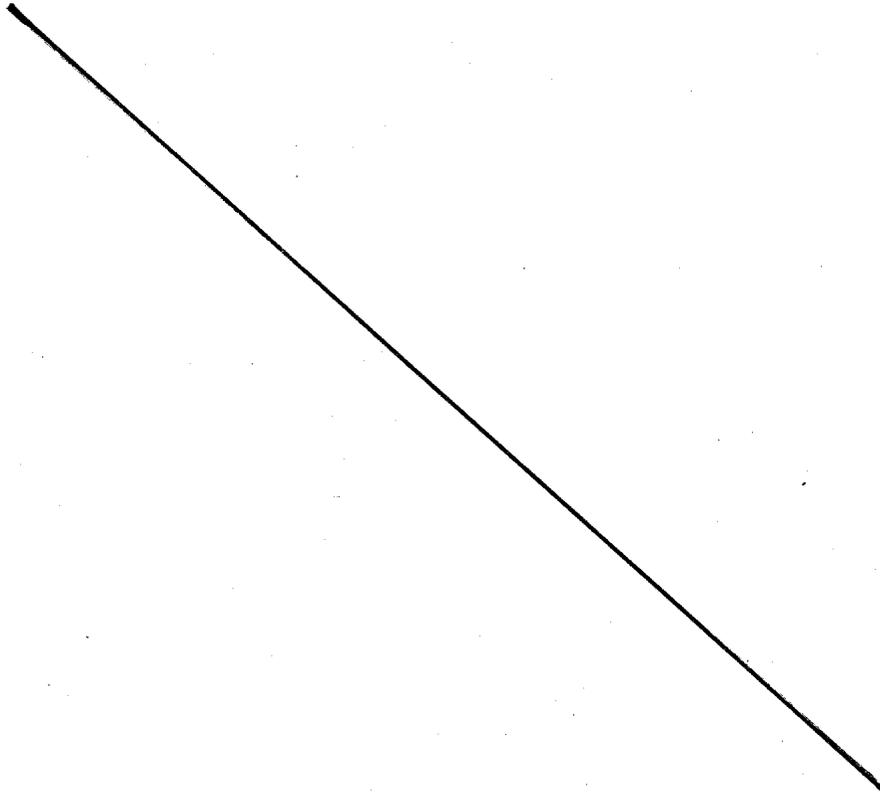
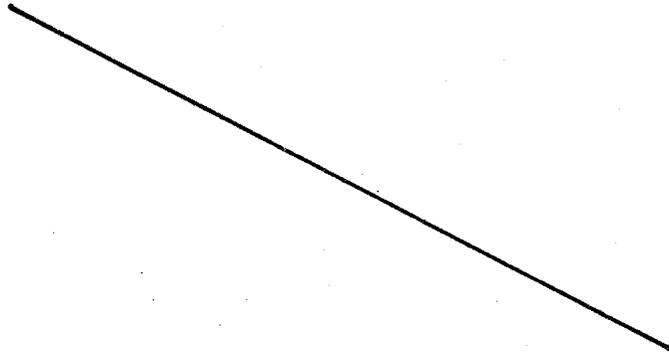


Figure 2-2



The dietary supplement containing the LFO dietary ingredient will be in capsule form. The LFO capsules will be clearly labeled and promoted as a dietary supplement. A description of the number of capsules per serving size will appear on the label, and each serving of the dietary supplement will contain _____ approximately 300 mg LFO). Consumption of up to three (3) servings per day will be suggested or recommended in the label directions, resulting in _____ approximately 900 mg LFO, or 15 mg/kg/day for a 60 kg body weight person).

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

Details of the manufacturing process for Kaneka LFO and the history of use of licorice and its components were reviewed in a prior submission. That submission, entitled "New Dietary Ingredient Notification for Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil" was dated April 27th, 2005 and was filed on May 5th, 2005 with FDA. A copy of that submission is included in this report as Appendix 1 and also is available from FDA's Division of Docket Management in docket number 95S-0316 (RPT 284). In addition to information provided in the previous document, an extensive review of nonclinical studies conducted by Kaneka Corporation supporting the safety of LFO _____

_____ are summarized in the current document. A discussion of published preclinical, genotoxicity, and clinical studies of licorice and its components, including glycyrrhizin (essentially absent from LFO) was included in the prior submission for the sake of completeness and to support the safety of Kaneka's LFO.

The information presented in Appendix 1 demonstrates that:

- (i) The chemical composition of LFO is well characterized, and the manufacturing process yields a product demonstrated to reproducibly meet compositional specifications.
- (ii) Nonclinical studies [*i.e.*, 90-day repeated dose toxicity, genotoxicity (reverse mutation, chromosomal aberration, bone marrow and liver micronucleus fraction test), rat medium-term liver bioassay for carcinogens] were conducted using LFO concentrate solution (3% glabridin) rather than LFO (1% glabridin), the diluted final product, in order to administer a higher dose to animals and create a worst-case exposure scenario.
- (iii) Based on the results of the subchronic toxicity study, _____

_____ the maximum recommended dose to consumers (600 mg/day LFO, equivalent to 10 mg LFO/kg b.w. for a 60 kg b.w. person) demonstrates the safety of Kaneka Glavonoid Rich Oil™.

(iv)

- (v) Reports available in the published scientific literature suggest that the main undesirable adverse effects of licorice stem from its mineralocorticoid activity. Specifically, 18 β -glycyrrhretinic acid, the active metabolite of glycyrrhizinic acid, inhibits the enzyme 11 β -HSD in the kidney. While these studies have no direct relationship to the safety of LFO, which is virtually devoid of glycyrrhizin, the results of these studies demonstrated that a daily intake of 10 mg of glycyrrhizin could be derived as a safety dose for most healthy adults. _____

_____ In this case, the margin of safety is more than 300-fold.

- (vi) Studies conducted with deglycyrrhizinated licorice showed that the test material generally had no adverse effects. Deglycyrrhizinated licorice contains no more than 3% glycyrrhizinic acid. Thus, the results of these studies are consistent with the _____

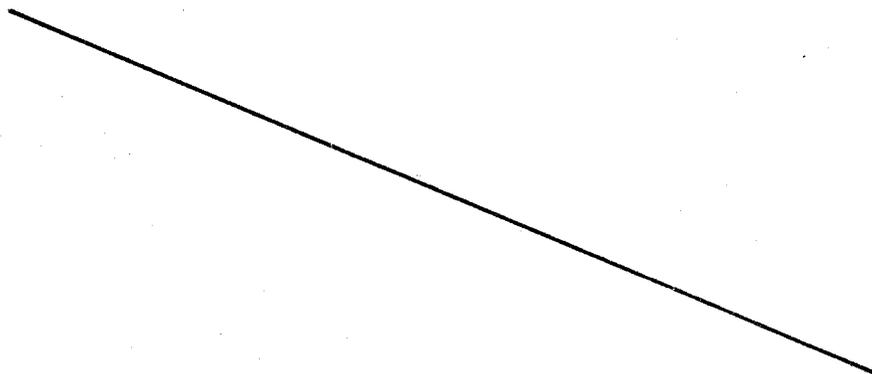
- (vii) Small quantities of LFO will be consumed as dietary supplements.

Based on this evidence, Kaneka concluded that the chronic use of LFO in dietary supplements at a level of 600 mg per serving (providing 60 mg licorice ethanol extract per serving and equivalent to 10 mg LFO/kg b.w. for a 60 kg b.w. person) was reasonably expected to be safe.

Kaneka now wishes to increase the recommended dose of LFO from 600 mg/day to 900 mg/day. _____

Although a large (80 to 120-fold) margin of safety still exists

between these NOAELs and the increased maximum recommended dose to consumers (900 mg/day LFO, equivalent to 15 mg LFO/kg bw for a 60 kg bw person), Kaneka has sponsored several clinical studies to provide additional evidence of safety at this higher dose. A more detailed discussion of these studies is presented herein in Sections 4.1 through 4.5.

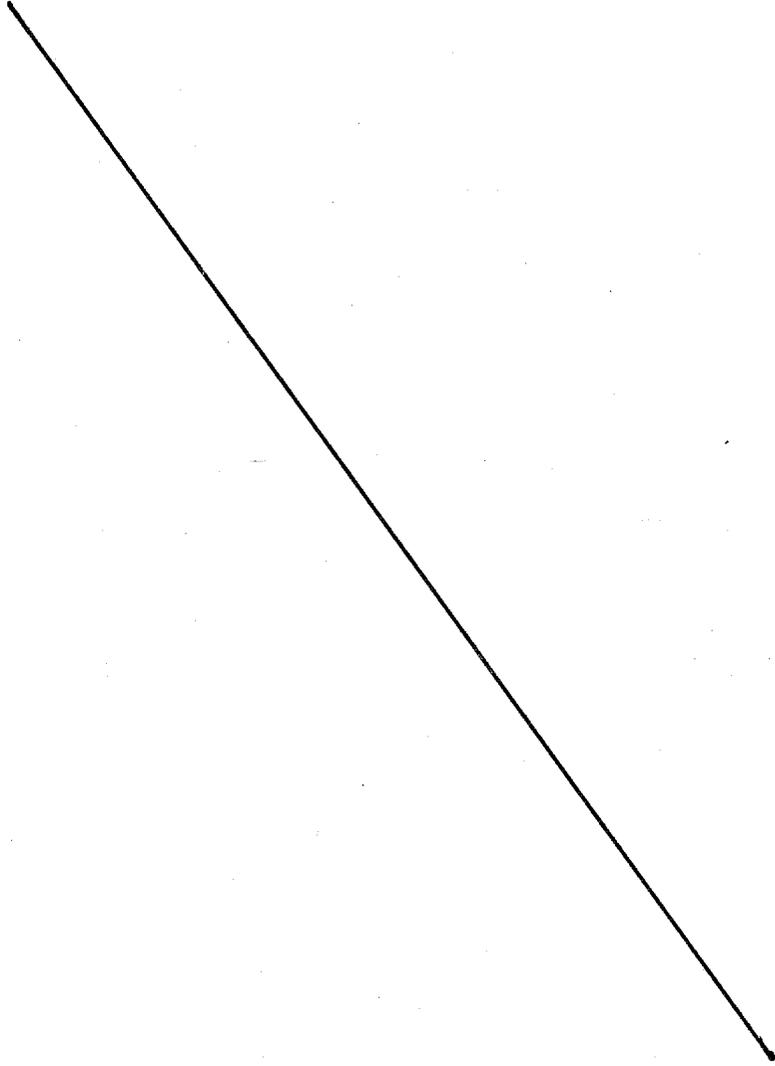


The remaining study data are available in the full reports, included as references.

It should be noted that an atypical statistical analysis was used in the clinical trials. Values within groups obtained during and after treatment were compared to those collected at the pre-ingestion evaluation rather than across groups (treatment groups vs. placebo group) at a given time point. In the one-week study (Section 4.2) for example, hematology values obtained on Day 8 in the 300 mg treatment group were compared to those obtained in the 300 mg group on Day 1 rather than to those values obtained from the placebo group on Day 8. As a result, analysis did not account for changes over time; although values within a group may have changed significantly compared to pre-ingestion values, they remain in agreement with values obtained from the placebo group at the same time point.

A discussion of the margin of safety between the recommended intake of 900 mg/day and published no-observed-adverse-effect levels (NOAELs) and Acceptable Daily Intake (ADI) values is presented in Section 4.7.

4.1 Single Dose Clinical Study

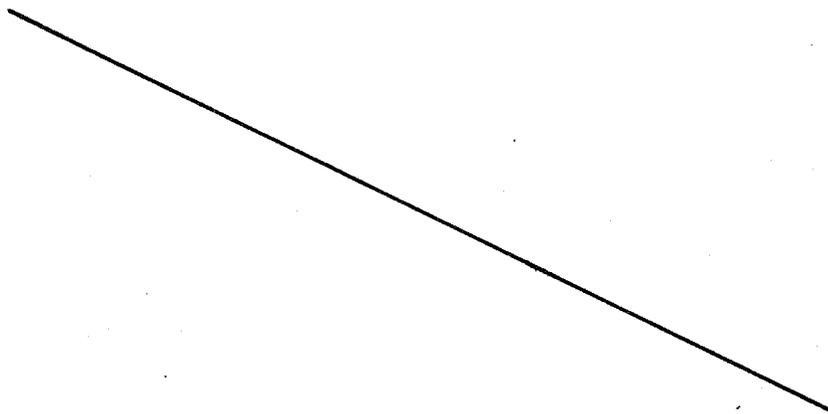


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INFORMATION

4.6 Overview of Clinical Data with Kaneka's LFO



4.7 Margin of Safety for Glycyrrhizinic Acid from Published Clinical Trials and Acceptable Daily Intake Values

In 1993, the European Commission's Scientific Committee on Food evaluated the available toxicological information for glycyrrhizinic acid and concluded that the data were inadequate to derive an ADI at that time (SCF, 1991). The Committee considered it prudent to advise however, that regular ingestion should not exceed 100 mg/day, while it was explicitly mentioned that this was a provisional figure.

Subsequent to that evaluation, new toxicological information, including data from human volunteer studies, was published. Two-repeated-dose studies in human volunteers were published from which two no-observed-adverse-effect levels (NOAELs) of 2 mg/kg bw/day (Bijlsma *et al.*, 1996) or 217 mg/person/day (Bernardi *et al.*, 1994) could be derived. The NOAEL obtained in the study by Bijlsma *et al.* (1996) was considered to be more appropriate because this study comprised larger groups of volunteers, including a placebo control group, and the exposure lasted for a longer period. At the next dose level above the NOAEL of 4 mg/kg bw/day (the highest dose tested), in 9 out of 11 volunteers, water retention, slight decreases in plasma potassium and suppression of the rennin-angiotensin-aldosterone axis was observed. In addition, in 1/11 volunteers clinical effects were observed as well.

Using a PBPK/PD model, Ploeger (2000) predicted that the intake of 100 mg/day, 4×10^4 exposed persons (95% confidence limits: 4.6 per 10^6 to 3 per 10^2) might show signs of "pseudohyperaldosteronism," but these should be considered as preliminary results, because they were largely dependent on one fairly small study with human volunteers, 100 mg/day was the intake provisionally established the Committee in 1991 that should not be exceeded on a regular basis. The model by Ploeger (2000) also provides insight into the determinants of differences in sensitivity in humans. Gastrointestinal transit time, sensitivity of the target enzyme 11- β -hydroxysteroid dehydrogenase-2 (11-BOHD-2) to glycyrrhetic acid and basal 11-BOHD-2 activity seem to be the most important determinants. Although there is no direct evidence from the biomedical literature, it is conceivable that the health of people with Cushing's syndrome, or other conditions related to hypertension, abnormal electrolyte or water homeostasis, may be adversely affected by exposure to glycyrrhizinic acid or its ammonium salt.

Using these new data, the SCF reevaluated its upper intake recommendation in April of 2003. Although these data provided a stronger basis for the upper limit for regular ingestion of glycyrrhizinic acid and ammonium glycyrrhizinate, the Committee concluded that an ADI could not be derived because the new human toxicity studies are too limited (small experimental groups, short duration). The Committee considered that the previous upper limit for regular ingestion of 100 mg/day provided a sufficient level of protection for the majority of the population while noting that this upper limit includes the intake of glycyrrhizinic acid *via* all products (e.g., licorice confectionery as well as glycyrrhizinic acid, or ammonium glycyrrhizinate-flavored products) (SCF, 2003).

At the same time, the Committee realized that there were subpopulations for which this upper limit might not offer sufficient protection. These subgroups comprise people with decreased 11-BOHD-2 activity, people with prolonged gastrointestinal transit time, and people with hypertension or electrolyte-related or water homeostasis-related medical conditions.

The Committee noted that for ammonium glycyrrhizinate as well as for glycyrrhizinic acid, used as chemically defined flavoring substances, the Upper Use Levels in food indicate that the Maximum Survey-Derived Intake (MSDI) exposure estimates (130 to 240 $\mu\text{g}/\text{person}/\text{day}$, respectively), may underestimate the intake for individuals who select to consume certain foods (e.g., foods flavored at the upper use levels).

The Committee noted that additional information was needed in order for a complete evaluation of glycyrrhizinic acid and ammonium glycyrrhizinate. However the provisional upper limit for regular ingestion of 100 mg/day, a level considered to offer a sufficient level of protection for the majority of the population, provides a basis for comparison for an acceptable glycyrrhizinic acid and ammonium glycyrrhizinate intake and the level of exposure to glycyrrhizinic acid from the use of Kaneka's Glavonoid Rich Oil™ Brand of Licorice Flavonoid Oil (LFO). Kaneka's

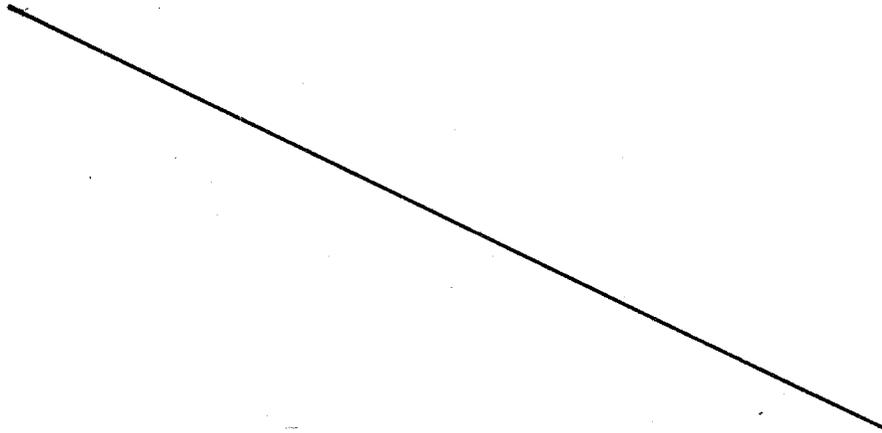
Glavonoid Rich Oil™ Brand of Licorice Flavonoid Oil (LFO)

is equivalent to a 2000-fold margin of safety from the provisional upper intake limit for regular ingestion of 100 mg/day established by the Scientific Committee on Food.

Based on observations from a clinical trial involving the administration of 0, 1, 2, or 4 mg glycyrrhizinic acid/kg b.w. for 8 weeks to healthy subjects, Van Gelderen *et al.* (2000) proposed a no effect level of 2 mg/kg. At intakes above this value, classic symptoms associated with 11 β -HSD inhibition (*i.e.*, reductions in serum aldosterone and ANP concentrations, and plasma rennin activity and potassium concentrations), as well as an increased incidence of headaches, nausea, and vomiting were observed. The authors applied a 10-fold safety factor to the no-observed effect level of 2 mg/kg to arrive at an acceptable daily intake of 0.2 mg/kg bw. This value is equivalent to the consumption of 12 mg glycyrrhizinic acid/day for a 60-kg person, or 6 g licorice/day (assuming the licorice contains 0.2% glycyrrhizinic acid). This limit suggested by van Gelderen *et al.* (2000) is below the ADI advised by the Dutch Nutrition Council of 200 mg glycyrrhizinic acid/day.

Thus, there exists a more than 2000-fold margin of safety between this intake and the Dutch Nutrition Council's ADI of 2000 mg/day and a more than 200-fold margin of safety from the ADI reported by van Gelderen *et al.* (2000).

SUMMARY



Thus, there is a more than 2000-fold margin of safety between the maximum glycyrrhizinic acid intake resulting from the consumption of 900 mg LFO and the Dutch Nutrition Council's acceptable daily intake value of 200 mg glycyrrhizinic acid /day from the provisional upper intake limit for regular ingestion of 100 mg/day established by the Scientific Committee on Food. There is more than a 200-fold margin of safety from the acceptable daily intake level of 0.2 mg/kg/bw derived from the no-effect level reported by van Gelderen *et al.* (2000).

CONCLUSION

Based on the clinical evidence summarized herein and the data presented in the prior submission (Appendix 1), Kaneka concludes that the chronic use of LFO in dietary supplements at a level of 900 mg per serving (providing 90 mg licorice ethanol extract per serving, or 15 mg/kg b.w. in a 60 kg person) is reasonably expected to be safe.

REFERENCES

Bernardi, M.; D'Intino, P.E.; Trevisani, F.; Cantelli-Forti, G.; Raggi, M.A.; Turchetto, E.; Gasbarrini, G. 1994. Effects of prolonged ingestion of graded doses of licorice by healthy volunteers. *Life Sciences* 55(11):863-872.

Bijlsma, J.A.; Van Vloten, P.; Van Gelderen, C.E.M.; Mensigna, Tj.T.; Mout, H.A.; Elvers, L.H.; Van Leeuwen, F.X.R.; Stolker, A.A.M.; Van Ginkel, L.A.; Looman, C.W.N.; Van der Maas, P.J.; Koomans, H.A.; Savelkoul, T.J.F. 1996. Onderzoek naar de effect van verschillende doseringen glycyrrhizine bij gezonde vrouwelijke vrijwilligers. [Study into the effects of different dosages of glycyrrhizin in healthy female volunteers; in Dutch]/ RIVM report 348801004, RIVM, Bilthoven, The Netherlands. Cited In: SCF, 2003.

Ploeger, B.A. 2000. Development of the use of a physiologically based pharmacokinetic-pharmacodynamic modal for glycyrrhizinic acid in consumer products. PhD thesis University of Utrecht. Cited In: SCF, 2003.

SCF. 1991. Reports of the Scientific Committee for Food (29th series). Commission of the European Communities, Food Science and Techniques. Report No EUR 14482, EN, CEC, Luxembourg. Cited In: SCF, 2003.

SCF. 2003. Opinion of the Scientific Committee on Food on Glycyrrhizinic Acid and its Ammonium Salt. SCF/CS/ADD/EDUL/225Final. 10 April 2003.

Van Gelderen, C.E.M.; Bijlsma, J.A.; van Dokkum, W.; Savelkoul, T.J.F. 2000. Glycyrrhizic acid: The assessment of a no effect level. *Human and Experimental Toxicology* 19:434-439.

CANTOX

HEALTH SCIENCES INTERNATIONAL

APPENDIX 1:

Revised New Dietary Ingredient Notification for Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil (LFO) at 600 mg/day

-Final-

Submitted by: CANTOX HEALTH SCIENCES
INTERNATIONAL
1011 U.S. Highway 22 West, Suite 200
Bridgewater, New Jersey
08807

On behalf of: Kaneka Corporation
Functional Foods Development Division
3-2-4, Nakanoshima, Kita-Ku
Osaka 530-8288, Japan

April 27, 2005

CANTOX Offices:

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905.542.2900

Reading, Berkshire, UK
+44 (0)118 935 7162

SECTION 1

The name and complete address of the manufacturer or distributor of the dietary supplement that contains a new dietary ingredient, or of the new dietary ingredient.

The manufacturer of the new dietary ingredient is:

Kaneka Corporation
Functional Foods Development Division
3-2-4, Nakanoshima, Kita-Ku
Osaka 530-8288, Japan

Direct correspondence to:

David H. Bechtel, Ph.D., DABT
Senior Scientific Consultant
CANTOX U.S. Inc.
1011 U.S. Highway 22, Suite 200
Bridgewater, NJ 08807
Phone: 908-429-9202
Fax: 908-429-9260

SECTION 2

The name of the new dietary ingredient.

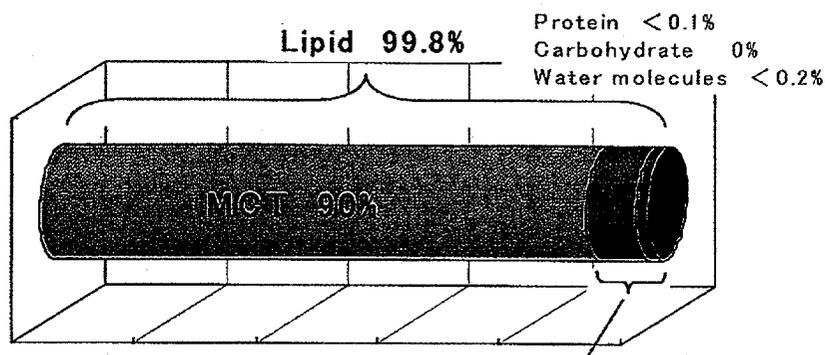
Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil (LFO)*.

* The term LFO appears throughout this NDI notification, and refers to Kaneka Glavonoid Rich Oil™.

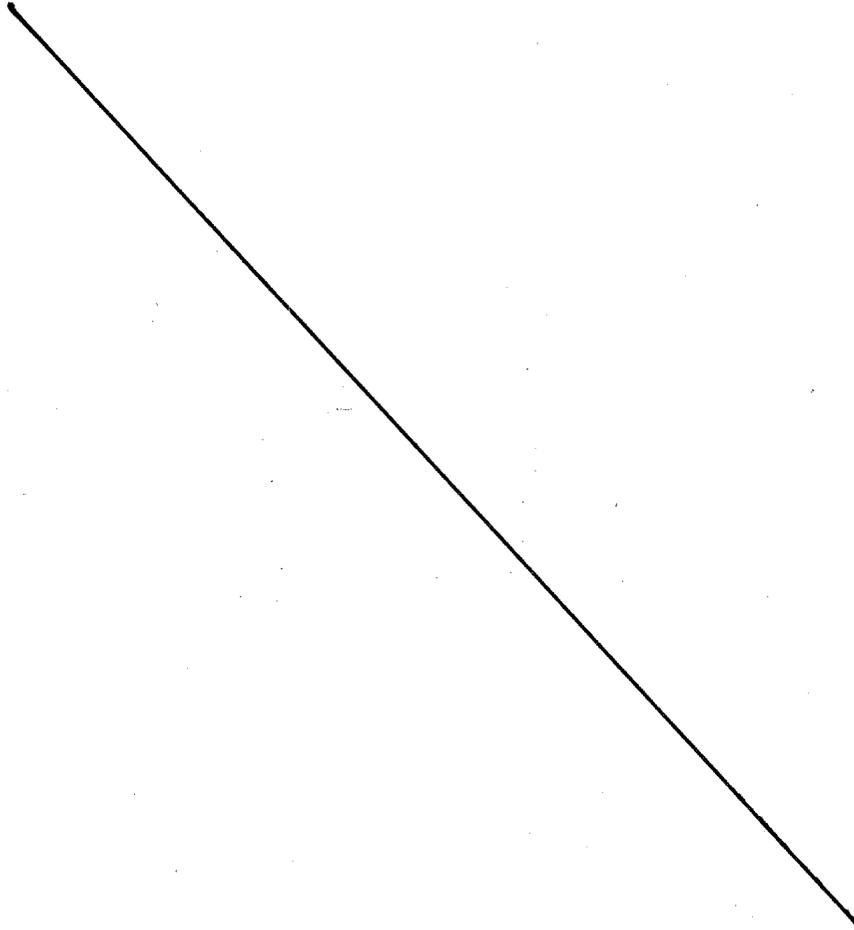
SECTION 3

Description of the dietary supplement or dietary supplements that contain the dietary ingredient including (i) the level of the dietary ingredient in the dietary supplement, and (ii) the conditions of use recommended or suggested in the labeling of the dietary supplement, or if no conditions of use are recommended or suggested in the labeling of the dietary supplement, the ordinary conditions of use of the supplement.

Figure 1 Composition of LFO



* The term LFO appears throughout this NDI notification, and refers to Kaneka Glavonoid Rich Oil™.



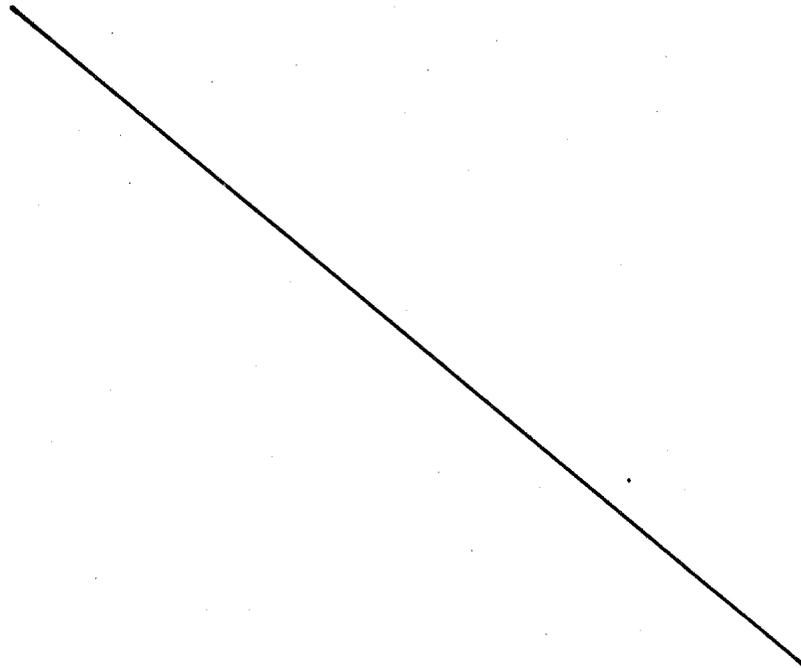
The dietary supplement containing the LFO dietary ingredient will be in capsule form. The LFO capsules will be clearly labeled and promoted as a dietary supplement. A description of the number of capsules per serving size will appear on the label, and each serving of the dietary supplement will contain _____ approximately 300 mg LFO). Consumption of up to 2 servings per day will be suggested or recommended in the label directions, resulting in _____ approximately 600 mg LFO, or 10 mg/kg/day for a 60 kg body weight person).

SECTION 4

The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the basis on which the distributor or manufacturer has concluded that the dietary supplement will reasonably be expected to be safe.

4.1 MANUFACTURE OF LFO

The flow chart shown in Figure 3 illustrates the manufacturing process for LFO*. A more detailed description of the manufacturing process for LFO is provided below.



* The term LFO appears throughout this NDI notification, and refers to Kaneka Glavonoid Rich Oil™.

PAGE 7 THROUGH PAGE 9

3 PAGES TOTAL

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CONTAINS

TRADE SECRET

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INFORMATION

4.2 SAFETY OF LICORICE AND ITS COMPONENTS

Glycyrrhizin and licorice water extract have been used as food and food additives in the United States as well as in Japan, where it has over a 1300-year history of use. The recent annual usage of licorice water extract in Japan was reported to be about 130 metric tons (in 1988). In the United States, licorice root, licorice root extract, and ammoniated glycyrrhizin are direct food substances considered Generally Recognized As Safe (GRAS) in accordance with 21 CFR 184.1408. These regulations allow for the use of these ingredients at a maximum use level, (expressed in terms of percent glycyrrhizin content of food as served, and shown in parenthesis) as a flavor enhancer and flavoring agents in various food categories: (1) Baked foods (0.05%); (2) Alcoholic beverages (0.1%); (3) Nonalcoholic beverages (0.15%); (4) Chewing gum (1.1%); (5) Hard candy (16.0%); (6) Herbs and seasoning (0.15%); (7) Plant protein products (0.15%); (8) Soft candy (3.1%); (9) Vitamin or mineral dietary supplements (0.5%), and; (10) all other foods except sugar substitutes (0.1%). The ingredient is not permitted to be used as a nonnutritive sweetener in sugar substitutes.

Considerable research has been conducted on the biological effects of licorice, its extracts and isolated components; data from these studies suggests that licorice exhibits several physiologic effects, including detoxification, antiulcer, anti-inflammation, anti-viral, antiatherogenic and anticarcinogenic (Wang and Nixon, 2001; Lutomski *et al.*, 1991). In addition to the abundance of data concerning the biological effects of licorice, its extracts and isolated components, a limited number of studies examining the safety of licorice and its components were also identified in the published scientific literature. Based on these reports, it appears the main undesirable adverse effects of licorice stem from its mineralocorticoid activity (Olukoga and Donaldson, 2000). Specifically, 18 β -glycyrrhretinic acid, the active metabolite of glycyrrhizinic acid, inhibits the enzyme 11 β -hydroxysteroid dehydrogenase (11 β -HSD) in the kidney (Shibata, 2000). As such, mineralocorticoid receptors are activated by cortisol, which thus acts as a potent mineralocorticoid; the result is a state of apparent mineralocorticoid excess (AME) (Walker and Edwards, 1994). AME is characterized by hypokalemic alkalosis, water and sodium retention with a tendency to hypertension, kaliuresis and suppression of the renin-angiotensin-aldosterone axis (Epstein *et al.*, 1977).

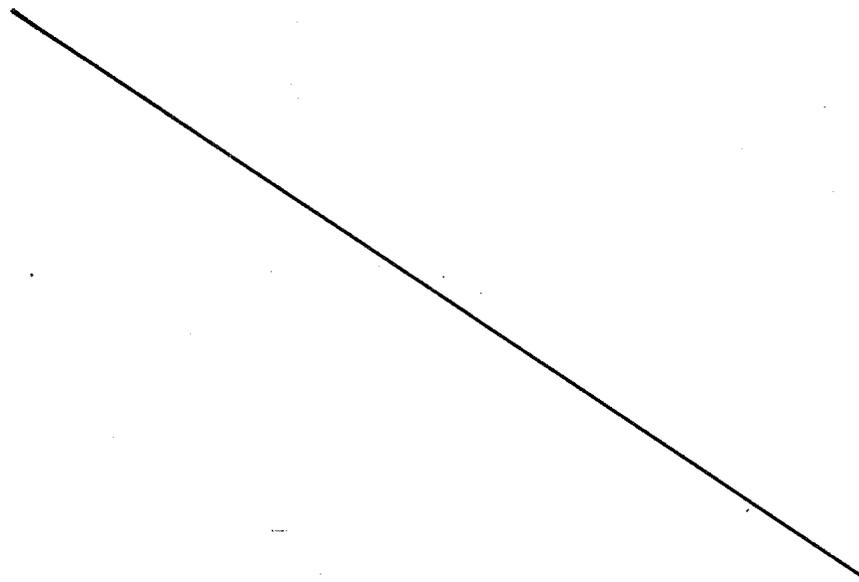
Susceptibility to the mineralocorticoid adverse effects of glycyrrhizinic acid varies greatly among individuals, and also largely depends on the amount and duration of glycyrrhizinic acid intake (Olukoga and Donaldson, 2000). Glycyrrhizinic acid is present in licorice root as the calcium or potassium salt [in which case it is referred to as glycyrrhizin] in concentrations ranging from 1% to 24%, depending upon a variety of factors, including source and botanical origin, agronomic

and environmental conditions and the nature and extent of subsequent processing and storage (Leung, 1980; Anonymous, undated; Lutomski *et al.*, 1991). The highly variable glycyrrhizinic acid content among licorice preparations can result in large variations in glycyrrhizinic acid intake and the susceptibility to its adverse effects.

At this level, the LFO product can be considered as safe even in case of extreme overdosing. The results of clinical safety studies shown in 4.2.3 demonstrated that a daily intake of 10 mg of glycyrrhizin could be derived as a safety dose for most healthy adults. The maximum recommended daily dose of LFO (600 mg) would result in a glycyrrhizin intake of 0.03 mg, assuming a glycyrrhizin content of 0.005%. In this case, the margin of safety is more than 300-fold.

Summaries of studies conducted specifically on Kaneka's LFO product are provided in Section 4.2.1. For the sake of completeness, published preclinical, genotoxicity, and clinical studies of licorice and its components, including glycyrrhizin, are summarized in Sections 4.2.2 and 4.2.3. While these studies are not directly related to the safety of LFO, LFO being virtually devoid of glycyrrhizin, it should be noted that:

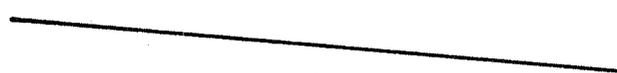
- (1) The safety studies performed by Kaneka in support of LFO involved the administration of experimental animals, rather than the diluted final product (LFO) itself, in order to create a worst-case exposure scenario.
- (2) The results of other published studies, summarized in Sections 4.2.2 and 4.2.3, are included in this notification to show that the main undesirable adverse effects of licorice stem is mineralocorticoid activity due to glycyrrhizinic acid. Most of the published studies detailed in these sections involved the administration of sweets with glycyrrhizin, glycyrrhizinic acid, or glycyrrhetic acid. Therefore, test substances used in the studies are different from "LFO" and "LFO concentrate solution" in terms of composition and manufacturing process, and have no direct relationship to the safety of LFO, which is virtually devoid of glycyrrhizin.
- (3) Clinical studies with deglycyrrhizinated extracts, summarized in Table 18 on page 54 of this notification demonstrate safety consistent with the observed safety of LFO. While specific analytical information related to the glycyrrhizinic acid content of the deglycyrrhizinated licorice test materials in these trails was not provided in these papers or identified elsewhere in the published scientific literature, Larkworthy and Holgate (1975) reported that deglycyrrhizinated licorice contains no more than 3% glycyrrhizinic acid (compared to the glycyrrhizinic acid or glycyrrhizin concentrations naturally present in licorice root, which may be as high as 24%). Thus, the results of these studies are consistent with the observed safety of LFO, which is virtually devoid of glycyrrhizin.



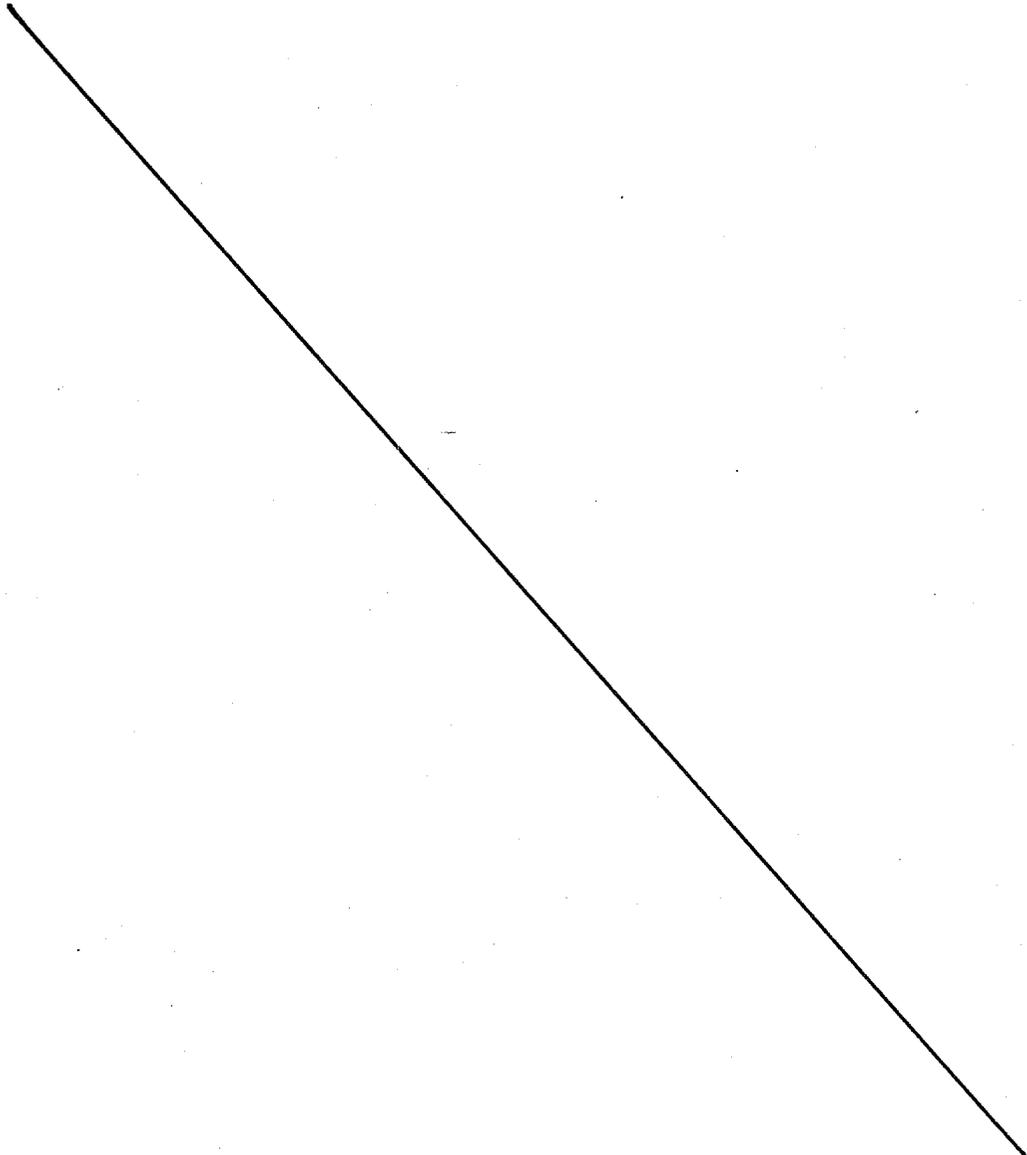
4.2.1 Non-clinical and Genotoxicity Studies Conducted with LFO Concentrate Solution

Nonclinical studies conducted by Kaneka Corporation supporting the safety of LFO* include a 90-day repeated dose toxicity study in rats, various genotoxicity studies (a reverse mutation assay, chromosomal aberration test, bone marrow micronucleus test, and liver micronucleus test), and a rat medium-term liver bioassay for carcinogens. These studies, summarized in Sections 4.2.1.1 through 4.2.1.3, utilized LFO concentrate solution rather than LFO. _____ This was done to create a worst-case exposure scenario.

4.2.1.1 Subchronic Toxicity



* LFO refers to Kaneka Glavonoid Rich Oil™.



* The maximum recommended dose to consumers is 600 mg/day LFO. This is equivalent to 10 mg LFO/kg b.w. for a 60 kg b.w. person. The doses in this rat study are equivalent to 120-, 180-, 240- and 480-times the human dose.

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4.2.2 Additional Non-clinical and Genotoxicity Studies in the Published Scientific Literature

For the sake of completeness, published preclinical and genotoxicity studies of licorice and its components, including glycyrrhizin, are summarized in Sections 4.2.2. While these studies are not directly related to the safety of LFO *per se*, LFO being virtually devoid of glycyrrhizin, it should be noted that these studies show that the main undesirable adverse effects of licorice stem is mineralocorticoid activity due to glycyrrhizinic acid. Most of the published studies detailed in these sections involved the administration of sweets with glycyrrhizin, glycyrrhizic acid, or glycyrrhetic acid. Therefore, test substances used in the studies are different from "LFO" and "LFO concentrate solution" in terms of composition and manufacturing process, and have no direct relationship to the safety of LFO, which is virtually devoid of glycyrrhizin.

4.2.2.1 Acute Toxicity

A number of LD₅₀ (lethal dose, 50 percent kill) values were provided in The Registry of Toxic Effects of Chemical Substances (RTECS) database for glycyrrhiza extract (CASRN 68916-91-6); Table 16 provides a summary of these values.

Table 16 Acute Toxicity Data for Glycyrrhiza Extract

Reference	Species	Route of Administration	LD ₅₀ Dose	Effect
Oyo Yakuri, 1967-	Mouse	i.p.	1,500 mg/kg	Convulsions or effect on seizure threshold, and changes in spleen.
Oyo Yakuri, 1967-	Rat	i.p.	1,420 mg/kg	Hypermotility of gastrointestinal tract and diarrhea. Changes were also observed in the kidney, ureter and bladder.
Oyo Yakuri, 1967-	Mouse	Oral	>7,500 mg/kg	N/R
Oyo Yakuri, 1967-	Rat	Oral	14,200 mg/kg	Hypermotility of gastrointestinal tract and diarrhea. Changes were also observed in the kidney, ureter and bladder.
Oyo Yakuri, 1967-	Mouse	s.c.	4,000 mg/kg	Convulsions or effect on seizure threshold, and changes in spleen.
Oyo Yakuri, 1967-	Rat	s.c.	4,200 mg/kg	Hypermotility of gastrointestinal tract and diarrhea. Changes were also observed in the kidney, ureter and bladder.

i.p. = intraperitoneal; N/R = Not Reported; s.c. = subcutaneous

In addition, Cantelli-Forti *et al.* (1997) conducted a single dose study to examine the pharmacokinetics of glycyrrhizin and its aglycone, glycyrrhetic acid, in bile due to oral administration of either pure glycyrrhizin, or licorice extract (LE) with a glycyrrhizin ammonium salt content of 7.64%, to male Sprague-Dawley rats. The study was prompted by reports indicating that while a number of adverse effects (e.g., cardiac dysfunction, edema, and hypertension) occurred in subjects following the administration of high doses of glycyrrhizin (Stormer *et al.*, 1993), few adverse effects were elicited by the administration of LE, containing glycyrrhizin (Cantelli-Forti *et al.*, 1994; Bernardi *et al.*, 1994). Analysis of rat bile showed significantly lower concentrations of glycyrrhizin in samples from animals treated with LE (6278 mg/kg b.w.) compared to pure glycyrrhizin (480 mg/kg b.w.). A significant choleric effect, which increased the excretion rate of glycyrrhizin, was also observed following both oral and intravenous (i.v.) administration of LE.

A report prepared by the Federation of American Societies for Experimental Biology (FASEB, 1974) entitled, *Evaluation of the Health Aspects of Licorice, Glycyrrhiza and Ammoniated Glycyrrhizin as Food Ingredients*, provided LD₅₀ values for various glycyrrhizin salts administered to mice; oral LD₅₀ values ranged from 1,220 mg/kg for monopotassium glycyrrhizinate (Klosa, 1957) to 12,700 mg/kg for crude ammonium glycyrrhizinate (Fujimura, undated). Intraperitoneal LD₅₀ values ranged from 1,050 mg/kg for crude ammonium glycyrrhizinate (Fujimura, undated) to 1,400 for dipotassium glycyrrhizinate (Fujimura and

Okamoto, undated); LD₅₀ values following the i.v., intramuscular (i.m.) and s.c. administration of monopotassium glycyrrhizinate was 412, 695 and 697 mg/kg, respectively (Klosa, 1957).

4.2.2.2 Subacute Toxicity

Al-Qarawi *et al.* (2002) examined the effects induced by a water freeze-dried extract of *Glycyrrhiza glabra* (licorice) on the pituitary-adrenal-kidney axis in male Wistar rats receiving 100, 250, and 500 mg/kg licorice *via* oral gavage for 15 consecutive days. Analysis of plasma concentrations of cortisol, adrenocorticotrophic hormone (ACTH), renin, aldosterone, sodium (Na) and potassium (K) showed that licorice treatment induced a dose-dependent decrease in cortisol, ACTH, aldosterone and K; in most cases, these decreases were significant when compared to control values. In addition, a concomitant dose-dependent increase in renin and Na concentrations were reported. On this basis the authors suggested that licorice exerts a strong and dose-dependent suppression of the adrenal-pituitary axis, accompanied by stimulation of rennin production from the kidney.

4.2.2.3 Subchronic Toxicity

A number of TDLo (lowest published toxic dose) values were provided in RTECS database for glycyrrhiza extract (CASRN 68916-91-6); Table 17 provides a summary of these values.

Table 17 Multiple Dose Data for Glycyrrhiza Extract

Reference	Species	Route/Duration of Administration	TDLo Dose	Effect
Food and Chemical Toxicology, 1982-	Mouse	Oral/90-day, continuous	2,700 mg/kg	Weight loss or decreased weight gain and changes in liver weight reported.
Oyo Yakuri, 1967-	Rat	Oral/30-day, continuous	37.5 mg/kg	Changes in liver and bladder weights reported. Changes in serum composition (e.g., bilirubin and cholesterol) also reported.
Oyo Yakuri, 1967-	Rat	Oral/13-week, continuous	114 mg/kg	Changes in liver, bladder and thymus weights reported.

Several additional studies examining the subchronic toxicity associated with licorice and its components were identified in the published scientific literature; brief summaries of these studies are provided below.

A 90-day feeding study was conducted by Mirsalis *et al.* (1993) to examine the effects of licorice root extract (LRE; 0.8, 2.5, 8 or 25%) on the activity of several phase I and phase II detoxification enzymes in male B6C3F₁ mice; the effects of the extract on clinical signs, weight gain, and survival were likewise reported. With the exception of mice in the lowest dose group, poor weight gain was observed in all animals receiving LRE. Significant increases in liver:body weight ratios were also noted in LRE groups. Treatment-related gross autopsy findings included, lesions of the kidney, liver, spleen and thymus. With respect to phase I and phase II detoxification enzymes, LRE induced a modest increase in UDP-glucuronyl transferase (UDPGT) activity, and a decrease in glutathione S-transferase (GST) activity. A significant increase in 7-ethoxycoumarin O-deethylase (7-ECOD) activity was also reported in LRE groups. In contrast, no treatment-related effects were observed on benzo[a]pyrene hydroxylase (BPH) and superoxide dismutase (SOD) activities. On this basis the authors concluded that LRE may alter the activities of several enzymes involved in the detoxification of chemical carcinogens (Mirsalis *et al.*, 1993).

Webb *et al.* (1992) conducted a study to examine the effect of dietary licorice root extract (0.38, 1.5 or 3.0% (wt/wt) on the levels of a series of enzymes indicative of increased or decreased risk for carcinogenesis in male Fischer (F344) rats; the authors also evaluated *in vivo* toxicity associated with dietary licorice root extract, after 1 and 3-months of feeding, by histopathological examination of a standard set of rodent tissues. Results showed that liver glutathione transferase, catalase, and protein kinase C, were significantly induced (up to 50%) by the administration of increasing doses of licorice extract. Only non-significant strain-related changes were noted upon histopathological evaluations of organs and tissues from animals treated with licorice root extract. Similarly, no licorice-related anatomic lesions or hematological changes were observed.

Wang *et al.* (1992) reported that no treatment-related effects on body weight, behavior or appearance were observed in A/J mice following the oral administration of a 1% water extract of licorice (LWE) for 42 weeks.

As part of its evaluation of licorice, glycyrrhiza and ammoniated glycyrrhizin for use as food ingredients, the FASEB (1974) reviewed several studies (Girerd *et al.*, 1958; Macabies *et al.*, 1963; Gordon 1974; Fujimura and Okamoto, undated) examining the subchronic toxicity of licorice (10 g/kg/day), glycyrrhizin (160 mg/day), glycyrrhizin salts (100 to 2000 mg/kg), glycyrrhetic acid [α and β isomers (300 mg/kg)], and deglycyrrhizinated licorice extract [extract containing 3 to 4% glycyrrhizin, as compared to 20 to 25% in the original extract (800 mg/kg)] following oral administration to rats. Upon review on these studies, which ranged in duration from 50 days to approximately 24 weeks, it was the opinion of the Select Committee of the FASEB that licorice and licorice derivatives possess a low order of toxicity. Furthermore, the Select Committee concluded that although the test materials were capable of eliciting a variety of physiologic effects (e.g., hypertensive and deoxycorticosteromimetic effects), these were observed only at levels considerably higher than are likely to be achieved in usual diets.

Miller *et al.* (1981) examined the subchronic effects of ammoniated glycyrrhizin when added with or without salt, to the diet of miniature swine. The results showed a decrease in weight gain in animals treated with ammoniated glycyrrhizin alone, or in combination with salt. In addition, administration of either ammoniated glycyrrhizin or salt alone elicited an increase in water consumption; polydipsia was observed when the test materials were administered in combination. A significant decrease in serum potassium was noted in animals treated with ammoniated glycyrrhizin alone, and a pronounced decline in serum potassium levels (approximately 50% of the control level) was observed upon administration of the combination. A rise in systolic and diastolic blood pressure was noted in animals receiving ammoniated glycyrrhizin alone; the effect was potentiated by simultaneous administration of salt. Administration of ammoniated glycyrrhizin in combination with salt was also associated with muscular weakness.

4.2.2.4 Reproductive and Developmental Toxicity

Wang and Nixon (2001) reviewed the available literature pertaining to licorice and its derivatives and reported that in 1 study (Mantovani *et al.*, 1988) the administration of glycyrrhetic acid (21.3 to 679.9 mg/kg b.w./day) in drinking water to female rats on gestation Days 7 to 17, resulted in a dose-related increase in embryo lethality and minor anomalies, especially in the sternebral variants. Renal ectopy increased significantly at the highest dose. Based on these findings, Wang and Nixon (2001) suggested that the possible embryotoxicity of aromatizing compounds should be considered.

In addition, as part of its evaluation of licorice, glycyrrhiza and ammoniated glycyrrhizin for use as food ingredients, the FASEB (1974) reviewed several studies (Food and Drug Research

Laboratories, Inc., 1972) examining the teratogenicity of ammonium glycyrrhizinate following administration to rats, mice, hamsters and rabbits, *via* oral intubation. It was concluded that ammonium glycyrrhizinate, at doses up to 1000 mg/kg, had no teratological effect, and it did not unfavorably influence maternal or fetal survival.

4.2.2.5 Neurotoxicity

Neurobehavioral profiles, including physiological parameters (blood pressure, heart rate, temperature) and indices of cognitive (conditioned avoidance, passive avoidance, fixed interval responding) and motor (exploration, general motility, rotorod activity) functions, were evaluated in male Sprague-Dawley rats exposed to dietary ammoniated glycyrrhizin (0, 2, 3 or 4%, resulting in effective dose levels of 0, 1.2, 1.9 and 2.6 g/kg/day) for 4 to 6 months (Sobotka *et al.*, 1981). Ammoniated glycyrrhizin-treated rats exhibited hypertension, bradycardia, polydipsia, increased relative weights of kidney and heart, and a slight decrease in body weight and growth. With respect to behavioral profiles, a specific effect on the conditioned avoidance response was observed; avoidance was facilitated at the 4% level, unaffected at the 3% level, and slightly depressed at the 2% level. No other changes in behavior were noted.

4.2.2.6 Genotoxicity

Three samples of licorice (2 alcoholic extracts and 1 tincture), at concentrations up to 100 μ L/plate, were reportedly non-mutagenic in the Ames assay with *Salmonella typhimurium* strains TA98 and TA100, both in the presence and absence of metabolic activation (Crebelli *et al.*, 1990).

Licorice powder, ammoniated glycyrrhizinate and monoammonium glycyrrhizin, at concentrations of 0.01 to 0.5 mg/mL, were non-mutagenic in the Ames assay with *S. typhimurium* strains TA97, TA98 and TA100, both in the presence and absence of metabolic activation (Cooper and Berry, 1988). Results of a subsequent experiment also showed that the test materials reduced the number of revertant colonies per plate with each *S. typhimurium* strain, compared to the mutagen (sodium azide, 2-aminofluorene or ICR-191) only plate counts. On this basis, the authors concluded that licorice powder, ammoniated glycyrrhizinate and monoammonium glycyrrhizin were antimutagenic, rather than mutagenic, in an *in vitro* assay system (Cooper and Berry, 1988).

Morimoto *et al.* (1982) examined the mutagenicity of 104 commercial crude drugs, including *Glycyrrhizae radix* (*Glycyrrhiza uralensis* Fischer), in a rec-assay with *Bacillus subtilis*; water and methanol extracts of *Glycyrrhizae radix* (100 g/L) reportedly both produced positive results in the absence of metabolic activation. The authors also examined the mutagenicity of *Glycyrrhizae radix* in a reversion assay with *S. typhimurium* strains TA98 and TA100; no mutagenicity was observed for water or methanol extracts either in the presence or absence of metabolic activation.

4.2.3 Clinical Safety

Clinical studies of licorice and its components, including glycyrrhizin, glycyrrhizic acid, and or glycyrrhetic acid, are summarized below. While these studies are not related to the safety of LFO *per se*, they are included here for the sake of completeness and because studies with deglycyrrhizinated extracts (which reportedly contain no more than 3% glycyrrhizic acid) show safety consistent with that of LFO, which is virtually devoid of glycyrrhizin.

In humans, prolonged ingestion of licorice and/or its active metabolites has been associated with an acquired form of apparent mineralocorticoid excess syndrome, characterized by sodium retention, potassium loss, elevated blood pressure, edema and suppression of the rennin-angiotensin-aldosterone system. In some cases, hypokalemia can be so severe as to induce myopathy (Revers, 1948; Molhuysen *et al.*, 1950; Ishikawa *et al.*, 1985; Shintani *et al.*, 1992; Stormer *et al.*, 1993; Wang and Nixon, 2001). Such mineralocorticoid effects have been attributed to glycyrrhetic acid, the active metabolite of glycyrrhizic acid resulting from glycyrrhizin de-glucuronation in the gastrointestinal (GI) tract (Stormer *et al.*, 1993; Sakiya *et al.*, 1979). Although the effects were once believed to be mediated by direct binding of glycyrrhetic acid to the mineralocorticoid receptor, it has since been demonstrated that glycyrrhetic acid inhibits the oxidation of cortisol *via* inhibition of the enzyme 11 β -hydroxysteroid-dehydrogenase (Walker and Edwards, 1991; Stormer *et al.*, 1993). Specifically, glycyrrhetic acid competitively binds the enzyme 11 β -dehydrogenase, which complexes with 11-oxoreductase to form 11 β -hydroxysteroid dehydrogenase (11 β -HSD). This complex is responsible for the interconversion of cortisol and cortisone, and as a result of its inhibition, cortisol is not degraded and thus, may exert its mineralocorticoid action in mineralocorticoid-selective tissues (*e.g.*, kidney, colon, and parotid gland) (Edwards *et al.*, 1988).

Due to the inhibition of cortisol oxidation, the mineralocorticoid receptors in the distal nephron, which are normally protected from cortisol by 11 β -HSD, are activated. Cortisol mimics aldosterone, stimulating the resorption of sodium from renal tubules and the secretion of potassium into the urine, causing a state of apparent mineralocorticoid excess. The increased sodium resorption depresses the renin-angiotensin-aldosterone axis, and as a reaction to increases in atrial stretch caused by fluid retention, the serum concentration of atrial natriuretic peptide (ANP) increases (van Gelderen *et al.*, 2000). Occurrence of arterial hypertension and edema indicate that the compensatory mechanisms counteracting the glycyrrhetic acid-induced sodium retention are overwhelmed. Recovery of the rennin-angiotensin system after discontinuation of licorice is delayed, due at least in part, to the slow clearance of the drug, and hence continued inhibition of 11 β -hydroxysteroid dehydrogenase (Schambelan, 1994).

In addition to the classical symptoms of hypertension, hypokalemia, and suppression of the rennin-aldosterone system, hypertensive encephalopathy, has been associated with the regular daily intake of low doses of licorice (Russo *et al.*, 2000). Glycyrrhizin and glycyrrhizic acid have

also reportedly reduced serum alanine transaminase and aspartate transaminase values, and in men, serum testosterone levels were reportedly reduced due to inhibition of 17 β -hydroxysteroid dehydrogenase and 17,20-lyase (Armanini *et al.*, 1999; Shibata, 2000). In general, it has been suggested that females are more sensitive to the effects of glycyrrhizic acid than males (Bernardi *et al.*, 1994; van Gelderen *et al.*, 2000).

It is not possible based on data currently available, to determine the minimum level of glycyrrhizin required to produce the described symptoms. This uncertainty stems, at least in part, from the grouping of different and often unspecified confectionary products containing glycyrrhizin as a sweetener and flavoring agent under the name licorice. In addition, the nature of the food containing glycyrrhizin may reportedly influence the likelihood of inducing mineralocorticoid-like untoward effects. Furthermore, Stormer *et al.* (1993) reported great individual variation in susceptibility to the effects of glycyrrhetic acid; in the most sensitive individuals, adverse effects may occur with regular daily intakes of no more than approximately 100 mg glycyrrhizin, corresponding to 50 g of licorice sweets (assuming a content of 0.2% glycyrrhizin). Despite these uncertainties, Stormer *et al.* (1993) suggested that by applying a 10-fold safety factor to the lowest observed adverse effect level (LOAEL) of 100 mg glycyrrhizin/day, a daily intake of 10 mg of glycyrrhizin could be derived as a safe dose for most healthy adults.

In this case, the margin of safety is more than 300-fold.

Similarly, based on observations from a clinical trial involving the administration of 0, 1, 2, or 4 mg glycyrrhizic acid/kg b.w. for 8 weeks to healthy subjects, Van Gelderen *et al.* (2000) proposed a no effect level of 2 mg/kg. At intakes above this value, classic symptoms associated with 11 β -HSD inhibition (*i.e.*, reductions in serum aldosterone and ANP concentrations, and plasma rennin activity and potassium concentrations), as well as an increased incidence of headaches, nausea, and vomiting were observed. The authors also applied a 10-fold safety factor to the no-observed effect level of 2 mg/kg to arrive at an acceptable daily intake of 0.2 mg/kg b.w. This value is equivalent to the consumption of 12 mg glycyrrhizic acid/day for a 60-kg person, or 6 g licorice/day (assuming the licorice contains 0.2% glycyrrhizic acid).

According to the RTECS database, the lowest published toxic dose for glycyrrhiza extract (CAS# 68916-91-6) is 79.9 mg/kg following 8 weeks (intermittent) of treatment; proteinuria was reportedly observed at this dose. In addition, Leung and Foster (1996) recommended that consumption of licorice extract be limited to a period of 4 to 6 weeks, due to potential adverse effects; the authors also noted that licorice extract use is contraindicated in cases of cholestatic liver disorders, cirrhosis, hypertonia, and pregnancy. Likewise, the potential for drug interactions reportedly exists between licorice and thiazide diuretics and digitalis glycosides.

Adverse effects observed in clinical studies conducted with licorice or its components are summarized in Table 18; most of these studies involved the administration of sweets with glycyrrhizin, glycyrrhizic acid, or glycyrrhetic acid. However, several studies were also conducted with deglycyrrhizinated licorice and showed that the test material generally had no adverse effects. While specific analytical information related to the glycyrrhizic acid content of the deglycyrrhizinated licorice test materials in these trials was not provided in these papers or identified elsewhere in the published scientific literature, Larkworthy and Holgate (1975) reported that deglycyrrhizinated licorice contains no more than 3% glycyrrhizic acid (compared to the glycyrrhizic acid or glycyrrhizin concentrations naturally present in licorice root, which may be as high as 24%). Thus, the results of these studies are consistent with the observed safety of LFO, which is virtually devoid of glycyrrhizin.

Table 18 Summary of Clinical Studies Conducted with Licorice and its Components

Reference	Test Material	Dose/Duration	Subjects	Results/Adverse Effects
Bernardi <i>et al.</i> (1994)	Glycyrrhizin (as licorice pills containing dried, aqueous extract of licorice root)	108, 217, 380, or 814 mg of glycyrrhizin daily for 4 weeks	6/dose	No significant adverse effects were noted at the 2 lowest dose levels. One subject at the 380 mg/day dose level experienced headache, leading to withdrawal from the study. Two subjects of the high-dose group (1 male with a family history of hypertension, and 1 female taking oral contraceptives) also withdrew from the study due to arterial hypertension, hypokalemia and peripheral edema. Adverse effects subsided within 24 to 48 hours following suspension of the protocol. A transient reduction in kalemia and an increase in body weight were also observed in high dose subjects after 1 and 2 weeks, respectively. A depression of plasma rennin activity occurred in the 380 and 814 mg/day dose groups. On this basis, the authors concluded that in healthy subjects, only the highest doses of licorice led to untoward effects, and these were favored by subclinical disease or oral contraceptives use. The authors also noted that the effects were less common and pronounced than what has been reported after intake of glycyrrhizin through its use as a flavoring agent in confectionary products.
Forslund <i>et al.</i> (1989)	Glycyrrhizin (as licorice candies)	700 mg of glycyrrhizin for 4 weeks	15 volunteers	Statistically significant changes in body weight, serum potassium and sodium concentrations, and arterial blood pressure were observed.
Epstein <i>et al.</i> (1977)	Glycyrrhizin (as licorice candies)	700 or 1400 mg of glycyrrhizin for 1 to 4 weeks	14 healthy volunteers	Plasma potassium levels fell by more than 0.3 mmol/L in 11 of the 14 subjects, including 4 who were eventually withdrawn from the study because of hypokalemia. Mild, transient generalized edema was noted in 4 subjects; two other subjects were withdrawn from the study due to uncomfortable edema of the face, hands, and ankles. Headaches and lethargy were reported in 3 and 4 subjects, respectively. Although blood pressure was not significantly affected, 10 people experienced a weight gain greater than 1 kg. Plasma sodium levels tended to rise during licorice ingestion, and after licorice withdrawal, a sodium diureis was observed in most subjects; these changes did not reach statistical significance. One or more components of the rennin-angiotensin-aldosterone system, in particular plasma rennin activity and urinary aldosterone concentrations, were considerably depressed in all subjects.

Table 18 Summary of Clinical Studies Conducted with Licorice and its Components (cont'd)

Reference	Test Material	Dose/Duration	Subjects	Results/Adverse Effects
Van Gelderen <i>et al.</i> (2000)	Glycyrrhizic acid (GA)	400 or 800 mg of GA/day for 4 weeks (Pilot study)	8/dose (4 males, 4 females)	Several volunteers (1 male and 2 females of the 800 mg/day dose group and 1 female of the 400 mg/day group) were withdrawn from the study due to edema, headache, and general discomfort. In total, 9 volunteers experienced edema after 4 to 7 days of ingestion. Serum potassium concentration decreased in all volunteers, especially in females. The aldosterone concentration also showed a considerable decrease, as did plasma rennin activity. The effects were reported to be more marked in women. Sensitivity to GA differed among individuals, and the dose of GA appeared to be of no influence on the severity of the symptoms in subjects.
	GA	0, 1, 2, or 4 mg GA/kg b.w. for 8 weeks	39 healthy female volunteers	One subject in the 2 mg/kg group was withdrawn from the study after 2 weeks because of a reduced plasma potassium concentration. One subject at the 4 mg/kg dose level was withdrawn after 6 weeks of ingestion because of concentration difficulties and general discomfort. A slight increase in blood pressure and body weight were also noted in this subject. Significant reductions in serum aldosterone concentrations and plasma rennin activity were seen at the 4 mg/kg dose level as compared to controls during the intake period; ANP concentration decreased significantly after discontinuation of GA. Blood pressure was increased relative to controls in the 2 and 4 mg/kg groups, however, this was due to a slight decrease in the control group rather than to changes in the experimental groups. No changes in body weight were observed. Plasma potassium concentrations were significantly reduced at the 4 mg/kg dose level as compared to controls in weeks 2 to 4 of ingestion; however, after reaching a minimum, potassium levels gradually increased to baseline values during the experiment. Dose-related increases in headaches, nausea, and vomiting were observed, with a significant difference between high-dose and control groups. A change in defecation pattern, a swollen face, and tickling in the arms and legs were also reported; the incidence of these effects differed in the 4 mg/kg compared to all other groups, however, there was no clear dose-effect relationship. The overall number of complaints decreased during the study in all dose groups.
Stewart <i>et al.</i> (1987)	Licorice sweet	580 mg of glycyrrhizin/day for 10 days	7 healthy subjects	Biochemical disorders, sodium retention/potassium loss, and effects on corticoid/aldosterone levels, and rennin-angiotensin levels were reported.
MacKenzie <i>et al.</i> (1990)	Glycyrrhizic acid	0.5 g glycyrrhetic acid/day for 3 to 10 days	10 healthy volunteers	All subjects showed symptoms of pronounced mineralocorticoid effects and changes in cortisol metabolism.
Smorenberg-Schoorl and Vree (1963)	<i>Succus Liquiritiae</i>	6 g of <i>Succus Liquiritiae</i> /day (1.56 g GA/day) (duration not specified)	17 healthy volunteers	All 17 subjects developed symptoms such as fluid retention and increased blood pressure. Thirteen subjects ceased test material intake after 1 week. Six of the volunteers were tested with half the dose, and less pronounced symptoms were observed. One of the most sensitive individuals experienced fluid retention after a daily dose of only 0.39 g GA, however, a dose of 0.13 g/day did not induce this symptom. The authors reported however, that this latter dose elicited a rise in blood pressure when taken over a long period.

Table 18 Summary of Clinical Studies Conducted with Licorice and its Components (cont'd)

Reference	Test Material	Dose/Duration	Subjects	Results/Adverse Effects
Anonymous, 1971	GA-reduced licorice	760 mg of GA-reduced licorice or placebo, 3 times daily for 6 weeks	90 men with relapse or chronic duodenal ulcer	No significant changes in weight, blood pressure, or serum electrolytes were observed.
Bardhan <i>et al.</i> (1978)	Deglycyrrhizinated licorice (DGL)	5 g/day deglycyrrhizinated licorice or placebo, for 28 days	96 patients with gastric ulcer	One patient reportedly experienced edema and hypokalemia while taking DGL, however, weight gain was minimal. With this exception, no abnormality occurred in any of the biochemical and hematological indices measured; in particular, hypokalemia was not recorded.
Hollanders <i>et al.</i> (1978)	Deglycyrrhizinated licorice	2.25 g/day deglycyrrhizinated licorice for at least 2 years	41 patients with benign chronic gastric ulceration	No clinical, biochemical, or hematological abnormalities were detected during treatment, and no evidence of long-term toxicity was found.
Larkworthy and Holgate, 1975	Deglycyrrhizinated licorice	3.8 g/day of deglycyrrhizinated licorice (variable duration)	32 patients with chronic duodenal ulcer	It was not stated whether subjects were monitored for adverse effects.
Fuhrman <i>et al.</i> , 2002	Licorice ethanolic root extract (GA-free)	0.1 g/day for 1 month followed by placebo for 1 month	12 hypercholesterolemic patients	Blood chemistry analysis showed no significant changes in markers for liver, kidney, or heart functions as measured by serum blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, bilirubin, and creatine phosphokinase. Serum electrolytes, including potassium and sodium, and serum alkaline phosphatase were not significantly affected in the licorice or placebo groups. A small (7%) but significant reduction in serum glucose, as well as a 10% reduction in serum amylase concentrations, was observed after licorice consumption; levels returned to baseline after the 1-month placebo treatment.
Serra <i>et al.</i> (2002)	GA	1 g/day of GA or placebo for 2 weeks	7 patients with anuria on chronic hemodialysis	The ratio of plasma cortisol/cortisone was increased in all patients after GA intake, indicative of 11 β -HSD inhibition. This increase was paralleled by a decline in the plasma potassium concentration in every patient. GA did not influence other plasma and hematology parameters. No changes in 24-hour blood pressure values or body weights were noted.

SUMMARY

The information presented herein shows that:

- (i) The chemical composition of LFO is well characterized, and the manufacturing process yields a product demonstrated to reproducibly meet compositional specifications.
- (ii) Nonclinical studies [*i.e.*, 90-day repeated dose toxicity, genotoxicity (reverse mutation, chromosomal aberration, bone marrow and liver micronucleus fraction), rat medium-term liver bioassay for carcinogens] were conducted using LFO concentrate solution _____ rather than LFO _____ the diluted final product, in order to administer a higher dose to animals and create a worst-case exposure scenario.
- (iii) Based on the results of the subchronic toxicity study, _____

_____ the maximum recommended dose to consumers (600 mg/day LFO, equivalent to 10 mg LFO/kg b.w. for a 60 kg b.w. person) provides support for the safety of Kaneka Glavonoid Rich Oil™ brand of LFO.
- (iv) _____

- (v) Reports available in the published scientific literature suggest that the main undesirable adverse effects of licorice stem from its mineralocorticoid activity. Specifically, 18β-glycyrrhretinic acid, the active metabolite of glycyrrhizinic acid, inhibits the enzyme 11β-HSD in the kidney. While these studies have no direct relationship to the safety of LFO, which is virtually devoid of glycyrrhizin, the results of these studies demonstrated that a daily intake of 10mg of glycyrrhizin could be derived as a safety dose for most healthy adults. _____

_____ In this case, the margin of safety is more than 300-fold.

- (vi) Studies conducted with deglycyrrhizinated licorice showed that the test material generally had no adverse effects. Deglycyrrhizinated licorice contains no more than 3% glycyrrhizinic acid. _____

- _____
- (vii) Small quantities of LFO will be consumed as dietary supplements.

CONCLUSION

Based on the evidence provided above, including results of preclinical safety studies conducted on LFO concentrate solution, the presence of a safety factor 120- to 180-fold that exists between the NOAELs from the 90-day repeated dose toxicity study and the maximum recommended dose to consumers (600 mg/day LFO, equivalent to 10 mg LFO/kg b.w. for a 60 kg b.w. person) and clinical studies of deglycyrrhizinated licorice, which like LFO, is virtually devoid of glycyrrhizin and generally had no adverse effects, Kaneka's concludes that the chronic use of LFO in dietary supplements at a level of 600 mg per serving (providing 60 mg licorice ethanol extract per serving) will be reasonably expected to be safe.

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