

CANTOX

HEALTH SCIENCES INTERNATIONAL

Revised New Dietary Ingredient Notification for Kaneka Glavonoid Rich Oil™ brand of Licorice Flavonoid Oil (LFO)

-Final-

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

April 27, 2005

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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
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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™.

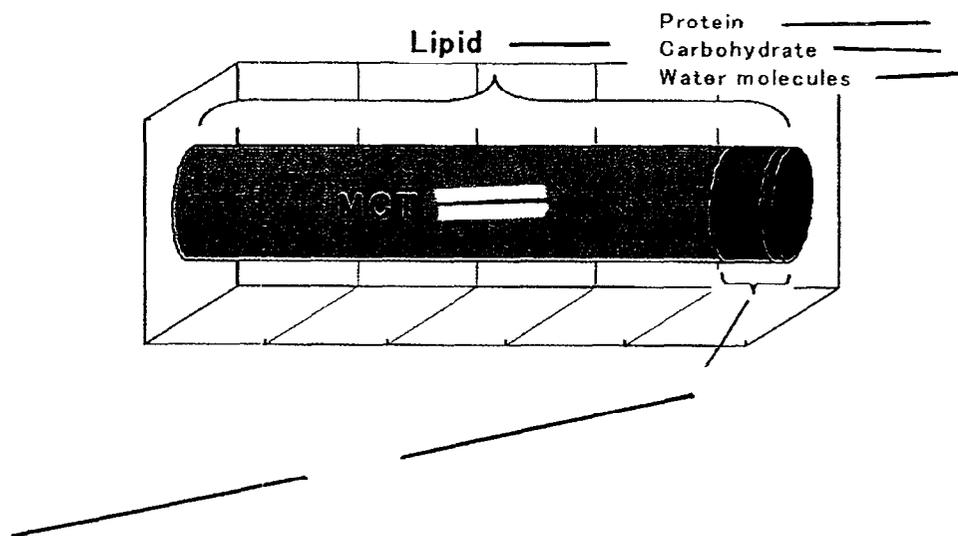
SECTION 3

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.

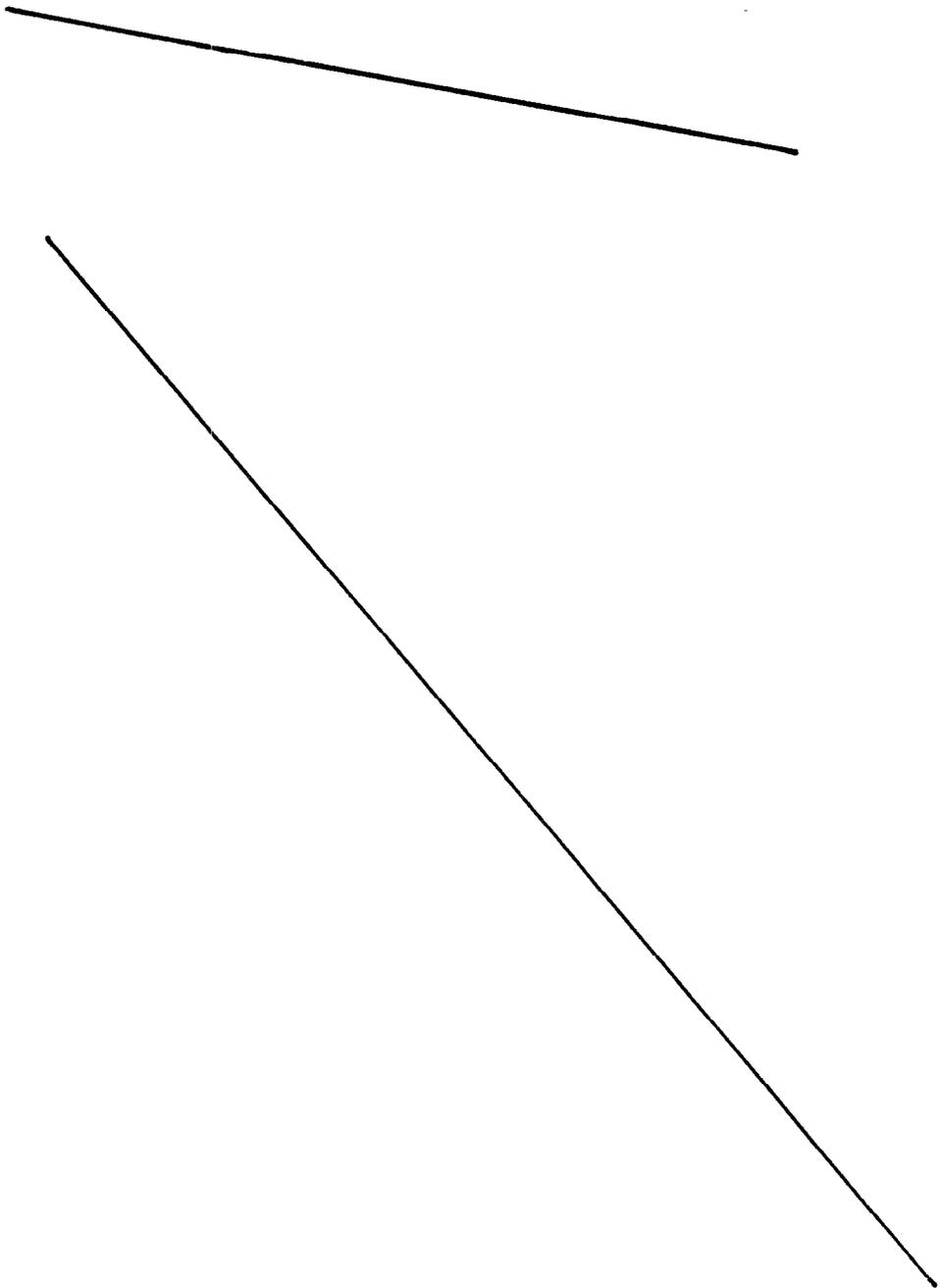
As shown in Figure 1, LFO*, a dark-brown colored oil derived from the root of *Glycyrrhiza glabra* L,



Figure 1 Composition of LFO



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



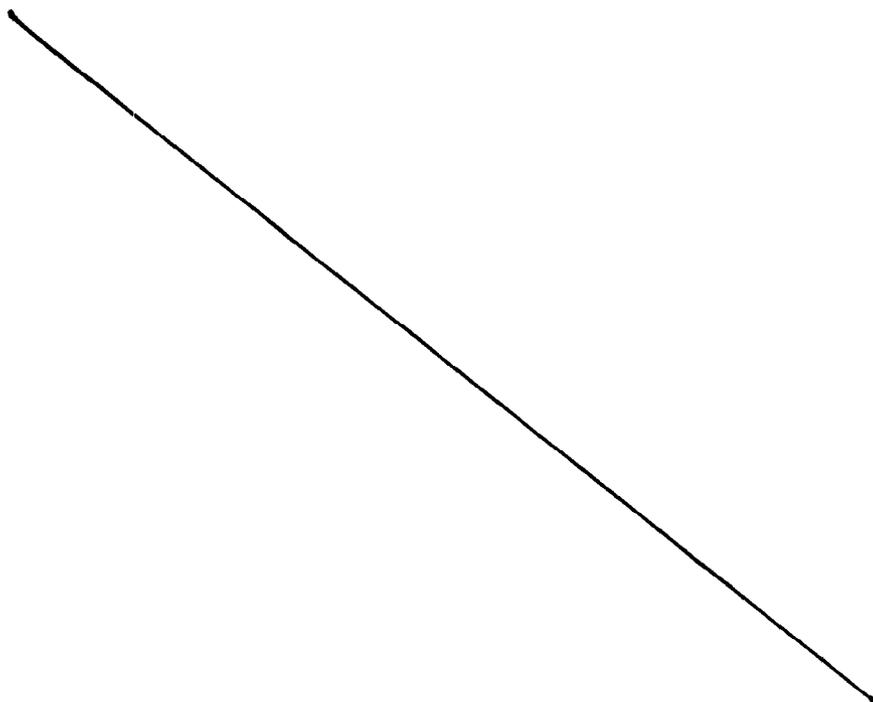
The dietary supplement containing the LFO dietary ingredient will be in capsule form. The LFO capsules will be clearly labeled and promoted as a dietary supplement. A description of the number of capsules per serving size will appear on the label, and each serving of the dietary supplement will contain _____ 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

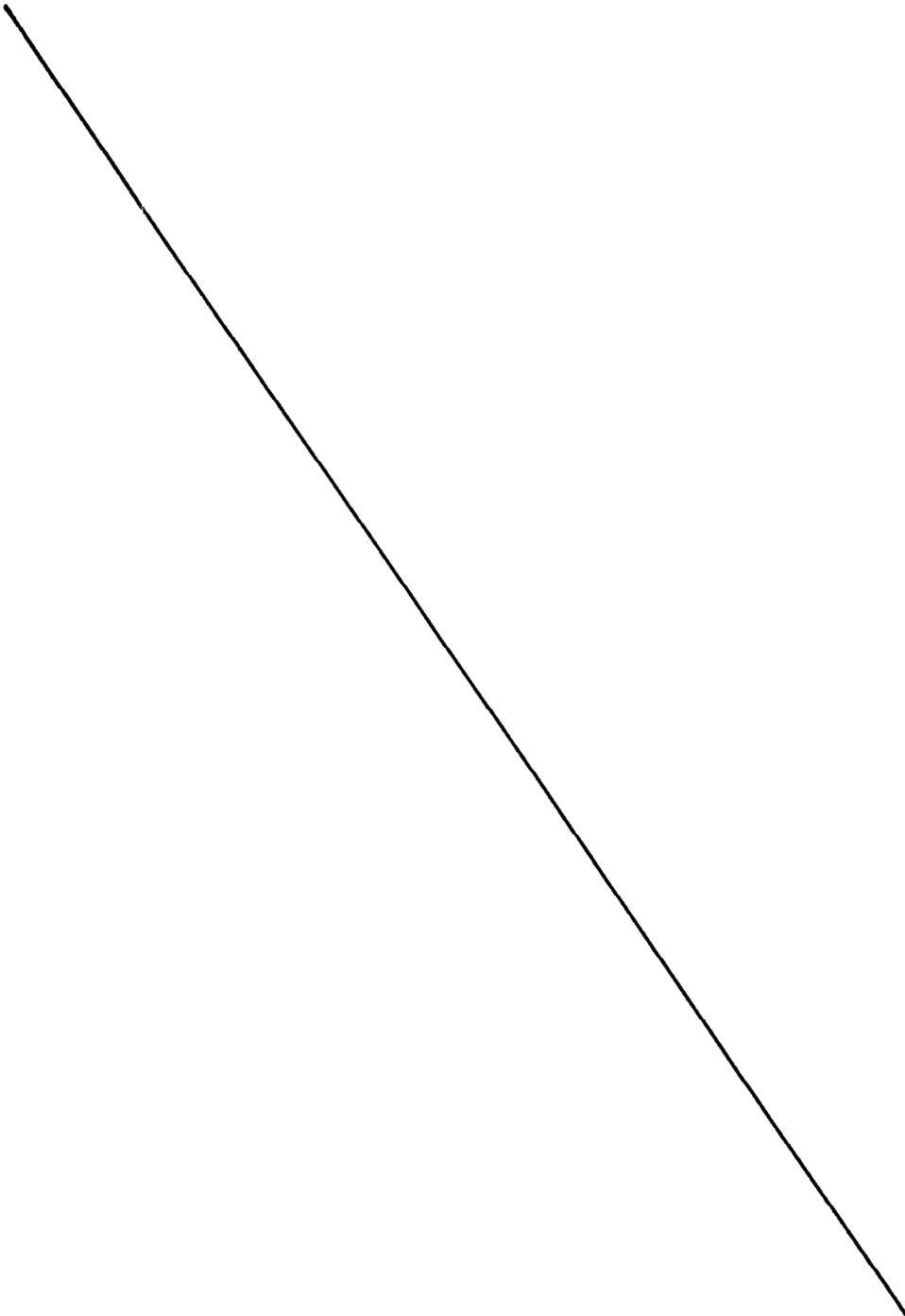
The history of use or other evidence of safety establishing that the dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe, including any citation to published articles or other evidence that is the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

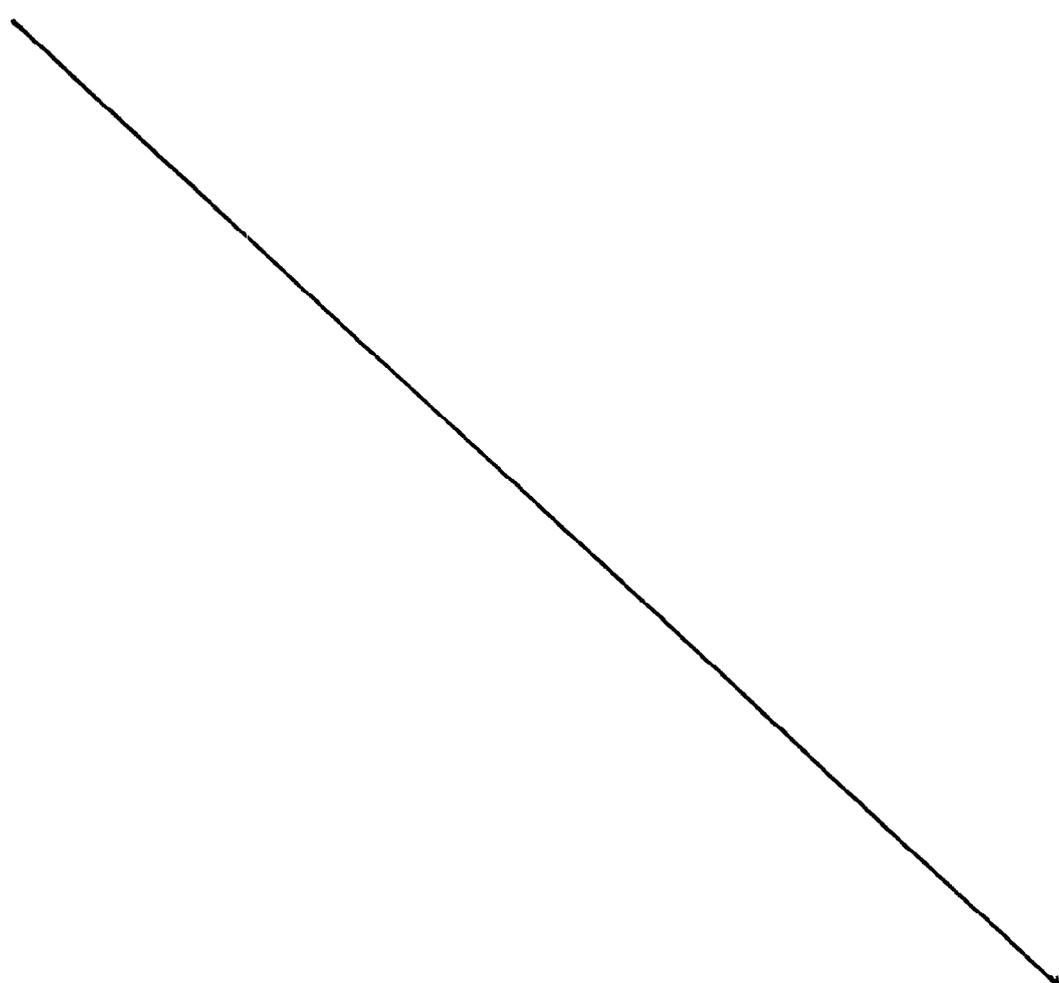
4.1 MANUFACTURE OF LFO

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.



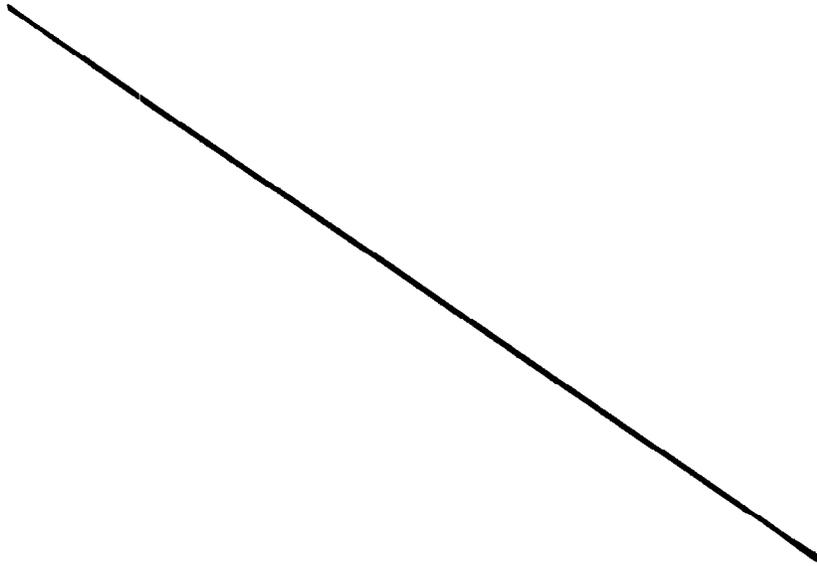
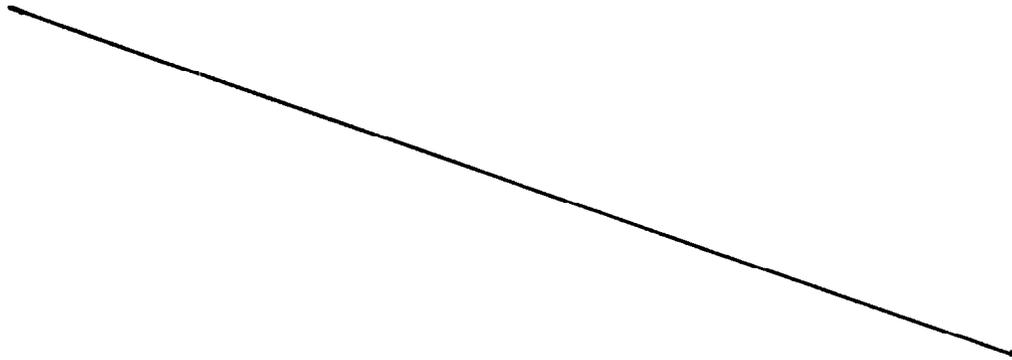
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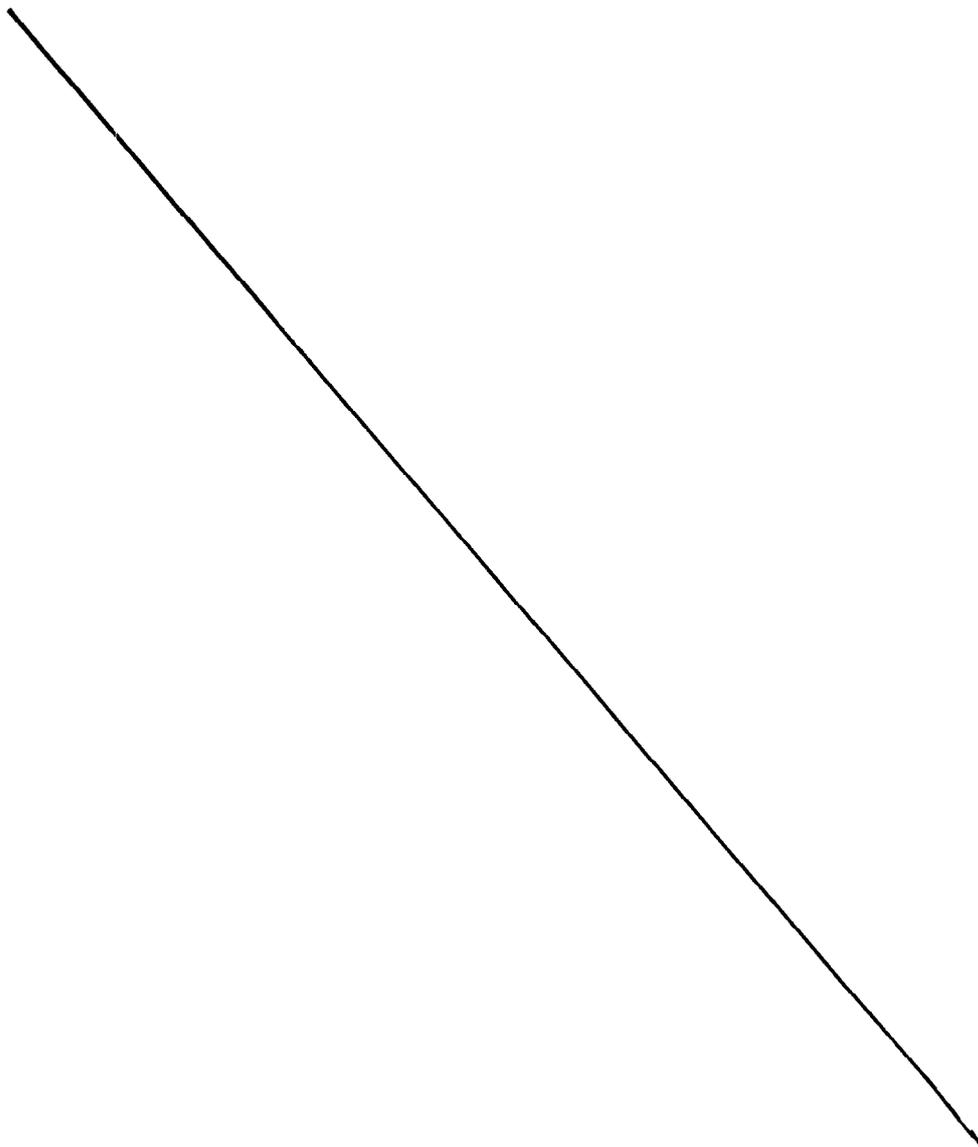
4.2 SAFETY OF LICORICE AND ITS COMPONENTS

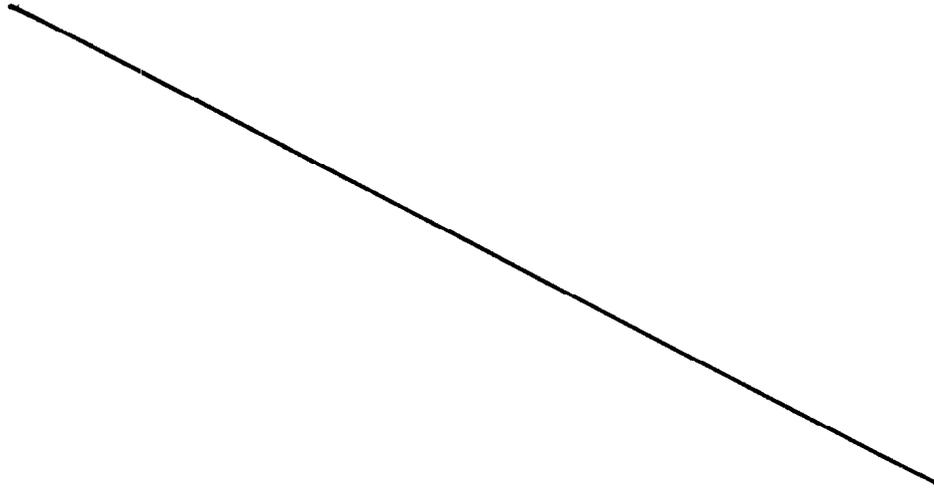
Glycyrrhizin and licorice water extract have been used as food and food additives in the United States as well as in Japan, where it has over a 1300-year history of use. The recent annual usage of licorice water extract in Japan was reported to be about 130 metric tons (in 1988). In the United States, 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).

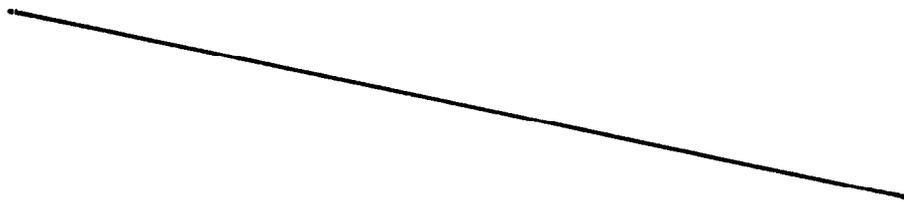
Susceptibility to the mineralocorticoid adverse effects of glycyrrhizinic acid varies greatly among individuals, and also largely depends on the amount and duration of glycyrrhizinic acid intake (Olukoga and Donaldson, 2000). Glycyrrhizinic acid is present in licorice root as the calcium or potassium 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.





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



4.2.1.1 Subchronic Toxicity



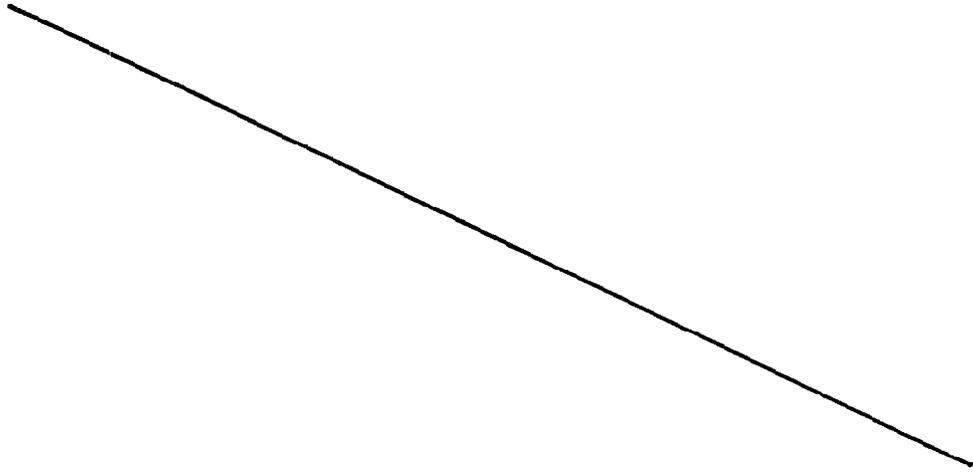
* LFO refers to Kaneka Glavonoid Rich Oil™.

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4.2.1.2

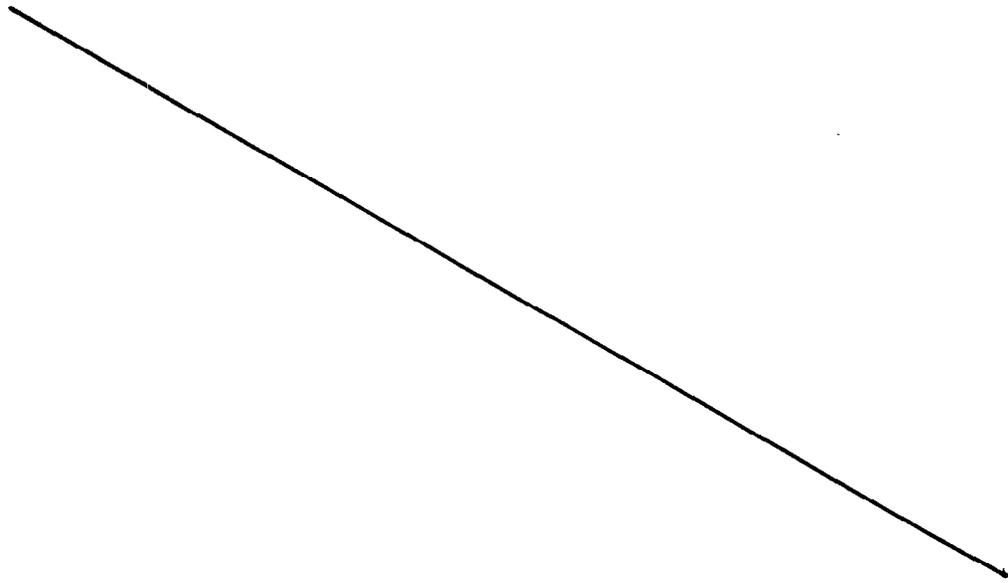
Genotoxicity



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4.2.1.3 Carcinogenicity



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

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	TDL _o 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 µL/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

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

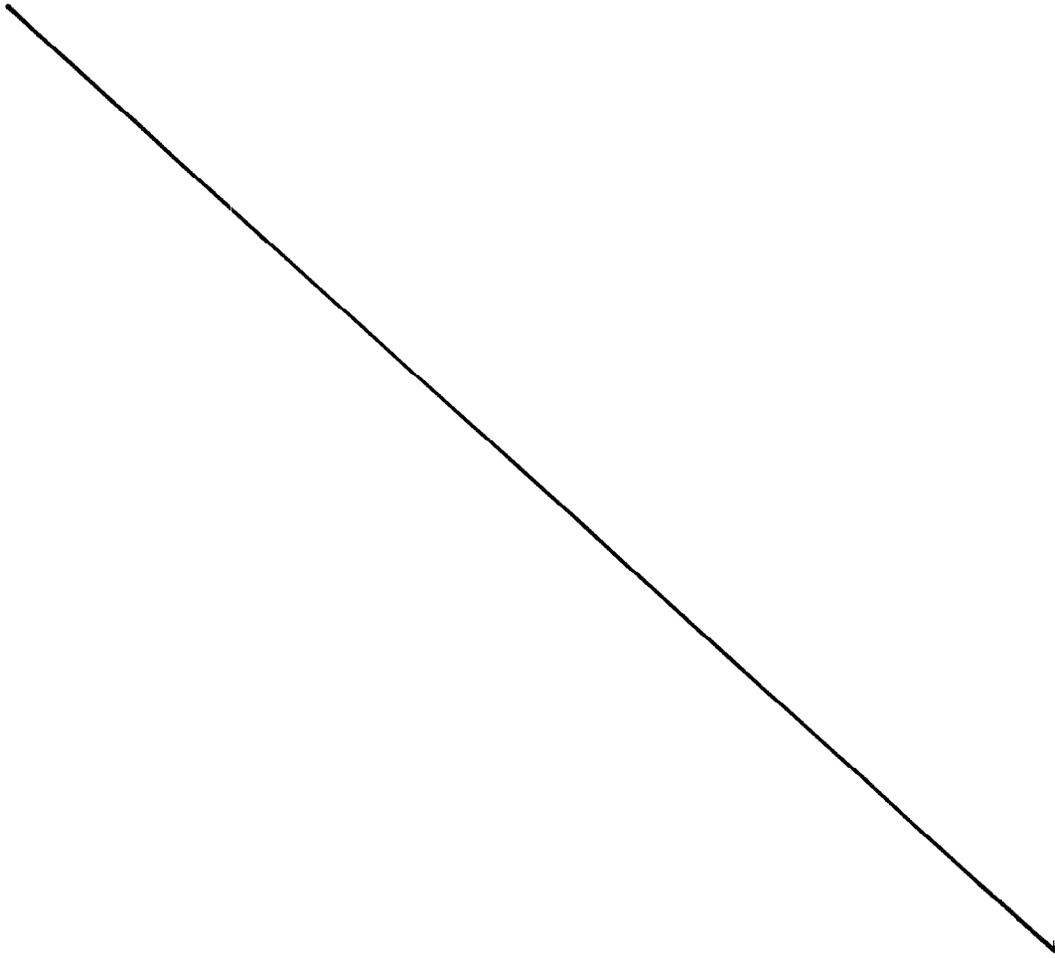
also reportedly reduced serum alanine transaminase and aspartate transaminase values, and in men, serum testosterone levels were reportedly reduced due to inhibition of 17 β -hydroxysteroid dehydrogenase and 17,20-lyase (Armanini *et al.*, 1999; Shibata, 2000). In general, it has been suggested that 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.

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.



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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, _____, 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. _____

- _____
- _____
- (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, _____ 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 _____ will be reasonably expected to be safe.

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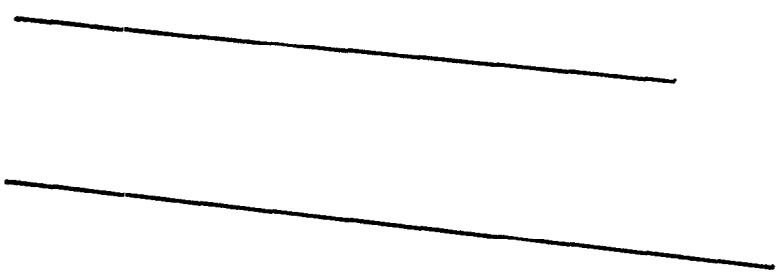
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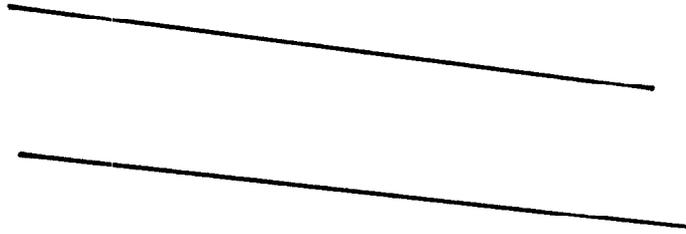
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