Bureau Nieuwe Voedingsmiddelen

Kalvermarkt 53, Den Haag
Tel 070 356 74 00
Fax 070 356 75 15
Postadres
Postbus 16229
2500 BE Den Haag

Internet
www.nieuwevoedingsmiddelen.nl

Novel Foods Unit

Kalvermarkt 53, The Hague
Tel +31 (0)70 356 74 00
Fax +31 (0)70 356 75 15
Correspondence address
P.O. Box 16229
2500 BE The Hague, The Netherlands

Internet
www.novel-foods.nl

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Uw brief

Uw kenmerk

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Onderwerp
Tweede beoordeling van een flavonoïdenrijk extract van zoethoutwortel als nieuw voedselingrediënt

Geachte mevrouw

Dit briefadvies betreft een tweede beoordeling volgens de Europese verordening 258/97, over het gebruik van een flavonoïdenrijk extract van de wortel van de zoethoutstruik *Glycyrrhiza glabra* L. (zoethoutwortel) als nieuw voedselingrediënt. De aanvraag is ingediend door de firma Kaneka Pharma Europe N.V. die dit nieuwe ingrediënt op de Europese markt wil brengen onder de naam Glavonoid. Deze tweede beoordeling is uitgevoerd door het Bureau Nieuwe Voedingsmiddelen (BNV) van het College ter Beoordeling van Geneesmiddelen. Het bureau heeft hiervoor de Commissie Veiligheidsbeoordeling Nieuwe Voedingsmiddelen geraadpleegd, hierna genoemd 'de Commissie VNV'.

De eerste beoordeling van de aanvraag voor markttoelating is verricht in België door de *Conseil Supérieur d'Hygiène* (CSH). De CSH concludeert dat de gezondheidsrisico's extreem laag zullen zijn als dit nieuwe product wordt toegelaten. De Belgische beoordelaars adviseren om de houdbaarheid van levensmiddelen, waaraan het flavonoïdenrijk extract is toegevoegd, te onderzoeken. Daarnaast bevelen zij marktmonitoring aan in verband met onzekerheden bij langdurige inname.

Het Nederlandse BNV interpreteert het oordeel van de CSH als een positief advies en is het hier niet mee eens. Het BNV baseert dit standpunt op de informatie uit het dossier, de eerste beoordeling door de CSH, en de bespreking van het dossier door de commissie VNV tijdens haar plenaire vergadering op 24 maart 2009. Hieronder licht het BNV haar belangrijkste argumenten toe waarom zij bezwaar heeft tegen autorisatie van het flavonoïdenrijk extract.

Productomschrijving

De productie begint bij de extractie van zoethoutwortel met ethanol. Dit extract wordt geconcentreerd en gezuiverd. Nadat er een speciale olie aan is toegevoegd worden de onoplosbare componenten

verwjderd. Het eindproduct bevat minder dan 0,5 % ethanol en wordt gestandaardiseerd op 3 % glabridine (het voornaamste flavonoïde in zoethoutwortel). De als verdunningsvloeistof gebruikte olie wordt geproduceerd uit kokosnootolie en palmpitolie. Deze olie bestaat uit triglyceriden met voornamelijk middellangeketen vetzuren en is van levensmiddelenkwaliteit.

Volgens de productspecificatie in het dossier bestaan de geëxtraheerde bestanddelen voor 80 % uit polyfenolen, mogelijk meer dan 70 verschillende verbindingen. De meesten hiervan behoren tot de chemische groep van flavonoïden. Hoewel op verzoek van de CSH een deel van de geëxtraheerde verbindingen geïdentificeerd zijn, blijft een belangrijk deel onbekend. Daarnaast merkt BNV op dat 20 % van de verbindingen uit de zoethoutwortel niet tot de polyfenolen behoren. De informatie in het dossier over deze andere stoffen is onvolledig. Het BNV meent dat deze fractie beter moet worden gespecificeerd.

Toepassing

De aanvraag betreft een brede toepassing. Naast het gebruik van het nieuwe product in voedingssupplementen stelt de aanvrager voor om het flavonoïdenrijke extract te verwerken in een breed assortiment levensmiddelen, bijvoorbeeld allerlei toetjes, *shakes*, graanrepen, smeerbare vetten, smeerkaas, dressings, soepen, maaltijdvervangers en verschillende soorten dranken. Informatie over portiegroottes en gehaltes van het extract in het eindproduct ontbreken. Het is de bedoeling dat consumenten dagelijks 100 à 300 mg van het flavonoïdenrijke extract innemen, maar het dossier bevat geen voedselconsumptiegegevens. Het lijkt onwaarschijnlijk dat de aanvrager zicht houdt op alle mogelijke toepassingen. Het BNV heeft daarom grote twijfels of duidelijke etikettering van de producten, waaraan het flavonoïdenrijke extract is toegevoegd, voldoende garantie biedt dat consumenten hun inname zullen beperken tot de hoeveelheid die de aanvrager wenselijk acht. Het is niet ondenkbaar is dat consumenten op één dag zowel supplementen als andere producten met het flavonoïdenrijke extract gebruiken.

Veiligheidsgegevens

Het dossier bevat de resultaten van een 90 dagen toxicologisch onderzoek bij ratten. Volgens de aanvrager is de dosering waarbij er geen klinisch relevante nadelige effecten zijn waargenomen 400 mg per kg lichaamsgewicht per dag voor mannetjesratten en 600 mg per kg lichaamsgewicht per dag voor vrouwtjesratten. De beoogde maximale inname bij mensen is ongeveer 100 maal lager (300 mg flavonoïdenrijke extract bij een lichaamsgewicht van 60 kg).

Het flavonoïdenrijke extract vertoont geen mutagene activiteit in bacteriële testsystemen, maar induceert wel chromosoomschade in zoogdiercellen *in vitro (*chromosoom aberraties). De aanvrager beschouwt deze laatste bevinding als niet relevant, omdat het wordt weerlegd door de negatieve resultaten van twee *in vivo* micronucleustesten bij ratten die het extract oraal kregen toegediend. Dit lijkt terecht, maar BNV vindt dat de aanvrager het protocol van de *in vivo* micronucleustest met rattenlevercellen nader moet toelichten omdat voor dit toxicologisch onderzoek geen OECD richtlijn bestaat. Aanvullend zou een *unscheduled DNA synthesis* (UDS) test met zoogdiercellen *in vivo* kunnen worden uitgevoerd om de negatieve resultaten te ondersteunen.

Een belangrijk punt van bezwaar is dat het flavonoïdenrijke extract een mengsel is van stoffen waarvan de samenstelling slechts gedeeltelijk gedefinieerd is. De meest bekende flavonoïden in zoethoutwortel zijn bepaalde, zogeheten prenylflavonoïden. Dit soort flavonoïden kunnen oestrogene eigenschappen hebben. Omdat een groot deel van de componenten in het extract niet gekarakteriseerd zijn, is het dus onbekend of het mogelijk prenylflavonoïden bevat met oestrogene werking. BNV constateert dat er geen zekerheid is over afwezigheid van schadelijke verbindingen. Zo omvat het proefdieronderzoek dat in het dossier wordt beschreven geen onderzoek naar de voortplanting en de ontwikkeling van de nakomelingen (reproductietoxicologie). Gezien het soort extract is het BNV in het bijzonder bezorgd over mogelijke gevolgen voor zwangere vrouwen. Zij meent dat aanvullend proefdieronderzoek noodzakelijk is om uit te sluiten dat ongewenste effecten in de embryonale en foetale ontwikkeling optreden.

Ook houdt BNV er rekening mee dat het flavonoïdenrijke extract jarenlang gebruikt gaat worden door de consument. Effecten van chronische blootstelling bij proefdieren zijn niet onderzocht conform internationale richtlijnen voor toxicologisch onderzoek. De aanvrager concludeert dat het nieuwe ingrediënt bij ratten niet carcinogeen is, maar BNV meent dat het beschreven onderzoek geen bewijs vormt voor afwezigheid van eventuele kankerverwekkende eigenschappen van het extract.

Het is bekend dat consumptie van producten afkomstig van de zoethoutstruik hypertensie kan veroorzaken. BNV benadrukt echter dat, door de specifieke bereiding, het product uit de voorliggende aanvraag nauwelijks glycyrrhizine bevat (ten hoogste 0,05 mg per gram). Deze verbinding is de karakteristieke zoetstof van de zoethoutwortel en wordt in ons maag-darmkanaal omgezet in glycyrrhetinezuur. Deze laatste kan een bloeddruk stijging teweegbrengen. Het BNV is het eens met de CSH dat de concentratie van glycyrrhizine voldoende laag is en geen aanleiding geeft tot bezorgdheid voor de volksgezondheid. Ook is het onwaarschijnlijk dat de glycyrrhizine-inname ongewenste effecten op de bloeddruk teweegbrengt bij grootverbruikers van producten waaraan het nieuwe ingrediënt volgens het dossier zou worden toegevoegd.

Samengevat concludeert het BNV dat er onvoldoende zekerheid bestaat over de veiligheid van het nieuwe ingrediënt bij jarenlang gebruik door de consument. Het BNV meent dat aanvullend toxicologisch onderzoek nodig is gezien de aard van het product, een mengsel van vele stoffen waarvan de chemische samenstelling onvoldoende bekend is.

Met vriendelijke groet,

Bureau Nieuwe Voedingsmiddelen (BNV) – Novel Foods Unit
The Hague

21 April 2009

Contact: Ref.: 070-3567586

Subject:

Second assessment of a flavonoid-rich extract of liquorice root as a novel food ingredient

Dear Ms

This letter concerns a second assessment under European Regulation 258/97 of the use of a flavonoid-rich extract of the root of the liquorice plant *Glycyrrhiza glabra* L. (liquorice root) as a novel food ingredient. The application was submitted by the company Kaneka Pharma Europe N.V., which wishes to place this new ingredient on the European market under the trade name Glavonoid. This second assessment was conducted by the *Bureau Nieuwe Voedingsmiddelen* (BNV) (Novel Foods Unit) of the *College ter Beoordeling van Geneesmiddelen* (Medicines Evaluation Board). To this end the Unit consulted the *Commissie Veiligheidsbeoordeling Nieuwe Voedingsmiddelen* (Novel Foods Safety Assessment Committee – VNV Committee).

The first assessment of the application for a marketing authorisation was made in Belgium by the *Conseil Supérieur d'Hygiène* (CSH), which concluded that the health risks of approving this new product were extremely low. The Belgian assessors recommended that the storage stability of foods containing the flavonoid extract be examined. They also recommended market monitoring in connection with uncertainty about long-term dietary exposure.

The Netherlands BNV interprets this CSH opinion as a positive assessment and disagrees with it. The BNV bases its view on information from the dossier, the first assessment by the CSH, and the discussion of the dossier by the VNV Committee at its plenary meeting on 24 March 2009. The BNV sets out below the main reasons why it opposes the authorisation of this flavonoid-rich extract.

Product description

Production begins with the extraction of liquorice root using ethanol. This extract is concentrated and purified. A special oil is added and the insoluble components are then removed. The end product contains less than 0.5% ethanol and is standardised at 3% glabridine (the main flavonoid in liquorice root). The oil used as a diluent is produced from coconut oil and palm kernel oil. It consists of triglycerides with medium-chain fatty acids predominating, and is of food quality.

According to the product specification in the dossier, 80% of the extracted components are polyphenols - possibly more than 70 different compounds. Most of these belong to the chemical group of the flavonoids. Although some of the extracted compounds were identified at the request of the CSH, a significant proportion remains unknown. Moreover, the BNV notes that 20% of the

liquorice-root compounds are not polyphenols. The information on these other substances in the dossier is incomplete. The BNV considers that this fraction should be specified.

Product applications

The request for marketing authorisation is for a variety of uses. In addition to the use of the new product in food supplements, the applicant intends to incorporate the flavonoid-rich extract in a wide range of foods, such as various desserts, shakes, cereal-based bars, spreadable fats, cheese spreads, dressings, soups, meal replacements and a variety of beverages. There is no information on portion sizes or on the content of the extract in the end product. The intention is that consumers consume 100-300 mg of the flavonoid-rich extract daily, but the dossier includes no food-consumption data. It appears unlikely that the applicant will monitor all possible applications. The BNV therefore has serious doubts as to whether the clear labelling of products to which the flavonoid-rich extract is added provides an adequate guarantee that consumers will restrict their intake to the quantity the applicant regards as appropriate. Consumers may well use both supplements and other products containing the flavonoid-rich extract in a single day.

Safety data

The dossier contains the results of a 90-day toxicological study in rats. According to the applicant, the dosage at which no clinically relevant adverse effects were identified was 400 mg per kg body weight per day for male rats and 600 mg per kg body weight per day for female rats. The desired maximum intake in humans is approximately 100 times less (300 mg flavonoid-rich extract for a body weight of 60 kg).

The flavonoid-rich extract shows no mutagenic activity in bacterial test systems, but it does cause chromosomal damage (chromosome aberrations) in mammalian cells *in vitro* . The applicant views the latter finding as irrelevant, since it is refuted by the negative results of two *in vivo* micronucleus tests in rats given the extract orally. This may be the case, but the BNV feels that the applicant must further clarify the record of the *in vivo* micronucleus test in rat liver cells, since there is no OECD guideline on this toxicological test. An unscheduled DNA synthesis (UDS) test on mammalian cells *in vivo* should also be conducted to confirm the negative results.

An important part of the objection is the fact that the flavonoid-rich extract is a blend of substances whose composition is stated only partially. The best-known flavonoids in liquorice root are specific prenylated flavonoids. This type of flavonoid may have oestrogenic properties. Since a high proportion of the components in the extract are not characterised, it is not known whether it contains prenylated flavonoids with oestrogenic effect. The BNV notes that the absence of harmful compounds cannot be guaranteed. The animal experiment described in the dossier includes no investigation of reproduction or of the development of progeny (reproduction toxicology). Given the type of extract, the BNV is particularly concerned about possible consequences for pregnant women. It considers that additional animal experiments are necessary to rule out undesirable effects on embryonic or foetal development.

The BNV also takes into account the fact that the flavonoid-rich extract would be used by consumers over many years. The effects of chronic exposure in experimental animals have not been investigated in accordance with international toxicological guidelines. The applicant concludes that the novel ingredient is not carcinogenic in rats, but the BNV considers that the experiment described constitutes no proof of the absence from the extract of possible carcinogenic properties.

It is known that the consumption of products derived from the liquorice plant may cause hypertension. The BNV stresses, however, that, given the specific preparation, the product in question contains almost no glycyrrhizine (0.05 mg per gramme at most). This compound is the

characteristic edulcorant of the liquorice plant and is converted to glycyrrhetinic acid in the human gastro-intestinal tract. This acid may cause blood pressure to rise. The BNV agrees with the CSH that the concentration of glycyrrhizine is sufficiently low to constitute no grounds for public health concerns. It is also unlikely that glycyrrhizine intake has undesirable effects on blood pressure in major consumers of products to which, according to the dossier, the novel ingredient is to be added.

The BNV concludes that there is insufficient certainty about the safety of the novel ingredient in long-term use by consumers. The BNV considers that further toxicological testing is necessary in view of the type of product, a blend of many substances whose chemical composition is insufficiently known.

Yours sincerely,

(signed)

Head of Novel Foods Unit

APPLICATION FOR THE APPROVAL OF GLAVONOID

October 18, 2007

SUMMARY

APPLICATION FOR THE APPROVAL OF GLAVONOID

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APPLICATION FOR THE APPROVAL OF GLAVONOID

1.0 ADMINISTRATIVE DETAILS

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2.0 GENERAL INTRODUCTION

KANEKA CORPORATION proposes to market GLAVONOID derived from the root or rootstock of *Glycyrrhiza glabra* L., for use as a nutritional food ingredient and food supplement in Europe. GLAVONOID is an important source of specific polyphenols, such as glabridin.

Although *Glycyrrhiza glabra* L., the licorice plant that serves as the source for GLAVONOID and licorice products derived from this plant have a long history of safe consumption, GLAVONOID has not hitherto been used for human consumption to a significant degree within the Community. GLAVONOID can be differentiated from other licorice products because it does not contain glycyrrhizinic acid, but only flavonoids. GLAVONOID is thus considered a novel food/food ingredient due to its manufacturing process.

KANEKA GLAVONOID™ falls under category (e) of Article 1(2) of Regulation EC 258/97 (European Parliament and the Council of the European Union, 1997) (foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices) and the European Commission's Scientific Committee on Food (SCF) Recommendations (Commission Recommendation 97/618/EC) (Commission of the European Communities, 1997), Class 2 as a "Complex Novel Food from non-GM source", since it is derived using conventional techniques, with no use of genetically modified organisms. The components of the final product (licorice flavonoids) and the source (*Glycyrrhiza glabra* L.) have a history of food use in the

Community. Accordingly, GLAVONOID would be further allocated under Sub-Class 2.1: "the source of the novel food has a history of food use in the Community".

I. SPECIFICATION OF THE NOVEL FOOD

The trade name KANEKA GLAVONOID™ will be used for marketing the food ingredient to food manufacturers. The proposed name for labelling purposes on final foods as presented to the consumers is "GLAVONOID from *Glycyrrhiza glabra* L.". The manufacturing process and extraction system is shown to produce material of a highly purified nature with a low level of glycyrrhizinic acid.

The specification has been well defined (Table I-1) and batch manufacture results have been provided to show that the products can consistently be manufactured in accordance with defined chemical and physical parameters.

Table I-1 Product Specifications for GLAVONOID

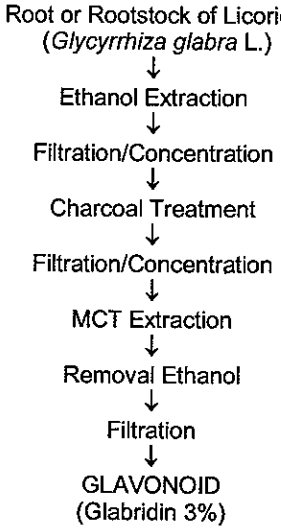
Test	Criterion
Appearance	Dark brown coloured liquid Distinct smell and taste
Identification	Correspond to the standard UV-VIS chart Correspond to the standard HPLC chart
Glabridin	3.0% ± 0.5%
Glycyrrhizinic Acid	< 0.005 weight %
Peroxide Value	≤ 0.5 meq/kg
Aerobic Plate Count	≤ 1,000 CFU/g
Coliforms	Negative

Only food grade ingredients and materials are used in the manufacture of GLAVONOID.

II. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

The production process for KANEKA GLAVONOID™ involves the ethanolic and medium-chain triglycerides (MCT) extractions of flavonoids from the root or rootstock of licorice (*Glycyrrhiza glabra* L.), followed by a series of concentrations, filtrations, and washings as shown in Figure II-1.

Figure II-1 KANEKA GLAVONOID™ Process Flow Diagram



III. HISTORY OF THE ORGANISM USED AS THE SOURCE OF THE NOVEL FOOD

Glycyrrhiza glabra L., the licorice plant that serves as the source for GLAVONOID, has a history of consumption for the past 6000 years (Mitscher *et al.*, 1986). Licorice roots have a long history of safe use as flavouring and sweetening agents, as well as demulcents and expectorants in western countries. Licorice root extracts have been used in Japan and China to treat allergic inflammation (Chandler, 1985; Hikino, 1985).

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 safe use. In the EU, dietary exposure to ammonium glycyrrhizinate and glycyrrhizinic acid occurs through the use of these substances as licorice confectionery, chemically defined flavouring substances, herbal teas, beverages, and chewing gum. In addition, glycyrrhizinic acid (in the form of licorice extract) is also added to tobacco products, including chewing tobacco (SCF, 2003).

IX. ANTICIPATED INTAKE/EXTENT OF USE OF THE NOVEL FOOD

GLAVONOID is best classified as a functional food ingredient that has positive physiological effects if consumed at a defined level. The following food categories are proposed as suitable vehicles for GLAVONOID:

- Desserts (dairy-based and others, portion ranges between 100 and 200 g)
- Desserts (instant powders for dilution in water e.g., eight sachets in a box)
- Shakes (instant powder for preparation or ready-to-drink, portions up to 250 ml)

- Biscuits, cookies (daily ration in one package, portions e.g., 75g)
- Cereal bars (daily ration in one package, 25 to 50 g)
- Yoghurt based health drinks (daily portions in one bottle of 65 or 100 ml)
- Spreads (cheese and fat-based)
- Salad dressings and mayonnaise-like dressings
- Soups (instant powders, portion if ready to eat: 250 ml)
- Ready-to-eat meals used as a meal replacement in a managed diet program
- Drinks (portions up to 500 ml)

GLAVONOID will also be sold as food supplements as capsules containing between 100 and 300 mg. The consumer's target window for a daily ingestion of GLAVONOID is between 100 and 300 mg. Total GLAVONOID intake from both food supplements and enriched foods should not exceed 300 mg/day. Product formulation, labelling, and advertisement will assure that higher intakes are avoided.

X. INFORMATION FROM PREVIOUS HUMAN EXPOSURE TO GLAVONOID FROM *GLYCYRRHIZA GLABRA* L.

Phenolic compounds and more specifically, flavonoids, present in GLAVONOID are widely distributed in the plant kingdom and are normal components of the diet.

A diluted form of GLAVONOID, termed Licorice Flavonoid Oil (LFO) is already available in the United States of America, where KANEKA CORPORATION has received FDA acceptance to sell it as a new dietary ingredient (NDI) (RPT 348 Volume 271, 95S-0316).

The dietary supplement containing the GLAVONOID dietary ingredient is in capsule form. Each capsule contains 30 mg licorice ethanol extract (equivalent to approximately 100 mg GLAVONOID). Consumption of up to 3 servings per day is suggested or recommended in the label directions, resulting in a maximum daily consumption of up to 90 mg licorice ethanol extract (equivalent to approximately 300 mg GLAVONOID, or 5 mg/kg/day GLAVONOID for a 60 kg bw person).

XI. NUTRITIONAL INFORMATION ON GLAVONOID FROM *GLYCYRRHIZA GLABRA* L. IN EUROPE

GLAVONOID will be added to foods as a nutritional ingredient and will be consumed as a food supplement. It is not expected to replace any foods in the diet. Clinical trials with a related product whose name is LFO, the dilution of GLAVONOID with MCT, demonstrate that the flavonoids present in GLAVONOID had nutritional benefits, including producing a decrease in body fat including visceral fat, resulting in weight reduction. In addition, consumption of LFO for

8 weeks resulted in lower total cholesterol and low-density lipoprotein cholesterol levels than at baseline.

XII. MICROBIOLOGICAL INFORMATION ON GLAVONOID FROM *GLYCYRRHIZA GLABRA* L.

As shown in Table XII-1, typical food borne microbes (*e.g.*, fungi, *Salmonella*, *Escherichia coli*) and mycotoxins (aflatoxins B_1, B_2, G_1, and G_2 and ochratoxin A) do not appear in the final product.

Table XII-1 Microbiology Test Results

Test	Specification	Lot Number	
		50605001	51217002
Microorganisms	$\leq 10^3$ CFU/g	$\leq$ 300 CFU/g	$\leq$ 300 CFU/g
Salmonella/Escherichia coli (cfu/25g)	Negative	Negative/0.22 g	Negative/0.22 g
Fungi	Negative	Negative/0.1 g	Negative/0.1 g
Mycotoxins			
Aflatoxin B_1	< 5 ppb	Not tested	Not detected
Aflatoxin B_2	< 5 ppb	Not tested	Not detected
Aflatoxin G_1	< 5 ppb	Not tested	Not detected
Aflatoxin G_2	< 5 ppb	Not tested	Not detected
Ochratoxin A	< 0.05 ppm	Not tested	Not detected

XIII. TOXICOLOGICAL INFORMATION ON GLAVONOID FROM *GLYCYRRHIZA GLABRA* L.

Licorice and licorice root extract derived from *Glycyrrhiza glabra* L. have a history of safe use, and information on these products can be used as a baseline to facilitate the toxicological assessment of GLAVONOID, particularly regarding the potential for mineralocorticoid activity seen with other licorice products.

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, 18β-glycyrrhretinic acid, the active metabolite of glycyrrhizinic acid, inhibits the enzyme 11β-hydroxysteroid dehydrogenase (11β-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. It should be noted, however, that specifications for GLAVONOID product limit the glycyrrhizinic acid content to < 0.005%, the quantification limit of the HPLC method in industrial use. At this level, the GLAVONOID product can be considered as safe even in case of extreme overdosing. No mineralocorticoid effects were observed in clinical trials with LFO, the diluted form of GLAVONOID with MCT, at up to 1200 mg/day (equivalent to 400 mg GLAVONOID/day) for 4 weeks. In addition, the results of published clinical safety studies demonstrated that a daily intake of 10 mg of glycyrrhizinic acid could be derived as a safe dose for most healthy adults (Størmer *et al.*, 1993). The maximum recommended daily dose of GLAVONOID (300 mg) would result in a maximum glycyrrhizinic acid intake of 0.015 mg/day, assuming a glycyrrhizinic acid content of 0.005%. In this case, the margin of safety is more than 600-fold.

KANEKA CORPORATION has conducted non-clinical studies and clinical trials to support the safety of GLAVONOID itself. The non-clinical studies 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 and peripheral blood micronucleus test), and a rat medium-term liver bioassay for carcinogens.

Based on the results of the subchronic toxicity study, the NOAEL of GLAVONOID was estimated to be 600 mg/kg/day for females. For males, it is concluded that the NOAEL is approximately 400 mg/kg/day. The large margin of safety (80- to 120-fold) that exists between these NOAELs and the maximum recommended dose to consumers (300 mg/day GLAVONOID, equivalent to 5 mg/kg/day GLAVONOID for a 60 kg bw person) provides support for the safety of GLAVONOID.

Results from a chromosome aberration test indicated that GLAVONOID had structural chromosomal aberration inducing ability in CHL/IU cells; however, GLAVONOID was non-mutagenic in a reverse mutation assay, bone marrow micronucleus assay, and liver and peripheral blood micronucleus test. The micronucleus assays were conducted *in vivo*, thus, they were considered a reliable predictor of carcinogenesis, as such, GLAVONOID was considered non-mutagenic. Furthermore, the rat medium-term liver bioassay for carcinogens (Ito's method) demonstrated that GLAVONOID was devoid of carcinogenic activity.

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 GLAVONOID, which is virtually devoid of glycyrrhizinic acid, the results of these studies demonstrated that a daily intake of 10 mg of glycyrrhizinic acid could be derived as a safe dose for most healthy adults. The maximum recommended daily dose of GLAVONOID (300 mg) would result in a glycyrrhizinic acid intake of 0.015 mg, assuming the glycyrrhizinic acid content of GLAVONOID was 0.005%. In this case, the margin of safety is more than 600-fold.

Studies conducted with deglycyrrhizinated licorice showed that the test material generally had no adverse effects. Deglycyrrhizinated licorice contains not more than 3% glycyrrhizinic acid. Thus, the results of these studies are consistent with the observed safety of GLAVONOID, which contains less than 0.005% of glycyrrhizinic acid; its use is not expected to elicit the mineralocorticoid adverse effects associated with glycyrrhizinic acid.

In addition, the clinical safety and pharmacokinetics of LFO, the diluted form of GLAVONOID with MCT, were evaluated in healthy subjects in a single dose trial and in one-, four-, and eight-week repeat dose ingestion studies. No clinically significant dose-related adverse effects were seen following the consumption of GLAVONOID at doses of up to 600 mg/day for 4 weeks and 300 mg/day for 8 weeks. These clinical data support the findings from preclinical studies and demonstrate safety in humans, including the lack of adverse effects on haematological parameters or evidence of mineralcorticoid effects.

OVERALL CONCLUSIONS

Based on the evidence provided above, including results of preclinical safety studies conducted on GLAVONOID, the presence of a safety factor 80- to 120-fold that exists between the NOAELs from the 90-day repeated dose toxicity study in rats and the maximum recommended dose to consumers (300 mg/day GLAVONOID, equivalent to 5 mg/kg/day GLAVONOID for a 60 kg bw person), the absence of adverse effects in clinical studies of GLAVONOID and in published clinical trials with deglycyrrhizinated licorice, which like GLAVONOID, is virtually devoid of glycyrrhizinic acid and generally had no adverse effects, KANEKA CORPORATION

concludes that the use of GLAVONOID at a level not to exceed 300 mg/day (providing 90 mg licorice ethanol extract per day) will be reasonably expected to be safe.

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