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ORIGINAL SUBMISSION

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June 20, 2000

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VIA HAND DELIVERY

Linda S. Kahl, Ph.D.
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W. (HFS-206)
Washington, D.C. 20204

Re: GRAS Notification for Cassia Gum

Dear Dr. Kahl:

On behalf of our client, B.F. Goodrich, pursuant to the Food and Drug Administration's April 17, 1997 proposed rule, we are submitting (in triplicate) the enclosed notification regarding the generally recognized as safe (GRAS) status of Cassia gum for use as a stabilizer (thickening and gelling agent) in food. If you have any questions regarding the notification, please contact me.

Respectfully submitted,

Melvin S. Drozen

Enclosures

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C. Street, S.W.
Washington, D.C. 20204

Re: GRAS Notification Procedure

1.0 Introduction

Plant-derived polysaccharides are excellent stabilizing and thickening agents and are common components of processed foods. For example, locust (carob) bean gum is listed as generally recognized as safe (GRAS) and is approved for use in food up to a maximum level of 0.75 % (21 CFR 184.1343). It is also approved for use as a thickening agent in Europe (EEC No. E410). Guar gum is listed as GRAS and is approved for use in food up to a maximum level of 2% (21 CFR 184.1339). Both are used in various foods including baked goods, cheese, and jams/ jellies.

Cassia gum, flour from the endosperm of *Cassia tora*, otherwise known as *Cassia obtusifolia*, is well suited for use in the manufacture of gels in combination with other colloids and has numerous food applications. It is used for various technical applications, including thickening, emulsification, foam stabilization, moisture retention, and texture improvement. Cassia gum is used at concentrations comparable to locust (carob) bean gum and guar gum.

The toxicological profile of Cassia gum and other data were assessed by an Expert Panel to determine GRAS status for use as a stabilizer (thickening and gelling agent) in food. The lack of toxicity of Cassia gum demonstrated in animals and the safe use of related gums indicate that the technological properties of Cassia gum may be safely utilized in food.

1.1 Notice of GRAS exemption claim:

Cassia gum is exempt from premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS) under conditions of intended use by experts qualified by scientific training and experience.

i) Notifier:

B.F.Goodrich
Performance Materials
Food & Beverage Ingredients Division
2235 Langdon Farm Road
Cincinnati, Ohio 45237-4790

Contact Person:

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ii) Common/usual name:

Cassia gum (Diagum CS™)

iii). Applicable conditions of use:

As discussed further below, based on the extant data and experts' conclusions, Cassia gum is believed to be GRAS at good manufacturing practice (GMP) levels for use in food. Specific examples of typical uses, not intended to be exhaustive, are set forth in the table below.

FOOD MARKET SEGMENT	MAXIMUM CONCENTRATION, PPM	REMARKS
Baked Goods	5,000	Water retention
Canned Goods	3,000 - 5,000	Gelling agent with carrageenan or xanthan
Soups,Sauces,Seasonings	2,000 5,000	Soft gels with xanthan Thickening agent
Deep Frozen Food	3,000 - 5,000	Only in combination with xanthan
Jams and Jellies	Up to 5,000	Gelling agent
Gelatins and Puddings	Up to 6,000	Depending on the desired gel strength or viscosity
Nut Products	2,000	Water retention
Dairy and Frozen Dessert	3,000 - 5,000	Depending on the desired gel strength or viscosity
Meat and Poultry Products and Sausages	3,000	Improvement of texture and water retention

iv) The basis of this GRAS determination is through scientific procedures (see section 4). This determination is supported by an expert panel review of the relevant toxicological data, as evidenced by the conclusions set forth in Appendix I.

v) The data and information for the basis of GRAS determination are available for the FDA's review and copying. A summary of the data included in this notification were originally published in 1997 in *Food and Chemical Toxicology* [Hallagan *et al.*, 1997]. All references are available through the contact person named above.

Respectfully submitted,
BF GOODRICH

By:

Melvin S. Drozen
Keller and Heckman, LLP
COUNSEL FOR THE NOTIFIER

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2.0 Chemical Identity

Cassia gum[1] (CAS No. 11078-30-1) is the purified flour from the endosperm of the seeds of *Cassia tora/obtusifolia,* which belongs to the leguminosae family. *Cassia tora/obtusifolia* grows wild in subtropical regions of the world and is occasionally cultivated. It is an annual ruderal plant that ripens after approximately 100 days.

Cassia gum is comprised of at least 75% polysaccharide consisting primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6 linked α-D galactopyranose units. The ratio of galactose to mannose is 1:5. The following composition was revealed in the sugar analysis conducted using HPLC: mannose (77.2-78.9%), galactose (15.7-14.7%) and glucose (7.1-6.3%).

Like most polysaccharides, the following formula applies:

$$(C_6H_{10}O_5)_n, H_20$$

Cassia gum is related to locust (carob) bean gum and guar gum in terms of structure and chemical properties. The specifications and purity of cassia gum and related gums are listed in Table 1.

Cassia galactomannan can be differentiated from other galactomannans by electrophoresis. Based on the molecular weights of the structurally related polygalactomannans, guar gum and locust (carob) bean gum [Klose and Glicksman, 1968; Leung and Foster, 1996], the molecular weight of cassia gum is estimated to be between 200,000 and 300,000 [Denkler, 1997]. The physical properties of cassia gum are reported in Table 2, and its structural properties as compared to other gums are reported in Table 3.

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[1] Cassia gum (trade name DIAGUM CS) is marketed in two forms:
a) 100% flour from the endosperm of the *Cassia tora/obtusifolia* seeds.
b)In a mixture with other gelling and thickening agents (e.g. carrageen, locust (carob) seed flour, guar gum, agar, xanthan gum and, where appropriate, other food additives), whereby DIAGUM CS comprises 10-90% of the mixture. Diagum CS was called Mucigel X 18 H during the temporary approval in France.

Table 1. Specifications and Purity of Cassia Gum and Related Gums for use in Foods

	CASSIA GUM[2]	CASSIA GUM	LOCUST (CAROB) BEAN GUM[3]	GUAR GUM[4]
	Typical Analytical Values	Proposed Limits	FCC Limits	FCC Limits
Galactomannan	82%	≥70%	≥75%	≥70%
Acid-Insoluble residue	0.40%	≤4%	≤4%	≤7%
Moisture	8.6%	≤12%	NR	NR
Ash	1.0%	≤1.2%	≤1.2%	≤1.5%
Proteins	6.0%	≤7%	≤7%	≤10%
Fat	1.5%	≤2%	NR	NR
Chrysophanic acid[5]	5 ppm	5 ppm	NR	NR
Heavy Metals:		≤0.002%	≤0.002%	≤0.002%
Lead	<1 mg Pb/kg	≤5 mg Pb/kg	≤5 mg Pb/kg	≤5 mg Pb/kg
Copper	1.2 mg Cu/kg	NR	NR	NR
Arsenic	<0.4 mg As/kg	≤3 mg As/kg	≤3 mg As/kg	≤3 mg As/kg
Zinc	<10 mg Zn/kg	NR	NR	NR
Mercury	<0.25 mg Hg/kg	NR	NR	NR
Impurities:				
Mycotoxins	<0.001 mg/kg	NR	NR	NR
Pesticides	≤0.001 mg/kg	NR	NR	NR
Total Germ Count:	≤5,000 g	NR	NR	NR
Coliforms (*E. coli*)	Negative	NR	NR	NR
Yeasts	≤100/g	NR	NR	NR
Molds	≤100/g	NR	NR	NR

Table 2. Physical properties of Cassia gum[6]

Appearance	Pale yellow powder without lumps
Odor	specific, fruity smell
Bulk density	0.6 kg/l
Particle size (sieve analysis)	250 µm (average) not less than 100% 100 µm (average) not less than 60%
Solubility	Disperses well in cold water and forms a colloidal solution
pH (5%)	6.5-7.5
Viscosity, 5% solution:	
Cold	5,000 +/- 2,500 mPas
Boiling	120,000 +/- 30,000 mPas
Gel-strength	80g +/- 10g

[2] Leimgruber R. and Marbot H., 1991; Woodson-Tenent Laboratories, 1998, Freedom Chemical Diamalt, 1998.
[3] Food Chemicals Codex, 1996.
[4] Food Chemicals Codex, 1996.
[5] A measure for the naturally occurring anthraquinone derivatives in the product
[6] Leimbgruber R. and Marbot H., 1991
NR= not reported

[redacted]

Table 3. Structural properties of Cassia Gum and related substances

Substance	Structure	Mannose:Galactose	Molecular Weight
Cassia Gum (CAS No. 11078-30-1)[7]	1,4-β-D-mannopyranose units with 1,6-α-D galactopyranose units.	5:1	200,000-300,000
Guar Gum[8] (CAS No. 9000-30-0)	1,4-β-D-mannopyranose units with 1,6-α-D-galactopyranose units attached to every alternate mannose.	2:1	220,000-250,000
Locust (Carob) Bean Gum[9] (CAS No. 9000-40-2)	1,4-β-D-mannopyranose units with 1,6-α-D-galactopyranose units attached to every fourth or fifth mannose.	4:1	310,000
Tara Gum[10] (CAS No. 39300-88-4)	1,4-β-D-mannopyranose units with side chains of 1,6-α-D-galactopyranose attached to approximately every third unit.	Approximately 3:1	Not Reported

3.0 Method of Manufacture

In the Country of Origin – ISO 9002 certified manufacturing facility

Indiamalt Private Limited
Post – Manjusar, Taluka – Savil
Dist. Vadodara 391 775, India

Cassia tora/obtusifolia is harvested when the plant is ripe (e.g., November-January) in India. Once the pods are harvested, they are threshed to obtain the seed grains, which are the raw material for cassia gum. Similar to the processing of cereals, separators are used to remove stones, dust and foreign bodies.

The endosperm is separated from husk and germ when hot. Non-comminuted seed grains are continuously fed into a rotation roaster, and heated to approximately 200 °C for about 30 seconds. The husks around the seeds are removed from the endosperm mechanically. Hulling machines pulverize the tender germ and husk when hot and separate them from the intact, hard endosperm. Two different fractions are obtained: one consists of the germ and husk (≈80% of the seed); and the other consists of the endosperm (≈20% of the seed). The endosperm is purified by sieving and put into sacks for later processing.

Processing

Cassia gum is processed by a physical procedure. The endosperm is fed into a sorting machine where any remaining germ and husk traces are removed. A special cleaning procedure completes the purification process. After milling and drying, the flour from the endosperm of the *Cassia tora/obtusifolia*

[7] CAS number for "galactomannan"
[8] Klose R..E., and Glicksman, M., 1968.
[9] Klose R..E., and Glicksman, M., 1968.
[10] NTP, 1982b.

[redacted]

seed is obtained. Several batches are mixed, in order to obtain a homogeneous product, and analyzed for purity. Discrepancies in the composition of the batches are rare because the manufacturing process is continuous. Careful selection of the raw material compensates for any crop-related differences.

4.0 Intended Use

Plant-derived colloidal polysaccharides are excellent stabilizing and thickening agents and are common components of processed foods. For example, locust (carob) bean gum is listed as generally recognized as safe (GRAS) by the U. S. Food and Drug Administration (FDA) and is approved for use in food up to a maximum level of 0.75% (21 CFR 184.1343). It is also approved for use as a thickening agent in Europe (EEC No. E 410). Guar gum is listed as GRAS by FDA and is approved for use in food up to a maximum level of 2% (21 CFR 184.1339). Both are used in various foods including baked goods, cheese, and jams/jellies. The establishment of acceptable daily intakes (ADIs) for locust (carob) bean gum and guar gum was deemed unnecessary by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) because the available data indicate that locust (carob) bean gum and guar gum do not represent a health hazard. Gum arabic, gum ghatti, guar gum, locust bean gum, sterculia gum, and gum tragacanth are all considered GRAS by FDA for use in animal feeds (21 CFR Sec. 582). Cassia gum is included in the Japanese 'List of Existing Food Additives' as a stabilizer and thickener. It is understood that this list is used as a list of naturally occurring additives that are permitted in foods generally, unless a restriction appears elsewhere. No restriction appears for Cassia gum. An application has also been submitted for the authorization of Cassia gum as a food additive in the European Union.

Cassia gum swells in water and forms high-viscosity aqueous colloids after it is boiled. When it is used in combination with other gelling or thickening agents such as carrageenan or xanthan gum in aqueous solution, it results in the formation of an aqueous gel that has potentially numerous food applications. It is used as a thickening and gelling agent in pet food and for other technical applications (e.g., emulsification, foam stabilization, moisture retention, and texture improvement) at concentrations comparable to locust (carob) bean gum and guar gum [Khan and Farooqi, 1991]. It has the potential for being used in human foods in combination with carrageenan or xanthan gum [Bayerlein *et al.*, 1989].

4.1 Evidence of self-limiting levels of use

Besides the properties thickening, emulsification, foam stabilization, moisture retention and texture improvement, the main application of Cassia gum is likely to be to create the range of soft to firm gels in synergism with kappa-carrageenan or xanthan. For example, neither xanthan nor Cassia, as a single additive, builds up gel, a viscoelastic and thermo-reversible gel is obtained by blending these two ingredients. Therefore, it is highly unlikely that the maximum level of use of Cassia gum would exceed levels of currently utilized gums, such as carrageenan or xanthan.

5.0 Estimated Exposure

Cassia gum is currently used in the manufacture of pet foods, but it has potential for being used in various human foods at concentrations up to 6,000 ppm. The annual volume of use in the U.S. of cassia gum is projected to be 1,000,000 kg. At least 60% of the volume will be used in pet foods. The intended use concentration of cassia gum in pet food is approximately 0.25% (w/w). Cassia gum is approved for use in canned pet foods in the U.S.A. and Europe up to a maximum concentration of 4,000 and 17,600 ppm (i.e., 4.0 and 17.6 g cassia gum/1 kg food), respectively [AAFCO, in press].

Based on a potential use volume of 400,000 kg (i.e., 40% of the total projected volume) for use in human foods, the daily per capita intake[11] of cassia gum by humans would be 0.076 mg/kg bw/d. If it is assumed that only 10% of the population consumes cassia gum (i.e., the 90th percentile), the intake would be 0.76 mg/kg bw/d[12].

The total annual volume of the related gums, locust (carob) bean gum, xanthan gum and guar gum, was 790,000 kg, 1,857,000 kg, and 2,630,000 kg, respectively, in the USA in 1995. This corresponds to estimated daily per capita intakes[11] of 0.150, 0.353, and 0.502 mg/kg bw/day, respectively.

6.0 Biological Studies

6.1 Absorption, Distribution, Metabolism and Excretion

Galactomannans are recognized as components of dietary fiber and are resistant to digestive enzymes in the gastrointestinal tract [Trowell *et al.*, 1976]. Galactomannans were shown to be stable in artificial gastric juice [Nurnberg and Bleimuller, 1981].

The biodisposition of cassia gum has not been studied, but is expected to be similar to structurally related galactomannans, including locust (carob) bean gum and guar gum, for which studies have been reported. In a digestibility study, groups of five male and five female rats (Purdue strain) were maintained on a mannose-free diet and provided 1% locust (carob) bean gum. Eighty-five (85) to 100% of the dose was excreted as mannose [Tsai and Whistler, 1975]. No evidence of hydrolysis was observed when locust (carob) bean gum (in solution or suspension) was incubated with human gastric juice, duodenal juice + bile, pancreatic juice, and *Succus entericus* with or without added rabbit small gut membrane enzymes [Semenza, 1975].

Ten (10) % (w/w) aqueous solution of guar gum was shown to decrease the rate of gastric emptying of rats [Shah *et al.*, 1986]. It was suggested that 5% aqueous solution guar gum decreased the efficiency of nutrient digestion and absorption, and increased secretion of digestive juices in rats [Ikegami *et al.*, 1990]. Guar gum at a concentration of 100 g/kg was fed to rats as part of a basal diet. Only 4% was excreted in the feces indicating that guar gum was almost completely fermented [Nyman and Asp, 1982].

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[11] Intake (mg/kg bw/day) calculated as follows: [(annual volume, kg) x (1 x 10^6 mg/kg)]/[240 x 10^6 persons x (60 kg bw/person) x 365 days].

[12] Intake (mg/kg bw/day) calculated as follows: [(annual volume, kg) x (1 x 10^6 mg/kg)/[24 x 10^6 persons x (60 kg bw/person) x 365 days].

It is expected that cassia gum would be excreted unchanged. Fermentation of cassia gum by gut microflora may occur to a small extent [Nyman and Asp, 1982]. Any hydrolyzed material would be absorbed and metabolized in normal biochemical pathways [Mathews and van Holde, 1990].

6.2 Toxicological Studies

(A summary of all available toxicity data is presented in tables 4 and 5)

Acute Oral Toxicity

Cassia gum exhibits very low acute toxicity. The oral (gavage) LD50 value of cassia gum was reported to be >5,000 mg/kg bw in male rats [Schering, 1986].

Repeated Dose Toxicity and Carcinogenicity

Cassia gum as a dietary admixture is well tolerated in animals and did not produce evidence of toxicity in three repeated dose studies in dogs, cats and rats. A decrease in food consumption was commonly observed and is attributed to the absorption of water by cassia gum within the gastrointestinal tract and subsequent increase in volume and bulk. The three studies summarized below were conducted according to the Organization for Economic Cooperation and Development Principles of Good Laboratory Practices [OECD, 1981] and were inspected by a Quality Assurance Unit.

Two groups of four male and four female Beagle dogs were provided with either 0.75% or 2.5% (w/w) cassia gum as part of a canned dog-food diet for 90 days. The average dose levels calculated on the basis of food consumption data were approximately 1000 and 3500 mg/kg bw/d [>1000-times the anticipated daily dietary exposure levels, respectively]. A control group received a similar diet containing 0.23% (w/w) carob (crushed locust beans) [Schuh, 1990].

Clinical, biochemical, hematological and urinary parameters were evaluated. Slight but statistically significant changes in some biochemical and hematological parameters were reported periodically in males and females in both dose groups. Since most of the changes were not dose- or time-dependent as compared to controls, they were not considered to be compound related. Bone marrow examinations, urinalysis, coagulation studies, and gross pathology including organ weights were conducted in all animals. Microscopic examination was performed on all major organs for all animals in the control and high dose groups. For animals in the low dose group, histological examination was limited to the liver, kidney, heart, and macroscopic findings. Survival was 100%. A dose-dependent increase in water consumption was observed in test animals and was most likely associated with water retention in the gastrointestinal tract by cassia gum. No compound-related adverse effects were reported [Schuh, 1990].

Three groups of five male and five female cats were provided with 0, 0.5% or 2.5% (w/w) cassia gum as part of a standard canned-food diet for 13 weeks. The dose levels were not available. Clinical, hematological, urinary, and biochemical parameters were evaluated and were in the normal range. There were no consistent treatment related dose-dependent adverse effects reported. Histological examination

was performed on all major organs in all animals. There were no histopathological findings. Survival was 100%. [Virat, 1984].

In a 28-day study, five groups of five male and five female Sprague Dawley rats were administered cassia gum as part of a powdered diet at concentrations of 0, 0.25%, 1.0%, 2.5%, or 5.0%. The dose levels were calculated [FDA, 1993] to be 0, 125, 500, 1250, or 2500 mg/kg bw/d. A sixth group, which was fed an untreated powdered diet, was treated intragastrically with cassia gum as a suspension in distilled water, two times a day at a dose of 1000 mg/kg bw/d.

Clinical, hematological, urinary, and biochemical parameters were evaluated. Generally, all hematological parameters examined were considered to be within the normal range of the background data for the species tested. The following minor changes were observed but were not considered treatment-related because they did not occur in the high oral dose group: a statistically significant decrease in the red blood cell count and packed cell volume of males and females at the 500 and 1250 mg/kg bw/d levels; a statistically significant decrease in mean hemoglobin values of females in the 500 and 1250 mg/kg bw/d groups; and a statistically significant increase in white blood cell count for males in the 500, 1250 and 2500 mg/kg bw/d levels.

Clinical chemistry findings included statistically significant increases in mean glucose concentrations of males and females in the 500 and 1250 mg/kg bw/d groups, and mean triglyceride concentrations of males only in the 500 and 1250 mg/kg bw/d groups. These changes are not considered dose-related because they were not observed at the highest dose. A decrease in blood urea nitrogen was statistically significant but not dose-dependent for males in the 500, 1250 and 2500 mg/kg bw/d groups, but was not statistically significant for females in the 1250 and 2500 mg/kg bw/d groups. Statistically significant, dose-dependent decreases in sodium were observed in males in the 500, 1250 and 2500 mg/kg bw/d groups, and the 1000 mg/kg bw/d intragrastic suspension group, and females at 1250 and 2500 mg/kg bw/d only. A statistically significant, dose-dependent increase in group mean triglyceride concentrations was reported in females in the 500 and 1250 mg/kg bw/d groups. Statistically significant but not dose-dependent reduced mean total protein (6.2%) and albumin levels (7.7%) of males in the 2500 mg/kg bw/d group, statistically significant elevated potassium levels (13.0%) of males in the 1250 mg/kg bw/d group, and decreased chloride levels (6.8%) of males treated 1000 mg/kg bw/d intragastrically were also observed. Body weight gain was statistically significantly reduced (20%) in males in the 2500 mg/kg bw/d group and in females (17% each) in groups fed 500 and 1250 mg/kg bw/d, and 1000 mg/kg bw/d intragastrically. These effects were observed when high dose levels of carbohydrate-based gums were administered to rats [Takahashi, 1994] and are not considered to be biologically significant.

All animals were necropsied and all tissues and organs were examined macroscopically. Histological examination was performed on major organs for animals in the control group, high dose group, and group treated intragastrically. Group mean absolute kidney weights were statistically significantly decreased in males fed 500 mg/kg bw/d (8.0%), 2500 mg/kg bw/d (15.3%), and 1000 mg/kg bw/d (6.9%) intragastrically. Group mean relative kidney weights were statistically significantly increased in females

[redacted]

fed 2500 mg/kg bw/d (11.3%). A dose-relationship was not reported. These changes in the kidney weights were not considered treatment-related since there were no changes in relative weight in males and in absolute weight in females and no histopathology was seen [Zühlke, 1990].

The structurally related galactomannan, guar gum did not produce evidence of compound-related toxicity when groups of 10 male and 10 female rats (strain not provided) were provided 0, 1%, 2%, or 5% guar flour as a dietary admixture for 90 days [Til *et al.*, 1974]; when two groups of 15 male and 15 female rats were provided either 0 or 5% guar flour in their diet for 24 months [Krantz *et al.*, 1948]; or when groups of 5 male and 5 female Sprague-Dawley rats were administered partially hydrolyzed guar gum by gavage for 28 days at 0, 500, or 2500 mg/kg bw/d [Takahashi, 1994]. When rats were provided guar gum in the diet for 91 days at levels of 1%, 2%, 4%, 7.5%, or 15%, a decrease in weight gain in females of all dose groups and males at the two highest levels was reported. Bone-marrow cellularity was reduced at the 15% level. A dose-related decrease in the absolute kidney weights of males was reported, but relative kidney weights were normal and no histopathology was seen[Graham *et al.*, 1981].

No compound related toxicological effects were reported when four groups of 5 male and 5 female Beagle dogs were fed 0, 1%, 5%, or 10% (dose levels were not available) of a precooked mixture containing guar and locust (carob) bean gum for 30 weeks [Cox *et al.*, 1974].

When two monkeys received 1 g of guar flour in their diet daily, growth, hematology, and overall health were normal. One monkey died after 16 months, and the other was sacrificed at 24 months. No abnormalities were noted upon gross examination and histopathology [Krantz, 1948].

Similarly, the structurally related galactomannan, locust (carob) bean gum, did not produce evidence of compound-related toxicity in groups of 10 male and 10 female rats given locust (carob) bean gum in the diet at levels of 0, 1%, 2% or 5% for 90 days [Til *et al.*, 1974].

There are no carcinogenicity studies on cassia gum. However, other gums including locust (carob) bean, guar, and tara when fed to rats and mice were not carcinogenic and were not mutagenic in appropriate test systems. The chemically related galactomannan locust (carob) bean gum did not induce cancer in F344/N rats or B6C3F1 mice [NTP, 1981], and is not mutagenic [Maxwell and Newell, 1972]. When guar gum was provided to F344/N rats or B6C3F1 mice as a dietary admixture at concentrations of 25,000 or 50,000 ppm (≈1250 mg/kg bw/d or 2500 mg/kg bw/d) for 103 weeks, it did not induce cancer [NTP, 1982a]. Similarly, when locust (carob) bean gum was provided to F344/N rats or B6C3F1 mice as a dietary admixture at concentrations of 25,000 or 50,000 ppm (≈1250 or 2500 mg/kg bw/day) for 103 weeks, it did not induce cancer [NTP, 1981]. F344/N rats fed diets containing 0, 25,000 or 50,000 ppm (0, 1250 or 2500 mg/kg bw/day) tara gum for two years exhibited no tumorigenic effects related to the test material [NTP, 1982b; Borzelleca *et al.*, 1993].

No adverse effects were reported when five volunteers ingested 1 gram of the structurally related substance guar flour in a capsule per day for 10 days [Krantz, 1948].

Table 4: Summary of Acute and Repeated Dose Toxicity Tests Performed on Cassia Gum (Diamalt CS™)			
Assay	Cassia Gum Test Dose(s)	Result(s)	Reference
Acute Oral Toxicity – Male Rats	5000 mg/kg bw/d by gavage	LD50 >5000 mg/kg	Schering 1986
Repeated Dose 28 day- Rats	0,125, 500, 1250 and 2,500 mg/kg bw/d as dietary admixture and 1000 mg/kg bw/d by gavage	NOAEL: 2,500 mg/kg bw/day oral or 1,000 mg/kg bw/d (gavage)	Zuhlke, 1990
Repeated Dose 90 days - Cats	0, 250 and 1,250 mg/kg bw/d as dietary admixture	NOAEL: 1,250 mg/kg bw/d	Virat, 1984
Repeated Dose 90 days - Beagle Dogs	1,000 and 3,500 mg/kg bw/d as dietary admixture	NOAEL: 3,500 mg/kg bw/d	Schuh, 1990

Reproductive and Developmental Toxicology

Four reproductive or developmental toxicology studies have been conducted including a one-generation study in cats, a two-generation study in rats, and teratology studies in rats and rabbits in which cassia gum was presented as a dietary admixture or administered by gavage. Cassia gum did not produce any evidence of embryotoxicity or teratogenicity. Only a slight increase in absolute and relative ovarian weights was noted at the high-dose level in the cat study, but was not accompanied by treatment-related histopathological changes and therefore was considered to have no biological relevance. In this study, high mortality of unknown origin was observed in the control group [Virat, 1989]. The four studies summarized below were conducted according to OECD Principles of Good Laboratory Practices [1981] and were inspected by a Quality Assurance Unit.

In a one-generation reproductive study, three parental groups (F_0 generation) of 10 male and 20 female cats were provided 0, 0.75%, or 2.5% (w/w) (doses were not available) cassia gum as part of the diet for at least 83 weeks. After at least 36 weeks, one male was mated with two females of the same dose group. At day 4 post-partum, all kittens in excess of 4 per litter were culled. The F_1 generation was terminated after at least 13 weeks and the F_0 generation was terminated after the 83-week treatment period [Virat, 1989].

Clinical, hematological, urinary, and biochemical parameters were evaluated. No treatment related effects on hematology parameters were noted. A slight, statistically significant decrease in activated pro-thrombin time (APTT) was reported in treated parental females in both groups at week 26, but was considered to have no biological significance because the changes were transient and within normal range. After 13 weeks of dosing, a statistically significant increase in bilirubin was noted in males and females in the 1250 mg/kg bw/d group, and females in the 375 mg/kg bw/d group. From week 26 onwards, this change was only observed in males and females in the 1250 mg/kg bw/d group. The values were transient, never reached a biologically significant range, and did not correlate with morphological alterations.

Therefore, the biological significance was considered unclear, but minor with respect to toxicity. Slight, statistically significant increases in clinical chemistry parameters were observed but were not dose-related and considered to have no biological significance.

Histological examination was performed on all tissues for all F_0 generation cats in the control and high dose groups, and on two kittens from each litter of the control and high dose groups. X-ray skeletal examination was performed on all kittens [Virat, 1989].

High mortality occurred in the control group after 2-3 weeks postpartum resulting in several total litter losses in this group. The mortality was preceded by several clinical signs but no consistent pattern of pathology was observed. Although treatment groups were unaffected, appropriate comparisons between control and treatment groups were impaired [Virat, 1989].

Some effects were observed in both generations, but were not clearly associated with the test compound. For example, females in the high-dose F_0 group exhibited a decrease in food consumption during late gestation and a slight increase in absolute and relative ovarian weights. The latter was not accompanied by evidence of histopathology. In the F_1 generation, the combined incidence of stillborn births and neonatal deaths was significantly higher in the high-dose group. No other findings were noted. The authors concluded that while there was no unequivocal evidence of reproductive toxicity at the highest dose level, the concentration of 375 mg/kg bw/d (w/w) cassia gum is considered a no observed adverse effect level (NOAEL) [Virat, 1989].

In a two-generation reproductive study using Sprague-Dawley rats, five groups of 25 males and 25 females were provided with 0, 5,000, 20,000, or 50,000 ppm of cassia gum, or 50,000 ppm of cassia gum-AW[13] as part of the diet. All parental rats (P generation) were treated for approximately 10 weeks prior to mating, during mating, gestation, and lactation. The dose levels were calculated [FDA, 1993] to be 0, 250, 1000, or 2500 mg/kg bw/d. Pregnant females were allowed to rear their offspring (F_{1a}) to weaning. Rats in both 2500 mg/kg bw/d dose groups exhibited low pregnancy rates and were mated again with the same males. They were allowed to litter, and the subsequent offspring (F_{1b}) were terminated on day 5-7 postpartum. Selected F_{1a} offspring were treated for a 10-week period of maturation, and during mating, gestation, and lactation. Pregnant F_{1a} females were allowed to rear their offspring (F_2) to weaning [McIntyre, 1990].

[13] In order to evaluate the potential for adverse effects associated with vegetable by-products that may be present in cassia endosperm flour, cassia gum-AW was highly purified in preparation for testing by thoroughly washing cassia endosperm flour with isopropanol followed by careful drying.

All P and F_1 animals were submitted for necropsy. Histopathological examination was performed on gross lesions and on the reproductive organs for all controls and animals fed 2500 mg/kg bw/d cassia gum. No compound related adverse effects were noted. The pregnancy rate of P females was slightly decreased at the first mating in the two 2500 mg/kg bw/d dose groups, but a subsequent mating revealed no evidence of a treatment-related effect on fertility. No adverse effects related to treatment with cassia gum were reported on gonadal function, mating behavior, conception, parturition, weaning and the growth and development of the offspring. There was a slight, but not statistically significant, decrease in the mean body weights of F_{1a} and F_2 pups in the group that received cassia gum-AW [McIntyre, 1990].

In a teratology study, three groups of 28 pregnant Sprague-Dawley rats were administered cassia gum by gavage in distilled water at concentrations of 0, 17.5, or 50 mg/ml (0, 350, or 1000 mg/kg bw/d) for 14 days from days 6 to 19 post-coitum (inclusive). The control animals received the vehicle (distilled water) alone. A fourth group containing 29 rats was administered cassia gum-AW by gavage in distilled water at a concentration of 50 mg/ml daily (1000 mg/kg bw/d) over the same period. All animals were sacrificed and examined on day 20 post-coitum [Muller, 1989]. No compound-related adverse effects were reported for pregnancy incidence, implantation, post-implantation loss, fetal defects upon necropsy. A statistically significant reduction in mean daily food consumption and mean body weight gain was observed in animals dosed at 1000 mg/kg bw/d of cassia gum or cassia gum-AW, and was considered to be a treatment related effect. However, these effects were not accompanied by any evidence of embryotoxicity or teratogenicity [Muller, 1989].

In a related teratology study, three groups of 20 pregnant New Zealand White rabbits were administered cassia gum by gavage in distilled water at concentrations of 0, 17.5 or 50 mg/ml (0, 350 or 1000 mg/kg bw/d) for 22 days from days 6 to 27 post-coitum (inclusive). The control animals received the vehicle (distilled water) alone. A fourth group of 20 pregnant rabbits was administered 1000 mg/kg bw/d cassia gum-AW by gavage in distilled water over the same period. All animals were sacrificed and examined on day 28 post-coitum [Muller, 1989].

No compound-related adverse effects were reported for clinical observations, mortality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. A reduction in mean daily food consumption was reported in animals administered 1000 mg/kg bw/d of cassia gum, and was accompanied by a reduction in mean fetal weights. However, these effects were not statistically significant. No indication of teratogenicity was reported [Muller, 1989].

[redacted]

Table 5: Summary of Reproductive and Develpomental (Teratological) Tests Performed on Cassia Gum (Diamalt CS™)			
Assay, Reference	Cassia Gum Test Dose(s)	Result(s)	Reference
One generation Reproduction in Cats	0, 375, 1,250 mg/kg bw/d	Transient effects on biochemical parameters at 1,250 mg/kg bw/d. No unequivocal evidence of reproductive toxicity at highest dose level. NOAEL considered to be 375 mg/kg bw/d.	Virat, 1989
Two generation Reproduction in Rats	0,250,100 and 2,500 mg/kg bw/d	No effects on gonadal function, mating behavior, conception, parturition, weaning and the growth and development of offspring. NOAEL considered to be 2,500 mg/kg bw/d.	McIntyre, 1990
Teratology – Rats	0, 350, 1000 mg/kg bw/d	No compound related adverse effects for clinical observations, mortality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. NOAEL considered to be 1000 mg/kg bw/d.	Muller, 1989
Teratology - Rabbit	0, 350, 1000 mg/kg bw/d	No compound related adverse effects for clinical observations, mortality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. NOAEL considered to be 1000 mg/kg bw/d.	Muller, 1989

Mutagenicity and Genotoxicity

Three different tests have been conducted evaluating the mutagenic activity of cassia gum. These include the reverse mutation assay (Ames test), the chromosome aberration assay, and an *in vitro* gene mutation assay. All three *in vitro* studies were conducted in compliance with the OECD Principles of Good Laboratory Practice [OECD, 1981] and were inspected by a Quality Assurance Unit.

Cassia gum was tested, with and without metabolic activation, in the Salmonella typhimurium reverse mutation assay (Ames test) with strains TA1535, TA1537, TA100, and TA98 at concentrations of 4, 16, 62.5, and 1000 μg/plate. Cassia gum precipitated on the plates at concentrations of 62.5 μg/plate and higher. Increases in the number of revertant colonies observed in strains TA1535, TA1537, and TA98 were seen only at precipitating concentrations and were within the historical control data range. The increase observed in strain TA100 was dose related and was outside the historical control data range, but were seen only at precipitating concentrations [Verspeek-Rip *et al.*, 1998a].

As a second experiment to the study described above, cassia gum was tested at concentrations of 1.6, 8, 40, 200, and 1000 μg/plate, in all the Salmonella typhimurium strains and in Escheria coli strain WP_2uvrA. . Cassia gum precipitated on the plates at concentrations of 200 μg/plate and higher. There was a negative response in strains TA1535, TA98, and WP_2uvrA. Salmonella strains TA1537 and

[redacted]

TA100 showed dose-related increases in the number of revertant colonies, but only at precipitating concentrations [Verspeek-Rip *et al.*, 1998a].

Based on the results from the two experiments, it was concluded that cassia gum is mutagenic in Salmonella typhimurium strain TA100, and not mutagenic in strains TA1535, TA1537, TA98, and E.coli.strain WP_2uvrA [Verspeek-Rip *et al.*, 1998a].

The ability of cassia gum to induce chromosome aberrations in cultured peripheral human lymphocytes was evaluated at concentrations of 1, 3, and 10 μg/ml, with and without metabolic activation. Cassia gum did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations [Bertens *et al.*, 1998].

The effect of cassia gum on the induction of forward mutations at the thymidine-kinase locus in L5178Y mouse lymphoma cells, with and without metabolic activation, was tested at concentrations up to 10 μg/ml. Cassia gum did not induce a dose-related increase in the mutant frequency, therefore, it was concluded that it is not mutagenic in the mouse lymphoma L5178Y test system [Verspeek et al., 1998b].

In the three separate mutagenicity assays that were conducted on cassia gum, the only positive result observed was in the Ames assay with strain TA100 [Verspeek-Rip *et al.*, 1998a]. Positive results were observed only at precipitating concentrations. Given the fact that the other Ames test strains (TA1535, TA1537, TA98, and WP_2uvrA) produced negative results only at precipitating concentrations, and no positive results were observed in any other test system, cassia gum is not considered mutagenic in appropriate test systems.

6.3 Other Studies

Hypersensitivity

Ten albino guinea pigs were intradermally injected with 0.2% concentration and epidermally exposed to a 50% concentration of cassia gum. Approximately 24 hours prior to epidermal exposure, each animal was challenged with 10% sodium dodecyl sulfate. Five control animals were similarly treated with the vehicle control (corn oil). Two weeks after the epidermal application all animals were challenged with a 50% cassia gum concentration and the vehicle. Skin reactions varying between grade 1 and 3 were observed in all experimental animals but were not evident in the control animals. These observations were indicative of sensitization by cassia gum [Busschers *et al.*, 1998].

Skin prick tests performed on 125 pharmaceutical workers previously exposed to *Senna* pods (*Senna obtusifolia* is a synonym for *Cassia obtusifolia/tora*) revealed that 15.3% were allergic to *Senna*. None of the 20 unexposed pharmaceutical workers also tested had a positive skin test to *Senna* [Marks *et al.*, 1991].

The allergenicity of 4 different species of *Cassia* (*auriculata, occidentalis, roxburghii, and siamea*), which were found to be dominant in India, was evaluated in 140 patients with a history of

respiratory allergy. Intradermal skin testing was performed by injecting the antigenic extraction of the 4 *Cassia* species into the patients' forearms. Twelve percent (12%) had a positive skin reaction [Satheesh *et al.*, 1994].

A study on workers health was conducted on 62 volunteers at the B.F. Goodrich Diamalt Inc., plant in Munich, Germany. The purpose of the study was to determine the risk to workers' health during the production of thickening agents, including ground Cassia, with emphasis on pulmonary function and predisposition towards allergic reactions. The study program included: 1) a detailed medical history, 2) a physical examination, 3) clinico-chemical laboratory tests, 4) a pulmonary function test, 5) several allergy tests, and 6) dust measurements from different production areas. In the medical reports 54.8 % reported irritation of the eyes, nose, lungs, or skin and 37% were suffering from acute cold symptoms. While several positive results and diagnoses were made, there was no statistical evidence of correlation between symptoms and occupational factors (including number of days exposure to Cassia powder) or personal factors (such as age, gender, occupational non-related previous diseases). However, participants who displayed sensitization to at least one common allergen were also more frequently sensitized to Cassia powder and/or store mites. Also, the exposure to powder dust exceeded the limit value that is currently set for the inhalable dust fraction. The investigators recommended that preventive health protection measures be implemented in the workplace [Steget *et al.*, 1999].

The results of the first three sensitization studies for Cassia would bear no effect on the use of cassia gum in human foods, and in some cases the *Cassia* species tested are not those used in the manufacture of Diagum CS. In the fourth study, although positive reactions were observed in humans, there was no evidence of correlation between exposure to the Cassia powder and incidence of allergic reactions. Moreover, this is an occupational health issue and would not bear significant relevance to the safety of the finished product in food.

Muscle toxicity

The presence of muscle toxins has been associated with certain species of *Cassia*. Toxicological data demonstrate that in animal models, the characteristic symptoms of toxicity associated with *Cassia* species are not present when animals consume cassia gum (*i.e.,* Diagum CS™). Additionally, basic medium such as sodium bicarbonate or sodium citrate has been shown[14] to remove muscle toxins from ground seeds of *Cassia occidentalis* (Hebert et al., 1983). As part of a purification procedure, cassia gum is extracted[15] at least once with a comparable basic medium comprised of 1) water 2) alkanol[16] and/or acetone, 3) an alkaline substance such as alkali carbonates, sodium hydroxide, potassium

[14] The presence of the muscle toxin(s) in the basic aqueous extract was confirmed as follows: 1) the extract showed the toxic effects typically observed from muscle toxins and 2) treatment of the base extract with a protein precipitating agent resulted in formation of a precipitate that produced the characteristic pattern of toxicity observed for muscle toxins.

[15] US Patent Number 4,840,811

[16] Preferably ethanol, isopropanol, or propanol (US Patent Number 4,840,811)

hydroxide, or ammonia and/or 4) a reducing agent such as dithionite, sulfite, or ascorbic acid. This purification procedure would remove muscle toxins, if present. Therefore, muscle toxins either are not present in Diagum CS™ after it has undergone the processing to manufacture it, or they are present at levels that are not toxicologically significant.

Muscle toxicity has been associated with the consumption of *Cassia occidentalis* by various animal species [Hebert *et al.*, 1983; Graziano *et al.*, 1983; Suliman and Shommein, 1986; O'Hara and Pierce, 1974; Barth *et al.*, 1994; Irigoyen *et al.*, 1991; Martin *et al.*, 1981; Colvin *et al.*, 1986], and to a lesser extent in non-food grade *Cassia obtusifolia* when it is administered at extremely high doses far exceeding possible human consumption [Nicholson *et al.*, 1977; 1986; Putnam *et al.*, 1988; Loung and McCoy, 1992]. There have been no reports of muscle toxicity associated with *Cassia tora.* While some physical and chemical properties of the muscle toxin(s) has been characterized, the identity of the muscle toxin(s) has not been elucidated [Hebert *et al.*, 1983; Nicholson, 1986; Putnam *et al.*, 1988; Loung and McCoy, 1992]; rather, evidence for the muscle toxin(s) in *Cassia* species is based on clinical observations and a characteristic pattern of lesions.

Therefore, the presence of muscle toxins in cassia gum may be evaluated biologically. The symptoms of toxicity associated with muscle toxins in *Cassia occidentalis* and *Cassia obtusifolia* are described below. Toxicology studies have been performed on cassia gum (Diagum CS™) and include testing of several of the endpoints used to characterize muscle toxin toxicity [Hallagan *et al.*, 1997]. By comparing the results of common toxicological endpoints in studies on cassia gum and studies on *Cassia occidentalis* and *Cassia obtusifolia,* the potential presence of muscle toxins in cassia gum can be assessed.

Non-Food Grade *Cassia obtusifolia*

Nine New Zealand white rabbits were fed diets of pelleted feed containing 50%, 75%, or 100% dried and ground *Cassia obtusifolia* for 15 to 51 days. Two rabbits were fed fresh aerial portions of *Cassia obtusifolia* for the same duration. Five control animals received rabbit chow alone. Gross effects were observed in some animals and included decreased body weight, muscle atrophy, and brown urine. The brown urine was negative for myoglobin in all but two rabbits, and may be associated with pigments in the plant or animal metabolism. Microscopic examination revealed lesions in heart, cerebrum, and striated muscles. Lesions included muscle fiber atrophy, hyalinization, flocullar degeneration, mixed inflammatory cell infiltrate in skeletal muscles, and vacuolar change of myocardial fibers. The muscle lesions found in rabbits fed the dried product were much less severe than in animals fed the fresh product. In the groups fed the pelleted diet containing dried *Cassia obtusifolia,* six rabbits exhibited mild to moderate multifocal periportal lymphocytic aggregates in the liver, and three rabbits showed mild to moderate multifocal cortical interstitial aggregates of lymphocytes and plasma cells in the kidneys. Creatine kinase (CK) and aspartate amino transferase (formerly glutamate oxaloacetate transaminase, GOT) levels were elevated in animals treated with fresh portions of *Cassia obtusifolia* [Loung and McCoy, 1992].

Fifteen Holstein bull calves were fed *Cassia obtusifolia* seed as part of the diet at concentrations of 0, 12.5% or 50% for 25 days. In the high-dose group, the concentration was reduced to 37.5% for days 10-24 in an effort to increase palatability. Effects in both dose groups included intermittent feed refusal, diarrhea, and decreased weight gain. Clinical observations and results of serum biochemical determinations (*i.e.,* serum aspartate aminotransferase, creatine phosphokinase, and lactic dehydrogenase activity) showed no indication of damage to skeletal or cardiac muscles. Necropsy and histologic examination was performed on one animal only (since myopathy was not observed) and revealed no gross or microscopic lesions of skeletal muscle, heart muscle, spleen, liver, or kidney [Putnam *et al.*, 1988].

Ingestion of *Cassia obtusifolia* by cattle caused ataxia, recumbency, paresis, severe liver damage, myoglobinuria, pale skeletal muscles, elevated levels of serum GOT (SGOT) and creatine phosphokinase[17] (CPK), striated muscle degeneration and necrosis, and death. Toxic levels were estimated to be 12 to 25 pounds of *Cassia obtusifolia* per cow per day [Nicholson *et al.*, 1977; 1986].

Non-food grade Cassia occidentalis

An unidentified toxin present in seeds of *Cassia occidentalis* has been associated with toxicity in chickens [Hebert *et al.*, 1983; Graziano *et al.*, 1983], goats [Suliman and Shommein, 1986], rabbits [O'Hara and Pierce, 1974], cattle [Barth *et al.*, 1994], horses [Irigoyen *et al.*, 1991; Martin *et al.*, 1981], and pigs [Colvin *et al.*, 1986]. Signs of toxicity include ataxia, depression, weight loss, rapid shallow breathing, shivering, muscle tremors, muscle weakness, staggering, tachycardia, dyspnea, moaning, salivation, diarrhea, myoglobinuria, increased levels of creatine kinase, aspartate transaminase, and gamma glutamyl transferase, recumbency leading to death, cardiac and muscular degeneration, damage to the heart, liver, kidneys, lungs, vascular system, and skeletal muscle.

The characteristic symptoms of toxicity associated with muscle toxins in non-food grade *Cassia occidentalis* and *Cassia obtusifolia* were not observed in studies of food grade cassia gum (Diagum CS™). Therefore, muscle toxins either are not present in Diagum CS™ after it has undergone the processing to manufacture it, or they are present at levels that are not toxicologically significant.

The literature demonstrates that the processes of manufacturing and purifying food grade cassia gum from the raw product *Cassia obtusifolia* significantly reduces or eliminates any existing muscle toxins that may be present. For example, lesions in rabbits fed fresh *Cassia obtusifolia* were much more severe than lesions in rabbits fed air dried *Cassia obtusifolia*, indicating that dehydration and storage of the plant compromises the activity of the toxin(s). Similarly, goats fed roasted beans of *Cassia occidentalis* exhibited milder (5 g/kg bw) or no (2.5 g/kg bw) toxic effects as compared to goats fed unroasted beans at the same dose levels, indicating that roasting reduces or eliminates the toxic activity of the muscle toxin(s). The production of food grade cassia gum from the endosperm of

[17] Same as creatine kinase (CK)

Cassia obtusifolia occurs *via* a heated (200°C) process, which would significantly reduce the activity of toxin(s) that may be present. Therefore, the manufacturing and purification processes used to produce food grade cassia gum from *Cassia obtusifolia* effectively reduce or eliminate muscle toxins that may be present in *Cassia* used to produce Diagum CS™. This conclusion is supported by animal studies on food grade cassia gum, which demonstrate an absence of toxicity characteristically associated with the presence of muscle toxins in some *Cassia* species.

* * *

The foregoing and attached information considered, it is respectfully submitted that, under the conditions of intended use cassia gum is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it is generally recognized as safe.

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APPENDIX I

CONCLUSIONS OF EXPERT PANEL

We the Expert Panel have, independently and collectively, critically evaluated the data and information summarized in the Notification for Cassia gum and conclude that Cassia gum produced in accordance with current good manufacturing practices and meeting the food grade specifications described therein is GRAS by scientific procedures for use as a stabilizer (thickening and gelling agent) in foods.

Joseph F. Borzelleca, Ph.D.
Professor, Medical College of Virginia
09 June 2000

Michael Pariza, Ph.D.
Professor, University of Wisconsin-Madison
15 June 00

[redacted]

SUBMISSION END

000035.001

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June 23, 2000

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VIA HAND DELIVERY

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Food and Drug Administration
200 C Street, S.W. (HFS-206)
Washington, D.C. 20204

2000 JUN 26 P 3: 35

Re: GRAS Notification for Cassia Gum

Dear Dr. Kahl:

As requested, the purpose of this letter is to confirm that the intent of the above-referenced GRAS notification submitted on June 20, 2000 was to indicate that, for the reasons stated in the notification, B.F. Goodrich has determined that Cassia gum is exempt from premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it is generally recognized as safe under the conditions of intended use. B.F Goodrich's conclusion is based upon the evaluation of Cassia gum under the intended conditions of use by experts qualified by scientific training and experience.

Respectfully submitted,

Melvin S. Drozen
Counsel for B.F. Goodrich

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September 20, 2000

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VIA HAND DELIVERY AND E-MAIL

2000 SEP 21 A 11:53

Rudaina Alrefai, Ph.D.
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W. (HFS-206)
Washington, D.C. 20204

Re: GRAS Notification for Cassia Gum (GRAS Notice No. 000051)

Dear Dr. Alrefai:

As requested, the purpose of this letter is to provide a written response to the two questions concerning the above-referenced GRAS notice that you conveyed during our September 8, 2000 telephone conversation.

First, you requested clarification of a reference to *C. obtusifolia* and *C. tora* in the discussion of muscle toxicity in the notice which appeared to suggest that *C. obtusifolia* and *C. tora* are different species of cassia. As stated in the introduction on page 1 of the notice, *Cassia tora* is otherwise known as *Cassia obtusifolia*. On page 17 of the notice, in the paragraph beginning "Muscle toxicity," it is stated that muscle toxicity in laboratory animals has been associated with high dose feeding of non-food grade *Cassia obtusifolia* but that there have been no reports of muscle toxicity associated with *Cassia tora*. This statement was not intended to imply a significant distinction between *C. obtusifolia* and *C. tora*, but rather between non-food grade *C. obtusifolia/tora* and food grade *C. obtusifolia/tora* or any other food grade Cassia gum. Thus, it would be more accurate to say that there have been no reports of muscle toxicity associated with food grade *Cassia tora/obtusifolia*. As the notice explains (see discussion of muscle toxicity on pp. 16-17), the purification process that is part of the production of the notifier's food grade Cassia gum (Diagum CS) eliminates or reduces to toxicologically insignificant levels any muscle toxins that might be present in the unprocessed Cassia.

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Second, in light of the Joint (FAO/WHO) Expert Committee on Food Additives' 2 ppm lead specification for gums and the notice's indication that typical analytical values for lead in Cassia gum are less than 1 ppm, you asked whether B.F. Goodrich would agree to lower its proposed lead specification from $\leq$ 5 ppm (the current *Food Chemicals Codex* limit for lead in locust bean gum and guar gum) to $\leq$ 2 ppm. B.F. Goodrich agrees to adopt a lead specification of $\leq$ 2 ppm for its Cassia gum for use in food.

We trust that this letter fully responds to your questions. If you have any further questions, please contact me.

Respectfully submitted,

Melvin S. Drozen
Counsel for B.F. Goodrich

AM

Alrefai, Rudaina H

From:
Sent: Thursday, September 28, 2000 5:31 PM
To: Alrefai, Rudaina H
Cc: Melvin Drozen
Subject: Dear Dr. Alrefai:

Dear Dr. Alrefai:
Per your request, here are references for two articles discussing the essential identity of C. tora and C. obtusifolia:

Proc. Indian Acad. Sci. (Plant Sci.), Vol 96, No.4, October 1986, pp. 321-326
Authors: S K Upadhyaya and V Singh
Title: Phytochemical evaluation of Cassia obtusifolia L. and Cassia tora L.

J. Adelaide Bot. Gard. 16: 55-58 (1995)
Author: B.R. Randell
Title: Taxonomy and Evolution of Senna Obtusifolia and S. Tora
(Senna is a synonym for Cassia)

If you would like copies of the articles, please let me know.
Best regards, John Foley

000042

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Washington, D.C. 20204

Re: GRAS Notification for Cassia Gum (GRAS Notice No. 000051)

Dear Dr. Alrefai:

As requested, enclosed are the two articles referenced in my September 28 email. As you will note, the Randell article reviews the taxonomy of *tora* and *obtusifolia*, noting that different botanists have taken the position that they are (1) the same species, (2) different species, or (3) infraspecific taxa within the same species. Although Randell ultimately concludes that *tora* and *obtusifolia* are best viewed as distinct species, the author states that it is universally acknowledged that *obtusifolia* and *tora* are closely related, with strong similarities in gross morphology, ecological requirements, and seed chemistry and that the constant differences between them "are few and relatively minor." The article by Upadhyaya and Singh compares phytochemical components of *obtusifolia* and *tora* and concludes that they are phytochemically distinct species with *tora* being derived from *obtusifolia* by the loss of certain phytochemical constituents. Notwithstanding the authors' view that they are separate species, the article tends to reinforce the fact that *tora* and *obtusifolia* are very closely related.

If you have any further questions, please contact me.

Cordially yours,

000043

John F. Foley

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Washington, D.C. 20204

Re: GRAS Notification for Cassia Gum; GRN No. 51

Dear Dr. Kahl:

On behalf of our client, BF Goodrich, we are writing to respond to four questions raised by the Agency regarding GRN No. 51 for cassia gum.[1]

1. What species is used as the source for the food-grade cassia gum?

BF Goodrich's food grade cassia gum is derived from the seeds of *Cassia tora* and/or *Cassia obtusifolia*. *C. tora* and *C. obtusifolia* closely resemble one another but have been classified by some botanists as distinct species.

2. How is C. tora harvested? Is it picked in the wild or cultivated?

The seed pods of *C. tora* and *C. obtusifolia* that are used in the production of the food-grade cassia gum are generally picked in the wild. *C. tora* and *C. obtusifolia* are harvested when the plant is ripe (e.g. November to January) in India. Once the pods are harvested, they are threshed to obtain the seed grains, which are the raw material for cassia gum.

---

[1] The specific questions were conveyed to us by you in a telephone message.

3. Does the manufacturer know whether C. occidentalis is likely to be included in the harvested seeds?

C. occidentalis does not grow in conjunction with *C. tora* or *C. obtusifolia* and hence, even by oversight, is unlikely to be collected together. The *C. occidentalis* pods look distinctly different from *C. tora* and *C. obtusifolia* and collectors do not collect *C. occidentalis*. Furthermore, BF Goodrich has a specification to limit the presence of *C. occidentalis* in the cassia seeds that are the raw material used to produce the food-grade Diagum CS. The specification limits *C. occidentalis* as well as black splits and foreign matter to a combined level of 2%. A copy of a Raw Material Specification Sheet (for "super grade" cassia splits) that BF Goodrich will apply for food-grade cassia gum[2] is enclosed together with the specific test standard for *C. occidentalis* (Test No. NC 02602).[3] (Tab 1) This specification applies to seeds prior to their undergoing an extensive cleaning process, which is described below in response to question 4. After cleaning, the level of *C. occidentalis*, if any, would be substantially lower.

4. Describe the steps taken during the manufacturing process to ensure that the food-grade gum will be of a suitable purity and not contain harmful levels of muscle toxins.

The seeds are subject to a cleaning process before splitting. The seed purification includes mechanical steps to remove stones, dirt and seeds other than *C. tora* and *C. obtusifolia*. Specifically, after removal of dust and coarse impurities with a round sieve, the seeds pass two separators and finally a gravity separator. At this stage, stones and seeds with a similar size but different weight compared to *C. tora* or *C. obtusifolia* seeds are removed. Any *C. occidentalis* seeds that remained at this point likely would be reduced further by the gravity separator since *C. occidentalis* has an average weight about 20% less than *C. tora* or *C. obtusifolia*.

After cleaning, the seeds undergo a splitting process to separate the endosperm used to produce cassia gum from the husk and germ. The splitting process starts with roasting, followed by two dehusking steps. The roasting involves continuously feeding non-comminuted seed grains

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[2] Goodrich produces different grades of cassia gum for use in animal feed and various non-food applications. The "super grade" cassia split specifications are currently applied to cassia gum intended for animal feed use and will be applied to food-grade gum when full-scale production of food-grade material begins. *Finished* food grade cassia gum is subject to specifications that are more stringent in several respects than the raw material specifications for "super grade" cassia splits (See response to question 4.)

[3] Each shipment of seeds is analyzed for compliance with the raw material specifications upon delivery. In the last collection season 1999/2000 on average only 0.02% *C. occidentalis* seeds were found. Based on testing of 325 samples, <<1% of *C. occidentalis* seeds was detected in 47 deliveries.

into a rotation roaster, where they are heated to approximately 200°C for about 30 seconds. After two cleaning steps with a separator and a round sieve, the splits pass again through two dehuskers. These splits are purified by a separator, two polishers and a brusher.

Cassia splits resulting from the above procedure are packed and transported to another facility for further processing into final cassia gum. Each split lot is subject to raw material control according to SOP XX 003 (copy enclosed Tab 2).

For food grade cassia gum, the splits are subject to the following additional purification steps before milling:

Metal cleaner: The splits pass a strong magnet to remove metal

- Polisher: Foreign material is removed which may come from the bags
- Aspirator: Removal of dust
- Polisher: Stoner: particles heavier than the average split weight are removed, for example splits which still include the germ.
- Color sorting equipment: detects all splits in color ranges not conforming to the desired split color. Dark splits can be removed nearly by 100%.
- Washing: the splits are washed with defined quantities of water.

The aspirator, stoner and the color sorting equipment were not part of the manufacturing procedure at the time that cassia gum was produced for the feeding tests 10 years ago.

Chrysophanic acid is the anthroquinone believed to be responsible for muscle toxicity effects associated with certain species of cassia pods. Because chrysophanic acid makes up approximately half of the anthroquinones in cassia plants, a 5 ppm specification for chrysophanic acid effectively establishes a 10 ppm specification for total anthroquinones.[4] The effectiveness of the processing in eliminating toxins is believed to be partly attributable to the heating step and partly to the thorough removal of husks since it is understood that most of the anthroquinones in the cassia seed reside in the husk.

In its quality control laboratory, BF Goodrich analyzes the chrysophanic acid content of each batch of both the raw material (splits) and final cassia gum (Diagum CS) using the same HPLC method.[5] The finished food grade gum is subject to a 5 ppm specification for

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[4] *See* Crawford L, McDonald GM, and Friedaman M (1990) Composition of sicklepod (cassia obtusifolia) toxic weed seeds. J. Agric. Food Chem. 38 (12) 2169-2175 (copy enclosed Tab 3).

[5] The splits are milled in the laboratory by a stone mill into a particle size similar to cassia gum before hydrolyzation.

chrysophanic acid. Enclosed (Tab 4) are copies of the finished product specifications, HPLC method and a certificate of analysis (from a pilot batch[6]) for finished food-grade Diagum CS confirming compliance with the 5 ppm specification.

The effectiveness of the manufacturing process in ensuring the safety of food-grade cassia gum is established by animal feeding studies which used as the test material commercial cassia gum obtained from the same sources and processed in the same way as the current food-grade product. Like the current product, the test material used in the toxicological studies was obtained from a combination of *tora* and *obtusifolia* plants collected in the wild and processed by dehusking, splitting, washing and roasting.

One notable difference between the current food-grade gum and the cassia gum used in the feeding studies is that the specification for chrysophanic acid at the time of the feeding studies was 10 ppm rather than 5 ppm. The current lower chrysophanic acid specification was made possible by the installation of additional polishers and a brusher in the dehusking and splitting process. Although the toxicology section of the GRAS notification (on p. 16) discusses the ability of a four step purification process involving extraction of cassia gum with "a . . . basic medium comprised of water, 2) alkanol and/or acetone, 3) an alkaline substance . . . and/or 4) a reducing agent" to eliminate any muscle toxins that may be present in cassia gum, we have now been advised by the company that none of this four-step process is employed in the current manufacturing process nor was it employed in the production of the test material used in the animal feeding studies. The four-step chemical cleaning procedure was developed at a time when it was not believed that comparable cleaning could be achieved through mechanical measures. The purity of the splits manufactured by the current mechanical process is equal to the chemical process. Furthermore, the new cleaning process causes less pollution, is environmentally beneficial and more economical than the chemical process.

We trust that the above information and enclosures fully answer the Agency's questions. If you require any further clarification, we will be happy to discuss such matters by telephone or in a meeting, as you prefer.

Respectfully submitted,

Melvin S. Drozen
Counsel for B.F. Goodrich

Enclosures

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6 Certificates of analysis for multiple lots of food-grade Diagum CS are not yet available because Goodrich has not begun large-scale production of food-grade Diagum CS.

List of Enclosures

Tab 1 - Raw Material Specification sheet for Super Grade Cassia Splits and Quality Assurance Test NC 02602

Tab 2 - SOP-IPXX 003

Tab 3 - Crawford et al. Composition of sicklepod (Cassia obtusifolia) toxic weed seeds

Tab 4 - Diagum CS (FG) specifications, certificate of analysis for Diagum CS Batch No. C-2000040521, HPLC method for chrysophanic acid

[redacted]

000065

Raw Material Specification
Natural Products Chemistry
Quality Assurance

Freedom Chemical Diamalt

NAME OF SUPPLIER:

[redacted]

RAW MATERIAL SPECIFICATION

CASSIA SPLITS / SUPER GRADE

GENERIC NAME	CASSIA TORA / SYN. C. OBTUSIFOLIA
CHEMICAL DESCRIPTION	CASSIA GALACTOMANNAN

TEST	SPECIFICATION	TESTMETHOD	TEST-No
APPEARANCE	ENDOSPERM SPLITS	visual	NC 00201
COLOUR	YELLOW TO SLIGHTLY REDDISH-BROWN	visual	NC 00301
MOISTURE	MAX. 8 %	IR-thermobalance	NC 00501
TOTAL COLOUR VALUE	MAX. 200 PPM	photometrical / wavelength 520 nm	NC 02501
CHRYSOPHANIC ACID	MAX. 10 PPM	HPLC	NC 02401
- BLACK AND DARK-BROWN SPLITS - FOREIGN MATTER - C.OCCIDENTALIS	MAX. 2 %	gravimetrical	NC 02602

Product-No.	Revision	Replaces	Authorized	Date	Signature
[illegible]	FS 11/96	17.11.96	Dr Kt/Qual Kon	25.05.98	

TESTING STANDARD

CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	**page 1 of 6**

CONTENTS

I Principle

II Definition

III Equipment

IV Sampling

V Test procedure

VI Calculation

VII Documentation

TESTING STANDARD

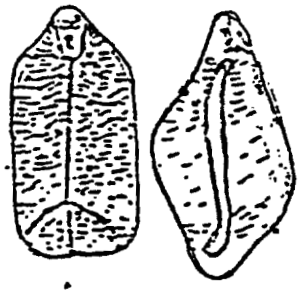
CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	**page 2 of 6**

I PRINCIPLE

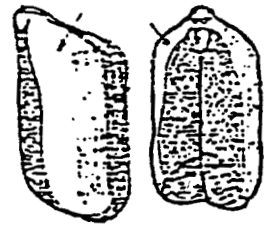
The seeds of Cassia tora / obtusifolia and of Cassia occidentalis are quite different in shape and size.They can be easily segregated by hand and the amount of C.occidentalis is determined gravimetrically.

II DEFINITION OF CASSIA SEEDS

C.obtusifolia



C.tora



Cassia obtusifolia is defined by a narrow and S-shaped aureole,while C.tora forms a broad and almost straight aurole.

C.occidentalis

C.occidentalis is a 4-5 mm long flat,brownisch disk

III EQUIPMENT

1. sampler
2. lab spoon (e.g. 150 mm)
3. weighing boat
4. bend pincer
5. magnifying glass
6. white sheet
7. balance (readings: 0,001 g)
8. calculator
9. plastic bags
10. sheet welder

IV SAMPLING

1. Select ($\sqrt{n} + 1$) of the bags at random from which 100 g - samples are drawn.These are thoroughly mixed to a representative sample and analysed for Cassia occidentalis,foreign matter and black seeds.

V TEST PROCEDURE

1 weigh 100 g of seeds and make thereof 10 groups with each 10.00 g (the numbering of groups is $G_1, G_2 \ldots G_{10}$)

2 pour 10 g of seeds onto a white sheet

3 segragate the sample by hand under a magnifying glass into following categories:

Cassia tora/obtusifolia
Cassia occidentalis
foreign matter
black seeds

4 weigh the separated categories

5 ***Partial measuring result:***

weight Cassia occidentalis	$= W_{co}$ [g]
weight foreign matter	$= W_{fm}$ [g]
weight black seeds	$= W_{bs}$ [g]

6 repeat step 2-5 for each 10 g - group ($G_1 \ldots G_{10}$)

VI Calculation

1 sum up each category of the ten groups ($G_1 ... G_{10}$) analysed

2 ***Partial measuring result:***
weight Cassia occidentalis:

$$\Sigma(W_{co}) = G_1(W_{co}) + G_2(W_{co}) \ldots G_{10}(W_{co}) \text{ [g]}$$

weight foreign matter

$$\Sigma(W_{fm}) = G_1(W_{fm}) + G_2(W_{fm}) \ldots G_{10}(W_{fm}) \text{ [g]}$$

weight black seeds

$$\Sigma(W_{bs}) = G_1(W_{bs}) + G_2(W_{bs}) \ldots G_{10}(W_{bs}) \text{ [g]}$$

3 ***Content Cassia occidentalis in percent***

$$\text{Percent Cassia occidentalis} = \frac{\Sigma(W_{co}) \text{ [g]} \times 100}{100 \text{ [g]}} \text{ [\%]}$$

4 ***Content foreign matter in percent***

$$\text{Percent Foreign Matter} = \frac{\Sigma(W_{fm}) \text{ [g]} \times 100}{100 \text{ [g]}} \text{ [\%]}$$

5 ***Content black seeds in percent***

$$\text{Percent black seeds} = \frac{\Sigma(W_{bs}) \text{ [g]} \times 100}{100 \text{ [g]}} \text{ [\%]}$$

6 ***Requirements:*** see specification

TESTING STANDARD

CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	**page 6 of 6**

VII Documentation

1 document results in lab journal

2 put segregated categories (c. occidentalis/foreign matter/black seeds) of all ten groups together and seal them in plastic bags

SOP

GENERAL WORKING ORDER

Title: Raw Material Handling

Valid since: 01.10.1997

This SOP replaces:

Policy: To describe the general process of the receipt of raw material, sampling, storage.

Responsibility: General Manager, Production Manager, Senior Officer Quality Control

Changes:

distribution: General Manager

Production Office

1. Policy:

This SOP describes the steps to be followed at the receipt of raw material in regard of handling, taking samples, and storage.

2. Area and Responsibility:

Loading platform, warehouse, laboratory

Responsible: General Manager
Production Manager
Administration Manager
Lab in charge

3. Regulations

3.1. Handling of the delivering truck

3.1.1. The truck has to be positioned at the forward platform, the canvas to be opened only under the roof during monsoon time to prevent rain water wetting of the gunny bags.

3.1.2. The freight bills have to be checked by the office to ascertain the conformity of the delivery with the raw material orders.

3.1.3. The delivery is labeled with a unique batch number as per usual standard to allow the verification of the material throughout the whole production.

3.1.4. The warehouse officer allots a suitable warehouse space and takes care of orderly storage, labeling, etc. during unloading.

3.1.5. The samples for quality control are taken by the lab in charge during unloading by statistical method, it is advised to take samples of the square root of bag numbers plus 1.
(That is for example at 180 bags $(|\sqrt{180}| + 1) = 14 + 1 = 15$, so samples from 15 bags, e.g. every 12th bag.
This samples are mixed, to get one statistic sample of the whole delivery.

For the analysis methods applicable refer to :

Quality Control Manual, Analysis Method NC 02602

3.2. Documentation

3.2.1. The delivery is entered into the stock register as per customs requirements.

3.2.2. The batch is also entered into the production stock register with all quality parameters for the purpose of production planning, blending of different qualities, etc.

The production stock register is to be seen also as the data source of suppliers quality supervision.

This register has to be kept on absolutely actual condition on a daily basis for incoming and outgoing quantities and qualities.

3.3. Raw Material Release

According to the content of Cassia Occidentalis, foreign matter and chrysophanic acid, the quality is classified into three different groups (see raw material specifications):

3.3.1. **Super Grade:**

Raw material of this quality will be manufactured for use in food products.

3.3.2. **First Grade:**

Raw material of this quality will be manufactured for use in pet food products.

3.3.3. **Technical Grade:**

Raw material of this quality will be manufactured for use in different technical applications, i.e. textile and paper industry.

Pages 000078 - 000084 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Goodrich
Performance Materials
CUSTOMER

BFGoodrich Diamalt GmbH

Preliminary Specification

DIAGUM CS (FG)

1. ORIGIN

Generic name	Cassia tora / C. obtusifolia
Chem. Identiy	high molecular galactomannan polysaccharide

TEST	*SPECIFICATION*	*TESTMETHOD*	*TEST-No*
2. Material Description			
Appearance	fine powder	Visually	NC 00201
Consistency	dry,free flowing,with no lumps	Visually	NC 00202
Colour	yellowish to reddish	Visually	NC 00301
Odour	fruity	Sensorically	NC 00302
Taste	almost neutral	Sensorically	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	5 % solution	NC 04201
4. Chem. and phys.	properties		
Loss on drying	Max. 12 %	105 °C / 4 h	FCC III
pH-Value	5,5 - 8,0	calibrated pH-meter	FCC III
Gel-strength	Min. 120 g	FIRA-Jelly Tester	NC 02101
Particle size <250µ	Min. 99 %	sieve DIN 3310	NC 01201
Colour Value	Max. 150 ppm	Photometer	NC 02501
Chrysophanic Acid	Max. 5 ppm	HPLC	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

Each production lot is subjected to a strict quality control in our analytical laboratories. A test report is documenting the results. A production lot is only released when all analytical tests are complying with the specification.

Product-No.	Revision	Replaces	Authorized	Date	Signature
2593025	FS 970901	01.09.97	Dr.Utz/Analytics	06/29/00	
Quality agreement accepted by Supplier					

Geschäftsführer Dr Helmut Wolf Sitz der Gesellschaft, München HRB München 109586 USt-IdNr DE 163077288
Deutsche Bank, Kto - Nr 1501220, BLZ 700 700 10, SWIFT DEUT DE MM

BFGoodrich
Performance Materials
CUSTOMER

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number **C- 2000040521**

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	properties		
Loss on drying	Max. 12 %	9,6 %	FCC III
pH-Value	5,5 - 8,0	7,2	FCC III
Gel-strength	Min. 120 g	142 g	NC 02101
Particle size <250µ	Min. 99 %	99,9%	NC 01201
Colour Value	Max. 150 ppm	133 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	4,8 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

Dr. Ferdinand Utz

000037

Chrysophanic acid

Principle - The high-molecular-weight polysaccharide matrix is depolymerized by hydrolysis, and the anthraquinone glycosides in it are split into aglycones and monosaccharides. The aglycones are extracted with n-hexane, and the chrysophanic acid is isolated selectively by solvent extraction on silica gel at pH 9. The purified isolate is quantified by HPLC on octadecylsilane, with an internal standard.

[Vertical/left margin:
Assay method
Chrysophanic acid]

Chrysophanic acid

Equipment

- Mettler ME 30 microbalance (readability: 0.00000 g)
- Mettler AK 160 balance (readability: 0.0000 g)
- Glass weighing dish 15 x 15 mm
- Glass boiling beads
- Hot plate with metal bath
- 250 ml round-bottom flask with ground joint
- Reflux condenser
- 1 ml vial with beaded edge
- Aluminum seal and septum
- Crimping tool
- Pipets (100 ml, 2 ml, 1 ml)
- Millipore suction flask with fritted glass filter holder
- High-pressure liquid chromatograph (HP 1084B) (Compact system with automatic sampling, fixed wavelength UV detector, 254 nm, and LC terminal 79850)

Reagents

- Distilled water
- 2 N sulfuric acid
- 1,8-dihydroxyanthraquinone, sublimed
- Chrysophanic acid (HPLC purity) Roth 7442
- n-Hexane, LiChrosolv, Merck 4391
- Methanol, LiChrosolv, Merck 6007
- Extrelut 1, Merck 15371 (= wide-pore silica gel sorbent)
- pH 9 buffer solution, Metrohm 62307020
- Millipore Norganic No. CC 1512000 (Trace organic removal cartridges for preparation of HPLC-grade water)

[Vertical/left margin:
Assay method
Chrysophanic acid]

Chrysophanic acid

Sample preparation

Hydrolysis	-	Accurately weigh 5.0000 g sample and 0.50 mg 1,8-dihydroxyanthraquinone (internal standard) into a 250 ml round-bottom flask.
	-	Add 100 ml 2 N sulfuric acid.
	-	Hydrolyze under reflux for 3 hours.
	-	Equilibrate in the water bath at 20°C.
Extraction	-	Mix the hydrolyzate with 100 ml hexane.
	-	Shake for 10 minutes.
	-	Let stand 2 hours for phase separation.
Clean-up	-	Condition Extrelut-1 column with 1 ml pH 9 buffer solution.
	-	Wait for 2 minutes.
	-	Pipet 1 ml sample solution from the hexane phase and apply it to the Extrelut column.
	-	Add 0.5 ml distilled water.
	-	Collect 0.5 ml eluate in a 1 ml vial.
	-	Seal the ampule with an aluminum cap.
	-	Sample solution ready for HPLC.
Standard solution	-	Accurately weigh 0.50 mg HPLC-pure chrysophanic acid and 0.50 mg 1,8-dihydroxyanthraquinone into a 250 ml round-bottom flask. Add 100 ml 2 N sulfuric acid and boil under reflux for 3 hours. Cool at 20°C. Extract with 100 ml hexane. Pipet 1 ml of the hexane solution from the sample solution. Elute it through an Extrelut column conditioned with pH 9 buffer solution. Collect the eluate in an ampule and seal. The standard solution is equivalent to 100 mg/kg chrysophanic acid with a 5.000 g sample and 100 ml hexane.

[Vertical/left margin:
Assay method
Chrysophanic acid]

Chrysophanic acid

HPLC conditions

HPLC model	-	Hewlett Packard HP 1084-B (compact system)
Injection system	-	HP automatic sampler (variable volume injector)
	-	Injection volume: 30 µl
Detector	-	HP fixed wavelength UV detector: 254 nm
	-	Attenuation: 5 (= $32 \cdot 10^{-4}$ AU/cm)
Column	-	Shandon (5 mm ID x 25 cm)
	-	Stationary phase: ODS-Hypersil, 5 µ
	-	Column temperature: 30°C
Mobile phase	-	Methanol/Water[1)] = 80-85%/20-15% (V/V)
	-	Flow rate: 2 ml/min
Pressure	-	150-200 bar
Integrator	-	HP-LC Terminal 79850
	-	Peak area evaluation by HP program "Internal Standard"
	-	Peak detection sensitivity: 0.1
	-	Paper speed: 0.5 cm/min

[1)] Purify distilled water through a Millipore Norganic cartridge.

[Vertical/left margin:
Assay method
Chrysophanic acid]

AM

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February 27, 2001

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VIA HAND DELIVERY

Linda S. Kahl, Ph.D.
Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W. (HFS-206)
Washington, D.C. 20204

Re: GRAS Notification for Cassia Gum; GRN No. 51

Dear Dr. Kahl:

Pursuant to our February 23, 2001 telephone conversation, on behalf of our client, BF Goodrich, we are withdrawing GRN No. 51 for cassia gum. As we discussed, we are taking this action with the understanding that we may, in the future, submit another GRAS notification or make another appropriate submission for cassia gum.

Respectfully submitted,

Melvin S. Drozen
Counsel for BF Goodrich

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September 12, 2001

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Office of Premarket Approval
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, S.W. (HFS-206)
Washington, D.C. 20204

Re: Pre-Submission meeting Regarding GRAS Notification for Cassia Gum

Dear Dr. Alrefai:

As you know, in advance of Noveon's (formerly BF Goodrich) submission of a new GRAS notification for cassia gum (tradename Diagum CS), we will be meeting on September 19, 2001 to discuss our efforts to address issues raised by FDA with regard to our previous GRAS notification submission for cassia gum. To help make this meeting productive, we have enclosed three copies of a draft GRAS notification and have identified below where in that document may be found information responding to the questions and concerns that the Agency has previously raised.

Safety of Cassia Gum—Muscle Toxicity

The primary question involved the fact that the seeds of certain species of cassia have been associated with muscle toxicity. Bound up in this issue are the questions of whether the origin of the muscle toxicity can be identified and whether a tolerance can be established to avoid any risk of muscle toxicity in the finished cassia gum. The primary issue with regard to the *general recognition* of the safety of cassia gum is whether or not the pivotal data supporting its safety are published in a peer-reviewed journal. The original GRAS notification indicated that the impurity that causes muscle toxicity in certain species of cassia was not known. Subsequent research has determined that there are reliable published data demonstrating that anthraquinones are responsible for the muscle toxicity effects of certain cassia species seeds. *See* Lewis, D.C. and Shibamoto, T. (1989) Effects of *Cassia obtusifolia* (sicklepod) extracts and anthraquinones on muscle mitochondrial function. *Toxicon.* **27**:(5), 519-529. These data and related data supporting the role of anthraquinones in the muscle toxicity of certain cassia species are discussed in detail in section 6.4 of the notification beginning on page 24. As is discussed in

section 6.4.8 (p. 24), the published data[1] on the muscle toxicity of cassia species permit the determination of a no observable adverse effect level (NOAEL) for anthraquinones.

On the basis of the NOAEL for anthraquinones, Noveon can assure the safety of its Diagum CS by controlling the levels of anthraquinones in its finished gum via finished product specifications for total anthraquinones. These specifications limit total anthraquinones to 150 parts per million (ppm) in the finished Diagum CS. As discussed on pages 9-10 of the notification (in section 3.0), total anthraquinones are quantified by the color value method of Koshioka and Takino. A copy of their article describing the use of this method is included with the notification. (See App. II, Tab 5.) Also included with the notification are copies of certificates of analysis for three batches of Diagum CS™ confirming compliance with the 150 ppm specification for finished cassia gum (App. II, Tab 5) and a report from Dr. Ferdinand Utz, Technical Manager for Noveon's Diagum CS, on analyses of four master batches of Diagum CS, which, among other things, also confirm compliance with the anthraquinone specification (App. II, Tab 4). In addition to a limit for chrysophanic acid,[2] Noveon has a raw material specification for the seeds of *C. occidentalis* (a cassia species with known muscle toxicity), which limits *C. occidentalis* as well as black splits and foreign matter to a combined level of 2%.[3] *C. occidentalis* is also limited by specification to 0.1% (w/w) of the total weight of the cassia "splits" (i.e., the purified endosperm of cassia seeds).

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[1] Specifically, Crawford L. and Friedman M. (1990) The effects of low levels of dietary toxic weed seeds (jimson weed) *Datura stramonium* and (sicklepod) *Cassia obtusifolia*, on the relative size of rat liver and levels and function of cytochrome P-450. *Toxicol. Letters* **54**:175-81, and Dugan G.M. and Gumbmann M.R. (1990) Toxicological evaluation of sicklepod and black nightshade seeds in short term feeding studies in rats. *Food Chem. Toxicol.* **28**:(2), 101-7.

[2] There are published data indicating that chrysophanic acid, one of the principal anthraquinones in cassia species, constitutes approximately 50% of *Cassia obtusifolia*. *See* Crawford L, McDonald GM, and Friedman M (1990) Composition of sicklepod (cassia obtusifolia) toxic weed seeds. J. Agric. Food Chem. 38 (12) 2169-2175. Other studies, however, suggest that the proportion of chrysophanic acid in the anthraquinones in cassia species may vary. Consequently, although Noveon has a 5 ppm specification for chrysophanic acid for quality control purposes, the company relies primarily upon total anthraquinone limits rather than chrysophanic acid limits to assure that the anthraquinone content of Diagum CS is maintained at safe levels.

[3] Each shipment of seeds is analyzed for compliance with the raw material specifications upon delivery. In the last collection season 1999/2000 on average only 0.02% *C. occidentalis* seeds were found. Based on testing of 325 samples, <<1% of *C. occidentalis* seeds was detected in 47 deliveries. The notification includes both the relevant Raw Material Specification Sheet and the specific test standard for *C. occidentalis* (Test No. NC 02602). (See App. II, Tab 1.)

Manufacturing Process

The current manufacturing process for producing Diagum CS from the cassia splits, including all cleaning and purification steps, is described in detail in section 3.0 (Method of Manufacture) beginning on page 7 of the draft notification and in SOP-IP XX 004 (App. II, Tab 4). Included with the notification are raw material specifications for the incoming cassia splits and SOPs (SOP XX 003) for the control of impurities (App. II, Tab 2.) For additional information on the production of cassia splits and Diagum CS, the notification includes two reports by Technical Manager Dr. Ferdinand Utz: (1) Report on the Production of Food Grade Cassia Splits (App. II, Tab 3)and (2) Report on the Production of Food Grade Diagum CS (App. II, Tab 4).

GRAS Panel Review of Manufacturing Process and Safety Rationale

A panel of food safety experts consisting of Drs. Joseph Borzelleca and Michael Pariza has reviewed the draft GRAS notification, including the description of the current manufacturing process and the conclusions regarding (1) the role of anthraquinones in inducing muscle toxicity in cassia seeds, (2) the NOAEL for anthraquinones, and (3) the ability of the manufacturing process to consistently produce a product that is safe vis-à-vis muscle toxicity. Their conclusion, which they will express formally when a new GRAS notification is submitted, is that Noveon's Diagum CS is GRAS for the intended uses described in the notification.

We look forward to meeting with you and your colleagues to ensure that the notification that we ultimately submit will be satisfactory to the Agency. If you have any questions prior to the meeting, please contact me.

Respectfully submitted,

Melvin S. Drozen
Counsel for Noveon

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Computer Technology Services, Inc.

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C. Street, S.W.
Washington, D.C. 20204

Re: GRAS Notification Procedure

1.0 Introduction

Plant-derived polysaccharides are excellent stabilizing and thickening agents and are common components of processed foods. For example, locust (carob) bean gum is listed as generally recognized as safe (GRAS) for use in food up to a maximum level of 0.75 % (21 CFR 184.1343). It is also approved for use as a thickening agent in Europe (EEC No. E410). Guar gum is listed as GRAS for use in food up to a maximum level of 2% (21 CFR 184.1339). Both are used in various foods including baked goods, cheese, and jams/ jellies.

Cassia gum, flour from the endosperm of *Cassia tora* and *Cassia obtusifolia*, is well suited for use in the manufacture of gels sometimes or always in combination with other colloids, and has several food applications. It is used for various technical applications, including thickening, emulsification, foam stabilization, moisture retention, and texture improvement. Besides the aforementioned technical applications, the main application of cassia gum is likely to be to create the range of soft to firm gels in synergism with kappa-carrageenan or xanthan. Cassia gum is used at concentrations comparable to locust (carob) bean gum.

The toxicological profile of cassia gum, including the potential for constituents of its source seeds to cause muscle toxicity when consumed at high levels, and other data were assessed by an Expert Panel to determine GRAS status for use in food. The lack of toxicity of cassia gum demonstrated in animals and the safe use of related gums indicate that the technological properties of cassia gum meeting finished product specifications may be safely utilized in food.

1.1 Notice of GRAS Exemption Claim:

Cassia gum is exempt from premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS) under conditions of intended use by experts qualified by scientific training and experience.

i) Notifier:

Noveon, Inc.
9911 Brecksville Road
Cleveland, Ohio 44141

Contact Person:

Melvin S. Drozen
Keller and Heckman LLP
1001 G. Street, N.W.
Suite 500 West
Washington, D.C. 20001
Telephone: (202) 434-4222

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Email: Drozen@khlaw.com

ii) Common/usual name:

Cassia gum (Diagum CS™)

iii) Applicable conditions of use:

As discussed further below, based on the extant data and experts' conclusions, cassia gum is believed to be GRAS for use in the applications set forth in the table below.

FOODS	MAXIMUM CONCENTRATION, PPM	INTENDED USES
Baked Goods—only cheese crème fillings in cakes	5,000	Water retention
Soups,Sauces,Seasonings —Dry soup mixes only	2,000 - 5,000	Thickening agent
Yogurt	3,000 - 5,000	Thickening agent
Meat and Poultry Products and Sausages	3,000	Texture improvement and water retention

iv) The basis of this GRAS determination is through scientific procedures (see section 4). This determination is supported by an expert panel review of the relevant toxicological data, as evidenced by the conclusions set forth in Appendix I.

v) The data and information that form the basis of the GRAS determination are available for the FDA's review and copying. All references are available through the contact person named above.

Respectfully submitted,
NOVEON

By: _____

Melvin S. Drozen
Keller and Heckman, LLP
COUNSEL FOR THE NOTIFIER

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2.0 Chemical Identity

Cassia gum[1] (CAS No. 11078-30-1) is the flour from the purified endosperm of the seeds of *Cassia tora* and *Cassia obtusifolia,* which belong to the *leguminosae* family. *C. tora* and *C. obtusifolia* closely resemble one another but have been classified by some botanists as distinct species. *Cassia tora* and *Cassia obtusifolia* grow wild in subtropical regions of the world and *Cassia tora* is occasionally cultivated. *Cassia tora* and *Cassia obtusifolia* are annual ruderal plants that ripen after approximately 100 days.

Cassia gum is comprised of at least 75% polysaccharide consisting primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6 linked α-D galactopyranose units. The ratio of galactose to mannose is 1:5. The following composition was revealed in the sugar analysis conducted using HPLC: mannose (77.2-78.9%), galactose (15.7-14.7%) and glucose (7.1-6.3%).

Like most polysaccharides, the following formula applies:

$$(C_6H_{10}O_5)_n, H_20$$

Cassia gum is related to locust (carob) bean gum and guar gum in terms of structure and chemical properties, although in terms of functionality cassia is more comparable to locust bean gum than to guar gum. The specifications and purity of cassia gum and related gums are listed in Table 1.

Cassia galactomannan can be differentiated from other galactomannans by electrophoresis. Based on the molecular weights of the structurally related polygalactomannans, guar gum and locust (carob) bean gum (Klose and Glicksman, 1968; Leung and Foster, 1996), the molecular weight of cassia gum is estimated to be between 200,000 and 300,000 (Denkler, 1997). The physical properties of cassia gum are reported in Table 2, and its structural properties as compared to other gums are reported in Table 3.

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[1] Cassia gum (trade name Diagum CS) is marketed in two forms:
a) 100% flour from the endosperm of the *Cassia tora* and *obtusifolia* seeds.
b)In a mixture with other gelling and thickening agents (e.g. carrageen, locust (carob) seed flour, guar gum, agar, xanthan gum and, where appropriate, other food additives), whereby DIAGUM CS comprises 10-90% of the mixture. Diagum CS was called Mucigel X 18 H during the temporary approval in France.

Table 1. Specifications and Purity of Cassia Gum and Related Gums for use in Foods

	CASSIA GUM	LOCUST (CAROB) BEAN GUM[2]	GUAR GUM[3]
	Proposed Limits	FCC Limits	FCC Limits
Galactomannan	≥70%	≥75%	≥70%
Acid-Insoluble residue	≤4%	≤4%	≤7%
Moisture	≤12%	NR[4]	NR
Ash	≤1.2%	≤1.2%	≤1.5%
Proteins	≤7%	≤7%	≤10%
Fat	≤2%	NR	NR
Chrysophanic acid	max. 5 ppm	NR	NR
Heavy Metals:	≤0.002%	≤0.002%	≤0.002%
Lead	≤5 mg Pb/kg	≤5 mg Pb/kg	≤5 mg Pb/kg
Arsenic	≤3 mg As/kg	≤3 mg As/kg	≤3 mg As/kg
Color value[5]	max. 150 ppm	NR	NR

2 Food Chemicals Codex, 1996.
3 Food Chemicals Codex, 1996.
4 "NR" means not reported.
5 Measure of naturally occurring anthraquinone derivatives in the product.

Table 2. Physical properties of Cassia gum[6]

Appearance	Pale yellow powder without lumps
Odor	specific, fruity smell
Bulk density	0.6 kg/l
Particle size **(sieve analysis)**	250 μm (average) not less than 100% 100 μm (average) not less than 60%
Solubility	Disperses well in cold water and forms a colloidal solution
pH (5%)	6.5-7.5
Viscosity, 5% solution:	
Cold	5,000 +/- 2,500 mPas
Boiling	120,000 +/- 30,000 mPas
Gel-strength	80g +/- 10g

6 Leimbgruber and Marbot, 1991.

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Table 3. Structural properties of Cassia Gum and related substances

Substance	Structure	Mannose:Galactose	Molecular Weight
Cassia Gum (CAS No. 11078-30-1)[7]	1,4-β-D-mannopyranose units with 1,6-α-D galactopyranose units attached to every fifth mannose.	5:1	200,000-300,000
Guar Gum[8] (CAS No. 9000-30-0)	1,4-β-D-mannopyranose units with 1,6-α-D-galactopyranose units attached to every alternate mannose.	2:1	220,000-250,000
Locust (Carob) Bean Gum[9] (CAS No. 9000-40-2)	1,4-β-D-mannopyranose units with 1,6-α-D-galactopyranose units attached to every fourth or fifth mannose.	4:1	310,000
Tara Gum[10] (CAS No. 39300-88-4)	1,4-β-D-mannopyranose units with side chains of 1,6-α-D-galactopyranose attached to approximately every third unit.	Approximately 3:1	Not Reported

3.0 Method of Manufacture

In the Country of Origin – ISO 9002 certified manufacturing facility

Indiamalt Private Limited

Post – Manjusar, Taluka – Savil

Dist. Vadodara 391 775, India

Cassia tora and *obtusifolia* are harvested when the plants are ripe (e.g., November-January) in India. The seeds that are used in the production of cassia gum are generally picked in the wild. *C. occidentalis,* a species of cassia which has been associated with muscle toxicity,[11]

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7 CAS number for "galactomannan"

8 Klose, and Glicksman, 1968.

9 Klose, and Glicksman, 1968.

10 NTP, 1982b.

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11 Muscle toxicity has also been associated with components of *C. obtusifolia*, although *C. occidentalis* appears to be somewhat more toxic. Steps taken to ensure that Diagum CS™ will

does not grow in conjunction with *C. tora* or *C. obtusifolia* and hence, even by oversight, is unlikely to be collected together. The *C. occidentalis* pods look distinctly different from *C. tora* and *C. obtusifolia* and collectors are educated not to collect *C. occidentalis*. Furthermore, Noveon has a specification to limit the presence of *C. occidentalis* in the cassia seeds that are the raw material used to produce Diagum CS™. The specification limits *C. occidentalis* as well as black splits and foreign matter to a combined level of 2%. A copy of a Raw Material Specification Sheet that Noveon will apply to food-grade cassia gum together with the specific test standard for *C. occidentalis* (Test No. NC 02602) is included in Appendix II at Tab 1. This specification applies to seeds prior to their undergoing an extensive cleaning process. After cleaning, the level of *C. occidentalis*, if any, would be substantially lower.[12]

Once the pods are harvested, the seeds are subject to a cleaning process before splitting. The seed purification includes mechanical steps to remove stones, dirt and seeds other than *C. tora* and *C. obtusifolia*. Specifically, after removal of dust and coarse impurities with a round sieve, the seeds pass two separators and finally a gravity separator. At this stage, stones and seeds with a similar size but different weight compared to *C. tora* or *C. obtusifolia* seeds are removed. Any *C. occidentalis* seeds that remained at this point likely would be reduced further by the gravity separator since *C. occidentalis* has an average weight about 20% less than *C. tora* or *C. obtusifolia*.

After cleaning, the seeds undergo a splitting process to separate the endosperm used to produce cassia gum from the husk and germ. The splitting process starts with roasting, followed by two dehusking steps. The roasting involves continuously feeding non-comminuted seed grains into a rotation roaster, where they are heated to approximately 200°C for about 30 seconds. After two cleaning steps with a separator and a round sieve, the splits pass again through two dehuskers. These splits are purified by a separator, two polishers and a brusher.

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not contain harmful levels of muscle toxins from *C. obtusifolia* or *C. tora* are discussed later in this section.

[12] Each shipment of seeds is analyzed for compliance with the raw material specifications upon delivery. In the last collection season 1999/2000 on average only 0.02% *C. occidentalis* seeds were found. Based on testing of 325 samples, <1% of *C. occidentalis* seeds was detected in 47 deliveries.

Cassia splits resulting from the above procedure are packed and transported to another facility for further processing into final cassia gum. Each split lot is subject to raw material control according to SOP XX 003 (see App. II, Tab 2).

For food grade cassia gum, the splits are subject to the following additional purification steps before milling:

Metal cleaner: The splits pass a strong magnet to remove metal

- Polisher: Foreign material is removed which may come from the bags
- Aspirator: Removal of dust
- Polisher: Stoner: particles heavier than the average split weight are removed, for example splits which still include the germ.
- Color sorting equipment: detects all splits in color ranges not conforming to the desired split color. Dark splits can be removed nearly by 100%.
- Washing: the splits are washed with defined quantities of water.

For additional details on the production of cassia splits, see the report by Technical Manager Ferdinand Utz of Noveon on the Production of Food Grade Cassia Splits (App. II, Tab 3).

The production of Diagum CS™ from cassia splits is described in SOP-IP XX 004 (App. II, Tab 4) and in the report of Dr. Ferdinand Utz on the Production of Diagum CS™ (App. II, Tab 4). As Dr. Utz explains in his report, the process involves four main steps: (1) final cleaning of the splits; (2) washing soaking, squeezing and drying of the flakes; (3) milling; and (4) blending of a batch and final pack.

As noted above, *C. obtusifolia* has also been associated with muscle toxicity in animal feeding studies. As discussed in section 6.4, there is clear evidence that anthraquinones are the cause of muscle toxicity resulting from the ingestion of certain species of cassia. Anthraquinones are not evenly distributed in the seed. While the average anthraquinone content in seeds is in the range of at least 10,000 ppm, Noveon has set a total anthraquinone specification of 150 ppm for Diagum CS™ as determined by the optical color value method of Koshioka and

Takino.[13] In its quality control laboratory, Noveon analyzes the anthraquinone content of each batch of both the raw material (splits) and Diagum CS™ using the color value method.[14] Included with this notification are copies of the finished product specifications, including color value; the color value method; and certificates of analysis for three batches of Diagum CS™ confirming compliance with the 150 ppm specification for finished cassia gum (App. II, Tab 5). Dr. Utz' report on the production of Diagum CS™ (App. II, Tab 4) also includes analyses of four recent master batches of Diagum CS™ which confirm compliance with the total anthraquinone specification. As discussed in section 6.4, compliance with this specification for anthraquinones ensures that Diagum CS™ will be of suitable purity and not contain harmful levels of muscle toxins.

4.0 Intended Use

Plant-derived colloidal polysaccharides are excellent stabilizing and thickening agents and are common components of processed foods. Cassia gum swells in water and forms high-viscosity aqueous colloids after it is boiled. When it is used in combination with other gelling or thickening agents such as carrageenan or xanthan gum in aqueous solution, it results in the formation of an aqueous gel that has potentially numerous food applications. It is used as a thickening and gelling agent in pet food. Examples of plant derived colloided polysaccharides include locust (carob) bean gum which is listed as generally recognized as safe (GRAS) by the U. S. Food and Drug Administration (FDA) for use in food up to a maximum level of 0.75% (21 C.F.R. § 184.1343). It is also approved for use as a thickening agent in Europe (EEC No. E 410). Guar gum is listed as GRAS by FDA for use in food up to a maximum level of 2% (21 CFR 184.1339). Both are used in various foods including baked goods, cheese, and jams/jellies. The establishment of acceptable daily intakes (ADIs) for locust (carob) bean gum and guar gum was deemed unnecessary by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) because the available data indicate that locust (carob) bean gum and guar gum do not represent a health hazard. Gum arabic, gum ghatti, guar gum, locust bean gum, sterculia gum,

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[13] Koshioka M. and Takino Y. (1978) Studies on the evaluation of crude drug I. Quantitative estimation of anthraquinones in cassia seeds. *Chem. Pharm. Bull.* 25(5) 1345-49.

[14] The splits are milled in the laboratory by a stone mill into a particle size similar to cassia gum before hydrolyzation.

and gum tragacanth are all considered GRAS by FDA for use in animal feeds (21 C.F.R. § 582). FDA's Center for Veterinary Medicine has also indicated that it does not object to the use of cassia gum in pet food at a level of 4,000 ppm.[15] Cassia gum is included in the Japanese "List of Existing Food Additives" as a stabilizer and thickener. It is understood that this list is used as a list of naturally occurring additives that are permitted in foods generally, unless a restriction appears elsewhere. No restriction appears for cassia gum. An application has also been submitted for the authorization of cassia gum as a food additive in the European Union.

4.1 Evidence of Self-Limiting Levels of Use

The intended use table in section 1.0 of this notification reflects the limited applications for which cassia gum is intended.

5.0 Estimated Exposure

The anticipated market for cassia gum is targeted to a very limited number of food categories. The food categories in the USDA data bases include a much wider variety of food items within their categories than are anticipated for cassia gum. We therefore estimate consumption by using the Flavor and Extract Manufacturers Association (FEMA) approach. (Adams *et al.*, 1996.) This takes the U.S. production volume for the substance used for food and divides by the population of the United States. Then it increases the result by 10, effectively assuming that one tenth of the population consumes all the cassia gum. This approach has been shown to give consumption estimates close to the PADI (possible average daily intake) approach for many food substances. The approach is appropriate when it is unlikely that a smaller population group will consume the substance. Since the products anticipated to contain cassia gum will likely have a wide and varied distribution, this assumption is reasonable in the present instance. The total annual U.S. production volume for cassia gum will be approximately 1/3 of the world production or 1,000, 000 kg. The U.S. per capita consumption is then 1,000,000 kg/240 x 10^6 persons or 4.17 g /p/yr = 11.4 mg/p/d. If it is assumed that only 10% of the population consumes cassia gum (i.e., the 90th percentile), the intake would be 114.1 mg/p/d or 1.89 mg/kg.bw/d.

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[15] Letter from Dr. George Graber, Center for Veterinary Medicine, to John Hallagan, January 19, 1999.

6.0 Biological Studies

6.1 Absorption, Distribution, Metabolism and Excretion

Galactomannans are recognized as components of dietary fiber and are resistant to digestive enzymes in the gastrointestinal tract (Trowell *et al.*, 1976). Galactomannans were shown to be stable in artificial gastric juice (Nurnberg and Bleimuller, 1981).

The biodisposition of cassia gum has not been studied, but is expected to be similar to structurally related galactomannans, including locust (carob) bean gum and guar gum, for which studies have been reported. In a digestibility study, groups of five male and five female rats (Purdue strain) were maintained on a mannose-free diet and provided 1% locust (carob) bean gum. Eighty-five (85) to 100% of the dose was excreted as mannose (Tsai and Whistler, 1975). No evidence of hydrolysis was observed when locust (carob) bean gum (in solution or suspension) was incubated with human gastric juice, duodenal juice + bile, pancreatic juice, and *Succus entericus* with or without added rabbit small gut membrane enzymes (Semenza, 1975).

Ten (10) % (w/w) aqueous solution of guar gum was shown to decrease the rate of gastric emptying of rats (Shah *et al.*, 1986). It was suggested that 5% aqueous solution guar gum decreased the efficiency of nutrient digestion and absorption, and increased secretion of digestive juices in rats (Ikegami *et al.*, 1990). Guar gum at a dose of 100 g/kg was fed to rats as part of a basal diet. Only 4% was excreted in the feces (primarily as rhamnose and arabanose) indicating that guar gum was almost completely fermented (Nyman and Asp, 1982).

It is expected that cassia gum would be excreted unchanged. Fermentation of cassia gum by gut microflora may occur to a small extent (Nyman and Asp, 1982). Any hydrolyzed material would be absorbed and metabolized in normal biochemical pathways (Mathews and van Holde, 1990).

6.2 Toxicological Studies

(A summary of all available toxicity data is presented in tables 4, 5 and 6)

6.2.1 Diagum CS™ test sample

The toxicological studies described below were generally conducted on Diagum CS™ test samples. These samples contain very low levels of anthraquinones, well below the level at

which anthraquinones have been found to induce muscle toxicity in animal studies (see discussion of muscle toxicity in Section 6.4). As noted under Section 3.0 Method of Manufacture, the aspirator, stoner and the color sorting equipment were not part of the manufacturing procedure at the time that cassia gum was produced for the feeding tests 10 years ago. Because the addition of this equipment improved the quality of the finished product, the test sample is believed to represent a worst case toxicological assessment

6.2.2 Acute oral toxicity

Cassia gum exhibits very low acute toxicity. The oral (gavage) LD50 value of cassia gum was reported to be >5,000 mg/kg bw in male rats (Schering, 1986).

6.2.3 Repeated dose toxicity and carcinogenicity

Cassia gum as a dietary admixture is well tolerated in animals and did not produce evidence of toxicity in three repeated dose studies in dogs, cats and rats. A decrease in food consumption was commonly observed and is attributed to the absorption of water by cassia gum within the gastrointestinal tract and subsequent increase in volume and bulk. The three studies summarized below were conducted according to the Organization for Economic Cooperation and Development Principles of Good Laboratory Practices (OECD, 1981) and were inspected by a Quality Assurance Unit.

Table 4: Summary of Repeated Dose Toxicity Tests Performed on Cassia Gum (Diagum CS™)

Assay	Cassia Gum Test Dose(s)	Result(s)	Reference
Repeated Dose 28 day-Rats	0,125, 500, 1250 and 2,500 mg/kg bw/d as dietary admixture and 1000 mg/kg bw/d by gavage	NOAEL: 2,500 mg/kg bw/day oral (dietary) or 1,000 mg/kg bw/d (gavage)	Zuhlke, 1990
Repeated Dose 90 days - Cats	0, 250 and 1,250 mg/kg bw/d as dietary admixture	NOAEL: 1,250 mg/kg bw/d	Virat, 1984
Repeated Dose 90 days - Beagle Dogs	1,000 and 3,500 mg/kg bw/d as	NOAEL: 3,500 mg/kg bw/d	Schuh, 1990

	dietary admixture		

Two groups of four male and four female Beagle dogs were provided with either 0.75% or 2.5% (w/w) cassia gum as part of a canned dog-food diet for 90 days. The average dose levels calculated on the basis of food consumption data were approximately 1000 and 3500 mg/kg bw/d (>1000-times the anticipated daily dietary exposure levels, respectively). A control group received a similar diet containing 0.23% (w/w) carob (crushed locust beans) (Schuh, 1990).

Clinical, biochemical, hematological and urinary parameters were evaluated. Slight but statistically significant changes in some biochemical and hematological parameters were reported periodically in males and females in both dose groups. Since most of the changes were not dose- or time-dependent as compared to controls, they were not considered to be compound related. Bone marrow examinations, urinalysis, coagulation studies, and gross pathology including organ weights were conducted in all animals. Microscopic examination was performed on all major organs for all animals in the control and high dose groups. For animals in the low dose group, histological examination was limited to the liver, kidney, heart, and macroscopic findings. Survival was 100%. A dose-dependent increase in water consumption was observed in test animals and was most likely associated with water retention in the gastrointestinal tract by cassia gum. No compound-related adverse effects were reported (Schuh, 1990).

Three groups of five male and five female cats were provided with 0, 0.5% or 2.5% (w/w) cassia gum as part of a standard canned-food diet for 13 weeks. The dose levels were not available. Clinical, hematological, urinary, and biochemical parameters were evaluated and were in the normal range. There were no consistent treatment related dose-dependent adverse effects reported. Histological examination was performed on all major organs in all animals. There were no adverse or compound-related histopathological findings. Survival was 100%. (Virat, 1984).

In a 28-day study, five groups of five male and five female Sprague-Dawley rats were administered cassia gum as part of a powdered diet at concentrations of 0, 0.25%, 1.0%, 2.5%, or 5.0% (Zulke, 1990). The dose levels were calculated (FDA, 1993) to be 0, 125, 500, 1250, or 2500 mg/kg bw/d. A sixth group, which was fed an untreated powdered diet, was treated by

gavage with cassia gum as a suspension in distilled water, two times a day at a dose of 1000 mg/kg bw/d.

Clinical, hematological, urinary, and biochemical parameters were evaluated. Generally, all hematological parameters examined were considered to be within the normal range of the background data for the species tested. The following minor changes were observed but were not considered treatment-related because they did not occur in the high oral dose group: a statistically significant decrease in the red blood cell count and packed cell volume of males and females at the 500 and 1250 mg/kg bw/d levels; a statistically significant decrease in mean hemoglobin values of females in the 500 and 1250 mg/kg bw/d groups; and a statistically significant increase in white blood cell count for males in the 500, 1250 and 2500 mg/kg bw/d levels.

Clinical chemistry findings included statistically significant increases in mean glucose concentrations of males and females in the 500 and 1250 mg/kg bw/d groups, and mean triglyceride concentrations of males only in the 500 and 1250 mg/kg bw/d groups. These changes are not considered dose-related because they were not observed at the highest dose. A decrease in blood urea nitrogen was statistically significant but not dose-dependent for males in the 500, 1250 and 2500 mg/kg bw/d groups, but was not statistically significant for females in the 1250 and 2500 mg/kg bw/d groups. Statistically significant, dose-dependent decreases in sodium were observed in males in the 500, 1250 and 2500 mg/kg bw/d groups, and the 1000 mg/kg bw/d intragrastic suspension group, and females at 1250 and 2500 mg/kg bw/d only. A statistically significant, dose-dependent increase in group mean triglyceride concentrations was reported in females in the 500 and 1250 mg/kg bw/d groups. Statistically significant but not dose-dependent reduced mean total protein (6.2%) and albumin levels (7.7%) of males in the 2500 mg/kg bw/d group, statistically significant elevated potassium levels (13.0%) of males in the 1250 mg/kg bw/d group, and decreased chloride levels (6.8%) of males treated 1000 mg/kg bw/d intragastrically were also observed. Body weight gain was statistically significantly reduced (20%) in males in the 2500 mg/kg bw/d group and in females (17% each) in groups fed 500 and 1250 mg/kg bw/d, and 1000 mg/kg bw/d intragastrically (gavage). These effects were observed when high dose levels of carbohydrate-based gums were administered to rats (Takahashi, 1994) and are not considered to be biologically significant.

All animals were necropsied and all tissues and organs were examined macroscopically. Histological examination was performed on major organs for animals in the control group, high dose group, and group treated intragastrically. Group mean absolute kidney weights were statistically significantly decreased in males fed 500 mg/kg bw/d (8.0%), 2500 mg/kg bw/d (15.3%), and 1000 mg/kg bw/d (6.9%) intragastrically. Group mean relative kidney weights were statistically significantly increased in females fed 2500 mg/kg bw/d (11.3%). A dose-relationship was not reported. These changes in the kidney weights were not considered treatment-related since there were no changes in relative weight in males and in absolute weight in females and no histopathology was seen (Zühlke, 1990).

The structurally related galactomannan, guar gum did not produce evidence of compound-related toxicity when groups of 10 male and 10 female rats (strain not provided) were provided 0, 1%, 2%, or 5% guar flour as a dietary admixture for 90 days (Til *et al.*, 1974); when two groups of 15 male and 15 female rats were provided either 0 or 5% guar flour in their diet for 24 months (Krantz *et al.*, 1948); or when groups of 5 male and 5 female Sprague-Dawley rats were administered partially hydrolyzed guar gum by gavage for 28 days at 0, 500, or 2500 mg/kg bw/d (Takahashi *et al.*, 1994). When rats were provided guar gum in the diet for 91 days at levels of 1%, 2%, 4%, 7.5%, or 15%, a decrease in weight gain in females of all dose groups and males at the two highest levels was reported. Bone-marrow cellularity was reduced at the 15% level. A dose-related decrease in the absolute kidney weights of males was reported, but relative kidney weights were normal and no histopathology was seen (Graham *et al.*, 1981).

No compound related toxicological effects were reported when four groups of 5 male and 5 female Beagle dogs were fed 0, 1%, 5%, or 10% (dose levels were not available) of a precooked mixture containing guar and locust (carob) bean gum for 30 weeks (Cox *et al.*, 1974).

When two monkeys received 1 g of guar flour in their diet daily, growth, hematology, and overall health were normal. One monkey died after 16 months, and the other was sacrificed at 24 months. No abnormalities were noted upon gross examination and histopathology (Krantz *et al.*, 1948).

Similarly, the structurally related galactomannan, locust (carob) bean gum, did not produce evidence of compound-related toxicity in groups of 10 male and 10 female rats given locust (carob) bean gum in the diet at levels of 0, 1%, 2% or 5% for 90 days (Til *et al.*, 1974).

There are no carcinogenicity studies on cassia gum. However, other gums including locust (carob) bean, guar, and tara when fed to rats and mice were not carcinogenic and were not mutagenic in appropriate test systems. The chemically related galactomannan locust (carob) bean gum was provided to F344/N rats or B6C3F1 mice as a dietary admixture at concentrations of 25,000 or 50,000 ppm (≈1250 or 2500 mg/kg bw/day) for 103 weeks, it did not induce cancer (NTP, 1981) nor was it mutagenic (Maxwell and Newell, 1972). When guar gum was provided to F344/N rats or B6C3F1 mice as a dietary admixture at concentrations of 25,000 or 50,000 ppm (≈1250 mg/kg bw/d or 2500 mg/kg bw/d) for 103 weeks, it did not induce cancer (NTP, 1982a).. F344/N rats fed diets containing 0, 25,000 or 50,000 ppm (0, 1250 or 2500 mg/kg bw/day) tara gum for two years exhibited no tumorigenic effects related to the test material (NTP, 1982b; Borzelleca *et al.*, 1993).

No adverse effects were reported when five volunteers ingested 1 gram of the structurally related substance guar flour in a capsule per day for 10 days (Krantz *et al.*, 1948).

6.2.4 Reproductive and developmental toxicology

Four reproductive or developmental toxicology studies have been conducted including a one-generation study in cats, a two-generation study in rats, and teratology studies in rats and rabbits in which cassia gum was presented as a dietary admixture or administered by gavage. Cassia gum did not produce any evidence of embryotoxicity or teratogenicity. (See Table 5.)

Table 5: Summary of Reproductive and Develpomental (Teratological) Tests Performed on Cassia Gum (Diagum CS™)

Assay, Reference	Cassia Gum Test Dose(s)	Result(s)	Reference
One generation Reproduction in Cats	0, 375, 1,250 mg/kg bw/d	Transient effects on biochemical parameters at 1,250 mg/kg bw/d. No unequivocal evidence of reproductive toxicity at highest dose level. NOAEL: 375 mg/kg bw/d.	Virat, 1989
Two generation Reproduction in Rats	0,250,100 and 2,500 mg/kg bw/d	No effects on gonadal function, mating behavior, conception, parturition, weaning and the growth	McIntyre, 1990

		and development of offspring. NOAEL: 2,500 mg/kg bw/d.	
Teratology – Rats	0, 350, 1000 mg/kg bw/d	No compound related adverse effects for clinical observations, mortality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. NOAEL: 1000 mg/kg bw/d.	Muller, 1989
Teratology - Rabbit	0, 350, 1000 mg/kg bw/d	No compound related adverse effects for clinical observations, mortality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. NOAEL: 1000 mg/kg bw/d.	Muller, 1989

Only a slight increase in absolute and relative ovarian weights was noted at the high-dose level in the cat study, but was not accompanied by treatment-related histopathological changes and therefore was considered to have no biological relevance. In this study, high mortality of unknown origin was observed in the control group (Virat, 1989). The four studies summarized below were conducted according to OECD Principles of Good Laboratory Practices (1981) and were inspected by a Quality Assurance Unit.

In a one-generation reproductive study, three parental groups (F_0 generation) of 10 male and 20 female cats were provided 0, 0.75%, or 2.5% (w/w) (doses were not available) cassia gum as part of the diet for at least 83 weeks. After at least 36 weeks, one male was mated with two females of the same dose group. At day 4 post-partum, all kittens in excess of 4 per litter were culled. The F_1 generation was terminated after at least 13 weeks and the F_0 generation was terminated after the 83-week treatment period (Virat, 1989).

Clinical, hematological, urinary, and biochemical parameters were evaluated. No treatment related effects on hematology parameters were noted. A slight, statistically significant decrease in activated pro-thrombin time (APTT) was reported in treated parental females in both

groups at week 26, but was considered to have no biological significance because the changes were transient and within normal range. After 13 weeks of dosing, a statistically significant increase in bilirubin was noted in males and females in the 1250 mg/kg bw/d group, and females in the 375 mg/kg bw/d group. From week 26 onwards, this change was only observed in males and females in the 1250 mg/kg bw/d group. The values were transient, never reached a biologically significant range, and did not correlate with morphological alterations. Therefore, the biological significance was considered unclear, but minor with respect to toxicity. Slight, statistically significant increases in clinical chemistry parameters were observed but were not dose-related and considered to have no biological significance.

Histological examination was performed on all tissues for all F_0 generation cats in the control and high dose groups, and on two kittens from each litter of the control and high dose groups. X-ray skeletal examination was performed on all kittens (Virat, 1989).

High mortality occurred in the control group after 2-3 weeks postpartum resulting in several total litter losses in this group. The mortality was preceded by several clinical signs but no consistent pattern of pathology was observed. Although treatment groups were unaffected, appropriate comparisons between control and treatment groups were impaired (Virat, 1989).

Some effects were observed in both generations, but were not clearly associated with the test compound. For example, females in the high-dose F_0 group exhibited a decrease in food consumption during late gestation and a slight increase in absolute and relative ovarian weights. The latter was not accompanied by evidence of histopathology. In the F_1 generation, the combined incidence of stillborn births and neonatal deaths was significantly higher in the high-dose group. No other findings were noted. The authors concluded that while there was no unequivocal evidence of reproductive toxicity at the highest dose level, the concentration of 375 mg/kg bw/d (w/w) cassia gum is considered a no observed adverse effect level (NOAEL) (Virat, 1989).

In a two-generation reproductive study using Sprague-Dawley rats, five groups of 25 males and 25 females were provided with 0, 5,000, 20,000, or 50,000 ppm of cassia gum, or 50,000 ppm of cassia gum-AW[16] as part of the diet. All parental rats (P generation) were treated

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[16] In order to evaluate the potential for adverse effects associated with vegetable by-products that may be present in cassia endosperm flour, cassia gum-AW was highly purified in

for approximately 10 weeks prior to mating, during mating, gestation, and lactation. The dose levels were calculated (FDA, 1993) to be 0, 250, 1000, or 2500 mg/kg bw/d. Pregnant females were allowed to rear their offspring (F_{1a}) to weaning. Rats in both 2500 mg/kg bw/d dose groups exhibited low pregnancy rates and were mated again with the same males. They were allowed to litter, and the subsequent offspring (F_{1b}) were terminated on day 5-7 postpartum. Selected F_{1a} offspring were treated for a 10-week period of maturation, and during mating, gestation, and lactation. Pregnant F_{1a} females were allowed to rear their offspring (F_2) to weaning (McIntyre, 1990).

All P and F_1 animals were necropsied. Histopathological examination was performed on gross lesions and on the reproductive organs for all controls and animals fed 2500 mg/kg bw/d cassia gum. No compound related adverse effects were noted. The pregnancy rate of P females was slightly decreased at the first mating in the two 2500 mg/kg bw/d dose groups, but a subsequent mating revealed no evidence of a treatment-related effect on fertility. No adverse effects related to treatment with cassia gum were reported on gonadal function, mating behavior, conception, parturition, weaning and the growth and development of the offspring. There was a slight, but not statistically significant, decrease in the mean body weights of F_{1a} and F_2 pups in the group that received cassia gum-AW (McIntyre, 1990).

In a teratology study, three groups of 28 pregnant Sprague-Dawley rats were administered cassia gum in distilled water by gavage at concentrations of 0, 17.5, or 50 mg/ml (0, 350, or 1000 mg/kg bw/d) for 14 days from days 6 to 19 post-coitum (inclusive). The control animals received the vehicle (distilled water) alone. A fourth group containing 29 rats was administered cassia gum-AW by gavage in distilled water at a concentration of 50 mg/ml daily (1000 mg/kg bw/d) over the same period. All animals were sacrificed and examined on day 20 post-coitum (Muller, 1989). No compound-related adverse effects were reported for pregnancy incidence, implantation, post-implantation loss, fetal defects upon necropsy. A statistically significant reduction in mean daily food consumption and mean body weight gain was observed in animals dosed at 1000 mg/kg bw/d of cassia gum or cassia gum-AW, and was considered to be a treatment related effect. However, these effects were not accompanied by any evidence of embryotoxicity or teratogenicity (Muller, 1989).

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preparation for testing by thoroughly washing cassia endosperm flour with isopropanol followed by careful drying.

In a related teratology study, three groups of 20 pregnant New Zealand White rabbits were administered cassia gum in distilled water by gavage at concentrations of 0, 17.5 or 50 mg/ml (0, 350 or 1000 mg/kg bw/d) for 22 days from days 6 to 27 post-coitum (inclusive). The control animals received the vehicle (distilled water) alone. A fourth group of 20 pregnant rabbits was administered 1000 mg/kg bw/d cassia gum-AW by gavage in distilled water over the same period. All animals were sacrificed and examined on day 28 post-coitum (Muller, 1989).

No compound-related adverse effects were reported for clinical observations, mortality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. A reduction in mean daily food consumption was reported in animals administered 1000 mg/kg bw/d of cassia gum, and was accompanied by a reduction in mean fetal weights. However, these effects were not statistically significant. No indication of teratogenicity was reported (Muller, 1989).

6.2.5 Mutagenicity and genotoxicity

Three different tests have been conducted evaluating the mutagenic activity of cassia gum. These include the reverse mutation assay (Ames test), the chromosome aberration assay, and an *in vitro* gene mutation assay. All three *in vitro* studies were conducted in compliance with the OECD Principles of Good Laboratory Practice (OECD, 1981) and were inspected by a Quality Assurance Unit.

Cassia gum was tested, with and without metabolic activation, in the *Salmonella typhimurium* reverse mutation assay (Ames test) with strains TA1535, TA1537, TA100, and TA98 at concentrations of 4, 16, 62.5, and 1000 μg/plate. Cassia gum precipitated on the plates at concentrations of 62.5 μg/plate and higher. Increases in the number of revertant colonies observed in strains TA1535, TA1537, and TA98 were seen only at precipitating concentrations and were within the historical control data range. The increase observed in strain TA100 was dose related and was outside the historical control data range, but were seen only at precipitating concentrations (Verspeek-Rip *et al.*, 1998a).

As a second experiment to the study described above, cassia gum was tested at concentrations of 1.6, 8, 40, 200, and 1000 μg/plate, in all the *Salmonella typhimurium* strains and in *Escheria coli* strain WP_2uvrA. Cassia gum precipitated on the plates at concentrations of 200 μg/plate and higher. There was a negative response in strains TA1535, TA98, and

WP_2uvrA. *Salmonella* strains TA1537 and TA100 showed dose-related increases in the number of revertant colonies, but only at precipitating concentrations (Verspeek-Rip *et al.*, 1998a).

Based on the results from the two experiments, it was concluded that cassia gum is mutagenic in *Salmonella typhimurium* strain TA100, and not mutagenic in strains TA1535, TA1537, TA98, and *E. coli* strain WP_2uvrA (Verspeek-Rip *et al.*, 1998a); however, the positive result in this strain was observed only at precipitating concentrations and therefore these results may be questionable.

The ability of cassia gum to induce chromosome aberrations in cultured peripheral human lymphocytes was evaluated at concentrations of 1, 3, and 10 μg/ml, with and without metabolic activation. Cassia gum did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations (Bertens *et al.*, 1998).

The effect of cassia gum on the induction of forward mutations at the thymidine-kinase locus in L5178Y mouse lymphoma cells, with and without metabolic activation, was tested at concentrations up to 10 μg/ml. Cassia gum did not induce a dose-related increase in the mutant frequency, therefore, it was concluded that it is not mutagenic in the mouse lymphoma L5178Y test system (Verspeek *et al.*, 1998b).

In the three separate mutagenicity assays that were conducted on cassia gum, the only positive result observed was in the Ames assay with strain TA100 (Verspeek-Rip *et al.*, 1998a). Positive results were observed only at precipitating concentrations. Given the fact that the other Ames test strains (TA1535, TA1537, TA98, and WP_2uvrA) produced negative results only at precipitating concentrations, and no positive results were observed in any other test system, cassia gum is not considered mutagenic in appropriate test systems.

6.3 Other Studies

Hypersensitivity

Ten albino guinea pigs were intradermally injected with 0.2% concentration and epidermally exposed to a 50% concentration of cassia gum. Approximately 24 hours prior to epidermal exposure, each animal was challenged with 10% sodium dodecyl sulfate. Five control animals were similarly treated with the vehicle control (corn oil). Two weeks after the epidermal application all animals were challenged with a 50% cassia gum concentration and the vehicle. Skin reactions varying between grade 1 and 3 were observed in all experimental animals but

were not evident in the control animals. These observations were indicative of sensitization by cassia gum (Busschers *et al.*, 1998).

Skin prick tests performed on 125 pharmaceutical workers previously exposed to *Senna* pods (*Senna obtusifolia* is a synonym for *Cassia obtusifolia*) revealed that 15.3% were allergic to *Senna*. None of the 20 unexposed pharmaceutical workers also tested had a positive skin test to *Senna* (Marks *et al.*, 1991).

The allergenicity of 4 different species of *Cassia* (*auriculata, occidentalis, roxburghii,* and *siamea*), which were found to be dominant in India, was evaluated in 140 patients with a history of respiratory allergy. Intradermal skin testing was performed by injecting the antigenic extraction of the 4 *Cassia* species into the patients' forearms. Twelve percent (12%) had a positive skin reaction (Satheesh *et al.*, 1994).

A study on workers health was conducted on 62 volunteers at the Noveon (formerly BF Goodrich) Diamalt Inc., plant in Munich, Germany. The purpose of the study was to determine the risk to workers' health during the production of thickening agents, including ground cassia, with emphasis on pulmonary function and predisposition towards allergic reactions. The study program included: 1) a detailed medical history, 2) a physical examination, 3) clinico-chemical laboratory tests, 4) a pulmonary function test, 5) several allergy tests, and 6) dust measurements from different production areas. In the medical reports 54.8 % reported irritation of the eyes, nose, lungs, or skin and 37% were suffering from acute cold symptoms. While several positive results and diagnoses were made, there was no statistical evidence of correlation between symptoms and occupational factors (including number of days exposure to cassia powder) or personal factors (such as age, gender, occupational non-related previous diseases). However, participants who displayed sensitization to at least one common allergen were also more frequently sensitized to cassia powder and/or store mites. Also, the exposure to powder dust exceeded the limit value that is currently set for the inhalable dust fraction. The investigators recommended that preventive health protection measures be implemented in the workplace (Steget *et al.*, 1999). Such measures have been implemented at the Indiamalt plant where cassia gum is now produced.

The results of the first three sensitization studies for cassia would bear no effect on the use of cassia gum in human foods, and in some cases the *Cassia* species tested are not those used in the manufacture of Diagum CS™. In the fourth study, although positive reactions were

observed in humans, there was no evidence of correlation between exposure to the cassia powder and incidence of allergic reactions. Moreover, this is an occupational health issue and would not bear significant relevance to the safety of the finished product in food.

6.4 Muscle Toxicity

Evidence for Anthraquinones as Muscle Toxicants

The toxicological studies discussed in section 6.2 were done on purified cassia gum(Diagum CS™) which contains extremely low levels of anthraquinones. None of the studies on cassia gum showed any evidence of muscle toxicity because the levels of anthraquinones were well below the NOAEL for muscle toxicity. To establish an adequate tolerance for anthraquinone muscle toxicity, we review below the literature on the subject and calculate an appropriate tolerance.

6.4.1 Mechanism of Action

Muscle toxicity from exposure to anthraquinones occurs within a matter of days. Studies with anthraquinones indicate that the short time to occurrence is the result of a biochemical mechanism involving the inhibition of NADH:cytochrome C oxidoreductase activity in the mitochondria. These biochemical interactions utimately result in symptoms such as anorexia and weakness and characteristic changes in the muscle tissue such as swelling and loss of the mitochondrial matrix.

6.4.2 Early literature on sicklepod toxicity

Henson *et al.* (1965) reported widespread skeletal myodegeneration in cattle apparently caused by grazing on *Cassia occidentalis* or *Cassia obtusifolia.* Both plants appear to be capable of causing the disease, but *Cassia obtusifolia* is reported to be slightly less toxic than *Cassia occidentalis.* All parts of the plant are toxic, especially the seed (McCormack and Neisler, 1980). Nicholson *et al.* (1977) reported toxic myopathy and death in cattle ingesting foliage heavily contaminated with immature sicklepod (*Cassia obtusifolia).* Poisoned cattle are weak, become recumbent, unable to rise, and their urine is often darkened due to the presence of the muscle pigment, myoglobin. Intake of sicklepod was estimated at 12 –25 lbs per cow for 8 to 12 days. Large amounts of sicklepod appear to be required to produce sicklepod toxicosis. The type

of sicklepod and the toxic principle, i.e., leaves, pod, seed or stems, were not indicated in the Nicholson article, but assuming all of the consumed amount was toxic, a crude, short term LD_{50} from this study would be 5 kg /500 kg.bw = 10 g /kg.bw/day. McCormack and Neisler reported deaths of dairy cows as a result of eating feed that was approximately 50% leaves, stems and seeds of sicklepod (McCormack and Neisler, 1980). The signature lesions of the disease are cardiac and skeletal muscle degeneration, e.g., rhabdomyoltic intoxication (rhabdomylotic refers to striated muscle fibers) (Hensen *et al.*, 1965; McCormack and Neisler, 1980; Nicholson *et al.*, 1977).

These toxic myopathies are characterized by impairment of mitochondrial function, including swelling, loss of mitochondrial matrix, fragmented cristae and glycogen depletion (O'Hara and Pierce, 1974a, b; Graziano *et al.*, 1983). A spectrum of acute toxic effects including: anorexia, diarrhea, hyperpnea, tachycardia and progressive muscle incapacitation and recumbency typically develop over a 5-7 day period. Such effects were observed in both cattle and goats at approximately the same dietary concentration from 2-12 g seed /kg bw/day. Previous clinical and laboratory data established that sicklepod produces the same or a very similar disease in chickens, rabbits and cattle (Nicholson *et al.*,1986; Hebert *et al.*, 1983). In addition to these effects, the liver appears vulnerable to large dietary doses of *Cassia* species. *Cassia occidentalis* produces liver lesions in chickens (Graziano *et al.*, 1983) and *Cassia obtusifolia* may produce enlarged livers in rats (Crawford and Friedman, 1990). Loung and McCoy (1992) found muscle lesions in rabbits after feeding large doses of the aerial portions of the plant. Because they fed as high as 100% plant some (but not all) of their results may be due to nutritional deficiencies. The lesions in striated muscle resembled those in cattle poisoned with *Cassia obtusifolia* and those in rabbits poisoned with *Cassia occidentalis.*

The earlier scientific papers on *Cassia obtusifolia* or *occidentalis* poisoning in farm animals speculated that the toxic principle in *Cassia occidentalis* might be a toxic albumen, a toxic alkaloid or an anthraquinone derivative. Hebert *et al.*(1983) extracted seeds of *Cassia occidentalis* and found that the aqueous extract contained the toxic principle typically bound to particulate matter in the extract. They did not characterize it, but speculated that its water solubility meant that it was a polar molecule and allowed the possibility it could be proteinaceous.

6.4.3 Lewis and Shibamoto (1989)

Gradually the focus of the search for the toxic principle in *Cassia* shifted to the anthraquinones. The occurrence of anthraquinones and related compounds in many *Cassia* species had been demonstrated (Takahashi *et al.*, 1978; Takido, 1958a, b 1960). Lewis and Shibamoto (1989) found that the extracts of *Cassia obtusifolia* and anthraquinones inhibited NADH:cytochrome C oxidoreductase activity of bovine heart mitochondrial particles. This study provides very strong evidence that the anthraquinones in *C. obtusifolia* can cause muscle toxicity in cows. Two studies were done: an *in vivo* study showed significant elevation in plasma creatine phosphokinase (CPK) in rats when exposed for 12 hours to both aqueous and organic extracts of *C. obtusifolia*. Elevation of plasma CPK activity is a widely used and accepted indication of rhabdomylosis-degeneration of striated muscle. Elevated CPK activities were obtained with (1) ground seed extract of *C. obtusifolia* in the diet, (2) an aqueous suspension by gavage, or (3) an organic extract by gavage.

Both the seed extract and individual anthraquinones inhibited NADH: cytochrome c oxidoreductase activity of bovine heart mitochondrial particles *in vitro* with comparable potency and in a dose dependent manner. Morgan-Hughes *et al.* (1979) have reported myopathy due to a genetic deficiency of mitochondrial NADH:CoQ oxidorecutase activity. The presence of the toxic principle in both aqueous and organic extracts indicated that the toxin was not a protein, but a more lipophilic compound that is bound strongly to plant proteins. The myotoxic organic extract was found to be a mixture of nine anthraquinones and three anthrones. Chrysophanic acid was one of the major anthraquinones identified. This study provides clear evidence that the active principle in *C. obtusifolia* muscle toxicity is a mixture of anthraquinones.

6.4.4 Levels of anthraquinones in *Cassia obtusifolia* seed

It is important to know the level of anthraquinones in the seed in order to calculate approximate anthraquinone NOAELs based the intake of seed or extracts. In 1978, Koshioka and Takino developed a quantitative method for measuring anthraquinones in cassia seeds based on optical color value. (Koshioka and Takino, 1978.) Using this method Koshioka and Takino measured total anthraquinone levels in Japanese and Formosan samples of *C. obtusifolia* and *C. tora* seeds ranging from 0.80% to 1.10%. The authors also measured free, bound and total anthraquinones in 14 samples of powdered seeds of *C. obtusifolia* or *C. tora* used as crude drugs

from Korea, China, Japan, Formosa and Vietnam. The results ranged from 0.01 to 0.04% for free anthraquinones, 1.01 to 1.29% for bound anthraquinones and 1.04 to 1.31 % for total anthraquinones. It is well established that most of the anthraquinones in *Cassia* are bound to sugars in the form of glycosides (with the aglycon occurring usually in a reduced form, e.g., anthrones, dianthrones, or oxanthrone). Using thin-layer chromatography in 1990 Crawford *et al.*, analyzed the composition of sicklepod and showed that the seeds contained 1-2% by weight of total anthraquinones. No alkaloids or saponins were present. The major portion of the anthraquinones exist as the di- or triglycosides. These include rhein, physcion, obstufolin, emodin, questin, lesser amounts of others, and chrysophanic acid. Chrysophanic acid made up virtually 50% of the total anthraquinones in his seed samples (Crawford *et al.*, 1990). Several authors have confirmed the 1990 work by Crawford *et al.*

6.4.5 Impact of admixture of *C. obtusifolia* with *C. tora*

The raw seed material used for Diagum CS™ typically will contain some *Cassia tora.* The *tora* and *obtusifolia* seeds grow in the same field and are difficult to distinguish visually; they are therefore harvested together. Poethke *et al.* (1968) concluded that Vietnamese *C. tora* is identical with *C. obtusifolia*. Other investigations have shown that the two are remarkably similar if not interchangeable (Crawford *et al.*, 1990). But there are some detectable chemical differences, in both amino acids and anthraquinone content (Upadhyaya and Singh, 1986). Koshioka and Takino (1978) and Upadhyaya and Singh (1986) suggest that *C. tora* is a phytochemically distinct species derived from *C. obtusifolia*. This is also the conclusion of Randell (1995), who discussed the taxonomy and evolution of the two species. However Regional differences in *C. obtusifolia* and *C. tora* cultivars may easily obscure the minor differences between these closely related species. The close similarity in seed chemistry, morphology and ecological requirements between the two plants are far more significant than the relatively minor differences between them. We therefore conclude that the presence of *C. tora* in the raw seed does not present any significant cause for concern.

A literature search has identified no reports of muscle or any other toxicity associated with *C. tora*. In the absence of any published specific toxicity data on *C. tora*, it is reasonable to conclude that while the pattern of anthraquinones may be slightly different in the two seeds, the

total anthraquinone levels are approximately the same, and thus toxicity of *C. tora* can be assumed approximately the same or less than that *C. obtusifolia*.

6.4.6 Effect of processing on anthraquinone content

In the production process of Diagum CS™ the endosperm is separated from husk and germ within a rotating roaster that reaches temperatures up to 200°C for approximately 30 seconds. Data from the literature show that the toxicity is also reduced by roasting. Suliman and Shommein (1985) observed that the ability of cassia to produce muscle toxicity in goats was virtually abolished when cassia beans were fed at levels of 2.5 g/kg.bw/day before and after roasting. Roasting had less effect on reducing the toxicity when the goats were given doses as high as 5 g/kg.bw/day. The observation of thermal degradation of the toxic principle in cassia was made on *C. occidentalis* rather than on *C. obtusifolia*. However, the toxic symptoms seen in *C. occidentalis*–fed goats were similar to that observed in cattle fed *C. obtusifolia* (Putnam *et al.*, 1988), indicating that the toxic agents responsible are the same or similar for the plant species.

6.4.7 Levels of anthraquinones in Diagum CS™

Analytical results from more than 2000 batch samples (from product marketed abroad) indicate that the level of anthraquinones in cassia splits used for the production of Diagum CS™ averaged 173 ppm. (See App. II, Tab 3.) The maximum specification for anthraquinones in finished Diagum CS™ is established at 150 ppm. The optical color analytical method of Koshioka and Takino (1978) for total anthraquinones is used. Included with this notification are copies of certificates of analysis for three batches of Diagum CS™ confirming compliance with the 150 ppm specification for finished cassia gum. Dr. Utz' report on the production of Diagum CS™ (App. II, Tab 4) also includes analyses of four recent master batches of Diagum CS which confirm compliance with the total anthraquinone specification

6.4.8 Chrysophanic acid specification

Noveon has a specification for chrysophanic acid, one of the anthraquinones found in the seeds of *C. obtusifolia* and other cassia species, which was established to assure quality characteristics of Diagum CS™, e.g., color, taste. The proportion of chrysophanic acid in *C. obtusifolia* seeds has not been proven to be stable so Noveon does not rely on the level of

chrysophanic acid as a primary indicator of control of total anthraquinones. Noveon has selected an optical absorbance analytical method that measures total anthraquinones as the method for confirming control of potential muscle toxins in Diagum CS. (Koshioka and Takino, 1978.)

6.4.9 Determination of NOAEL for anthraquinone-induced muscle toxicity

6.4.9.1 Crawford and Friedman

Crawford and Friedman (1990) showed that despite its toxicity in rats and to range animals at high doses, sicklepod weed seeds do not produce any discernable indication of toxicity in the liver when fed at 0.5% of ground seeds in the basal diet. This study and some of the others allows an estimate to be made of the toxicity of the anthraquinones if we assume approximately 1% anthraquinones in the seeds (Koshioka and Takino, 1978; Crawford *et al.* (1990)). A dietary level of 0.5% equates to 5000 ppm or 500 mg/kg.bw/day for younger rats. If approximately 1.0% of the seed is a mixture of anthraquinones, the NOAEL for the anthraquinones is 5,000 ppm x 0.01 = 50 ppm in the diet or about 5 mg anthraquinone/kg bw/day for the young rat.

6.4.9.2 Putman *et al.*

Putnam *et al.* (1988) fed 15 bull calves, 5 calves per dose group, three doses of *C. obtusifolia* seed (0, 0.45, and 1.81 kg/day with their daily ration for 9 days). The effects of the low and high *C. obtusifolia* seed contamination were intermittent feed refusal, and diarrhea, and decreased weight gain in some animals. Average daily weight gains for the control, low dose and high dose groups were 0.76, 0.53 and 0.31 kg/day, respectively. The control group consumed all 3.63 kg of ration each day. The average daily feed consumption for the low exposure group was 3.45 kg (1.68-1.63 range) and 2.54 kg (0.68-3.63 range) for the high exposure group. No change was observed in serum aspartate aminotransferase (AST) creatinine phosphokinase (CPK) activity, lactic dehydrogenase activity (LDH), and histologic examination showed no evidence of muscle necrosis. The calculated estimate of average daily consumption of *C. obtusifolia* seed was 0, 0.36 and 0.70 kg/day or 0, 2.4 and 4.7 g/kg.bw/day. When a substantial amount of the daily quantity of seed presented was eaten, the toxic effects were transient diarrhea and anorexia. The authors concluded that at these average levels of *C. obtusifolia* seed consumption, the most serious effect, the decrease in weight gain, was probably due to impalatability rather than toxicity. The NOAEL for *C. obtusifolia* seed from this study was therefore

4.7 g/kg.bw/day or using 1% for seed-to-anthraquinone conversion, the NOAEL for anthraquinone toxicity from this study would be approximately 47 mg/kg.bw/day.

6.4.9.3 Lewis and Shibamoto

Lewis and Shibamoto (1989) fed adult male Sprague-Dawley rats an extract of ground *C. obtusifolia* seeds 3 days after which control diet was given. The anthraquinone extract was present in powdered rat chow at a concentration of 0.3%. Plasma creatine kinase (CPK) was significantly increased in the treated rats and returned to normal after a normal diet was reintroduced. There was also a temporary anorexia and loss in weight gain. The dosing equates to an effect level for the anthraquinones of 202 mg/kg.bw/day (0.3% x 26 g feed/day = 78 mg of extract per day /387g bw/day = 202 mg/kg.bw/day).

6.4.9.4 Dugan and Gumbmann

Diets containing sicklepod at 1, 2, 4, 8, 16 and 32% were fed to groups of 3 –5 Sprague-Dawley rats in a series of short term (8-9 days) toxicity studies (Dugan and Gumbmann, 1990). In addition, groups of five female Sprague-Dawley rats were fed the same dosages to compare the effect on body weight, feed and water consumption. There was 100% mortality in the 32% group and one death in the female 16% group. Assuming approximately 20% was a half-lethal dose the LD_{50} for a short term exposure was 200,000 ppm x 0.05 = 10.0 g/kg.bw/day. Sicklepod fed at 8% or less did not result in adverse clinical signs. There was a marked weight gain decrement clearly related to dose and this was barely detectable at the 2% dose and significant at 4%. The testes of rats fed 8% or more sicklepod seed exhibited a significant incidence of hypospermia. Histological evaluation of the liver did not reveal any pathology and liver weight was not increased. The lowest NOAEL (weight loss) for any toxic effect in the Sprague-Dawley rat fed sicklepod seed was 2%. (2% = 20,000 ppm x 0.05 = 1000 mg/kg.bw/day. If approximately 1.0% of the seed is a mixture of anthraquinones, the NOAEL for the anthraquinones is 10.0 mg/kg.bw/day. Despite significant weight loss, and the detection of testicular lesions in rats, there was no mention of muscle degeneration in rats. CPK levels were not measured, and glutamic-oxalacetic transaminase (GOT) levels increased only at doses of 16%. The seed in these studies was milled, and there is some evidence that the potency of the ground seed can decrease with storage time (O'Hara and Pierce, 1974a).

6.4.9.5 Friedman and Henika

Sicklepod methanol extracts were found to be mutagenic in the Ames *Salmonella* test without activation by S9, with TA 98 and TA 2637 being the most sensitive tested strains (Friedman and Henika, 1991). The extracts require hydrolysis in order to be maximally effective (a 15-fold increase in activity vs unhydrolysed). The authors concluded that "exposure to sicklepod seeds carries a risk for genotoxicity as well as for myotoxicity." However, mutagenic anthraquinones are not thought to pose a high cancer risk because of their inability to bind covalently with rat liver DNA (Bosch *et al.*, 1987). Emodin, an important anthraquinone in sicklepod is also present in rhubarb extracts and partially responsible for the mutagenicity of these extracts (Van der Hoeven *et al.*, 1983). The extracts of many commonly consumed fruits and vegetables are mutagenic (NAS, 1996).

Table 6 lists the results from the various field and laboratory studies from which it is possible to derive NOAEL values. The effect data from Lewis and Shibamoto are also shown for comparison.

Table 6

Listing of Field Observations and Toxicity Studies on C. Obtusifolia Seeds or Extracts

Study	NOAEL	Duration of Study	Toxic Effect	Animal
	Anthraquinone basis* (mg/kg.bw/day)			
Crawford and Friedman	5.0	unstated	No effect	Sprague-Dawley rat
Putman *et al.*	47	9 days on ration	Weight loss	Holstein calves
Lewis and Shibamoto	effect at 202	3 days on diet	Muscle toxicity	Sprague-Dawley rat
Dugan and Gumbmann	4.0	8-9 days on diet	Weight loss	Sprague-Dawley rat
Noveon studies (NOAEL = 2.5%)	0.5	90 days	No effect	Several studies

*_ The nominal composition of 1% anthraquinones in seed was used to convert seed-based level to an anthraquinone-based level.

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In a series of laboratory studies conducted by the Noveon Company (then BF Goodrich) (reviewed by Hallagan *et al.,* 1977), cassia gum was shown to have a low order of toxicity comparable to other gums, e.g., locust bean and carob. The reported NOAELs from the studies ranged from 0.75% to 5% cassia gum in the diet. Using a 2.5% NOAEL for purposes of calculation and the anthraquinone level of 150 ppm in the cassia gum, the diet therefore contained 3.75 ppm anthraquinones (0.025 x 150 ppm = 3.75 ppm). This corresponds to approximately 0.4 mg/kg.bw in young rats. Based on the results from the other studies, this dose was far too low to produce a muscle toxicity effect and also well below the capacity of anthraquinones to produce weight loss.

6.4.10. Risk assessment and characterization

6.4.10.1 Calculation of estimated daily intake (EDI) for Cassia gum and anthraquinones

As discussed in section 5.0 above, we calculate the dietary intake of cassia gum from the estimated U.S. production volume. This yields an EDI of 114.1 mg/p/d or 1.89 mg/kg.bw/d for cassia gum, which, assuming 150 ppm anthraquinone content, yields an EDI of 17.10 μg/p/d or 0.30 μg/kg.bw/d. for total anthraquinones. For the reasons discussed in section 5.0, the FEMA approach produces a more realistic estimate of likely intake of cassia gum and exposure to anthraquinones so it is reasonable to use this EDI in evaluating the safety of the anticipated intake of anthraquinones.

6.4.10.2 ADI and margin of safety for Cassia gum

The approximate NOAEL for anthraquinones was derived from Crawford and Friedman. Ground sicklepod (*C. obtusifolia*) seeds fed in the basal diet of rats at 0.5% (5000 ppm). If approximately 1/100 of this is anthraquinones in the sample,[17] the NOAEL for the anthraquinones is 5,000 ppm/100 = 50 ppm anthraquinone in the diet or about 5 mg /kg bw/day. This is close to the NOAEL that may be determined from the Dugan and Gumbmann study.

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[17] As discussed in section 6.4.4, the studies of Kohioka and Takino (1978) and Crawford *et al.* (1990) indicate that the total anthraquinone levels in seeds of *C. obtusifolia* and *C. tora* are likely to be approximately one percent.

Neither of these two studies exhibited muscle toxicity at these doses; the effect on weight gain decrement appears the more sensitive effect.

The ADI from the Crawford and Friedman study, using a 1000 fold safety factor, would be 5 mg/kg.bw/day/1000 = 0.005 mg/kg.bw/day = 5 μg/kg.bw/day. The FEMA EDI is 0.39 μg/kg.bw/day, about 10 times lower than the ADI. These data verify the safety of cassia gum containing levels of anthraquinones at 150 ppm, when used at the anticipated levels in the foods specified in the intended use table in section 1.0, above.

* * *

The foregoing and attached information considered, it is respectfully submitted that, under the conditions of intended use cassia gum is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because it is generally recognized as safe.

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APPENDIX I

CONCLUSIONS OF EXPERT PANEL

We the Expert Panel have, independently and collectively, critically evaluated the data and information summarized in the Notification for cassia gum and conclude that cassia gum produced in accordance with current good manufacturing practices and meeting the food grade specifications described therein is GRAS by scientific procedures for use as a stabilizer (thickening and gelling agent) in the specified foods.

Joseph F. Borzelleca, Ph.D.	Michael Pariza, Ph.D.
Professor, Medical College of Virginia	Professor, University of Wisconsin-Madison

APPENDIX II

SPECIFICATIONS, ANALYTICAL METHODS, CERTIFICATES OF ANALYSIS

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TAB 1

Raw Material Specification sheet for Super Grade Cassia Splits
and Quality Assurance Test NC 02602

Raw Material Specification
Natural Products Chemistry
Quality Assurance

Freedom Chemical Diamalt

NAME OF SUPPLIER:

[redacted]

RAW MATERIAL SPECIFICATION

CASSIA SPLITS / SUPER GRADE

GENERIC NAME	CASSIA TORA / SYN. C. OBTUSIFOLIA
CHEMICAL DESCRIPTION	CASSIA GALACTOMANNAN

TEST	*SPECIFICATION*	*TESTMETHOD*	*TEST-No*
APPEARANCE	ENDOSPERM SPLITS	visual	NC 00201
COLOUR	YELLOW TO SLIGHTLY REDDISH-BROWN	visual	NC 00301
MOISTURE	MAX. 8 %	IR-thermobalance	NC 00501
TOTAL COLOUR VALUE	MAX. 200 PPM	photometrical / wavelength 520 nm	NC 02501
CHRYSOPHANIC ACID	MAX. 10 PPM	HPLC	NC 02401
- BLACK AND DARK-BROWN SPLITS - FOREIGN MATTER - C.OCCIDENTALIS	MAX. 2 %	gravimetrical	NC 02602

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Product-No.	Revision	Replaces	Authorized	Date	Signature
[illegible]	FS 11/86	17.11.86	Dr Kt/Qual Kon	25.05.98	

TESTING STANDARD

CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	page 1 of 6

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I Principle

II Definition

III Equipment

IV Sampling

V Test procedure

VI Calculation

VII Documentation

TESTING STANDARD

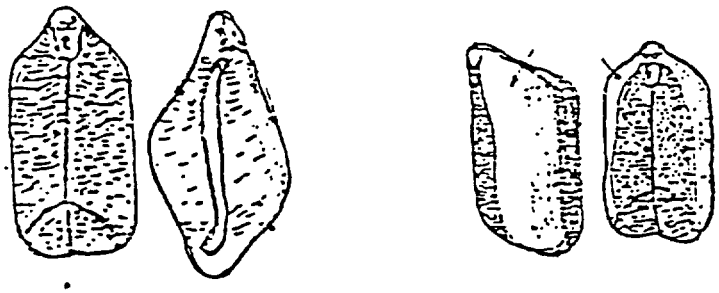
CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	page 2 of 6

I PRINCIPLE

The seeds of Cassia tora / obtusifolia and of Cassia occidentalis are quite different in shape and size.They can be easily segregated by hand and the amount of C.occidentalis is determined gravimetrically.

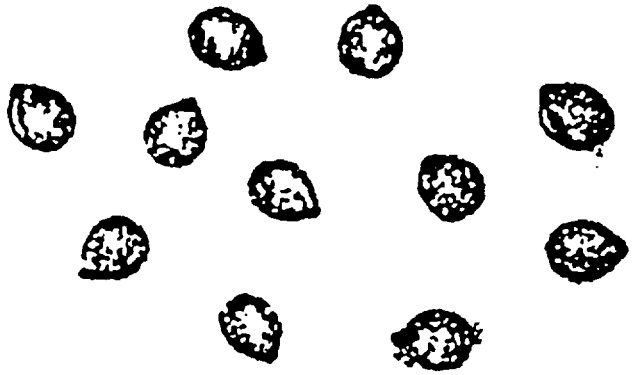
II DEFINITION OF CASSIA SEEDS

C.obtusifolia **C.tora**



Cassia obtusifolia is defined by a narrow and S-shaped aureole,while C.tora forms a broad and almost straight aurole.

C.occidentalis



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C.occidentalis is a 4-5 mm long flat,brownisch disk

TESTING STANDARD

CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	**page 3 of 6**

III EQUIPMENT

1 sampler

2 lab spoon (e.g. 150 mm)

3 weighing boat

4 bend pincer

5 magnifying glass

6 white sheet

7 balance (readings: 0,001 g)

8 calculator

9 plastic bags

10 sheet welder

IV SAMPLING

1 Select ($\sqrt{n} + 1$) of the bags at random from which 100 g - samples are drawn.These are thoroughly mixed to a representative sample and analysed for Cassia occidentalis,foreign matter and black seeds.

TESTING STANDARD

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CONTAMINANT: CASSIA OCCIDENTALIS	**page 4 of 6**

V TEST PROCEDURE

1 weigh 100 g of seeds and make thereof 10 groups with each 10.00 g (the numbering of groups is $G_1, G_2 .... G_{10}$)

2 pour 10 g of seeds onto a white sheet

3 segragate the sample by hand under a magnifying glass into following categories:

Cassia tora/obtusifolia
Cassia occidentalis
foreign matter
black seeds

4 weigh the separated categories

5

Partial measuring result:	
weight Cassia occidentalis	$= W_{co}$ [g]
weight foreign matter	$= W_{fm}$ [g]
weight black seeds	$= W_{bs}$ [g]

6 repeat step 2-5 for each 10 g - group ($G_1 ... G_{10}$)

TESTING STANDARD

CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	**page 5 of 6**

VI Calculation

1 sum up each category of the ten groups ($G_1 ... G_{10}$) analysed

2 ***Partial measuring result:***
weight Cassia occidentalis:

$$\Sigma(W_{co}) = G_1(W_{co}) + G_2(W_{co}) .... G_{10}(W_{co}) \; [g]$$

weight foreign matter

$$\Sigma(W_{fm}) = G_1(W_{fm}) + G_2(W_{fm}) .... G_{10}(W_{fm}) \; [g]$$

weight black seeds

$$\Sigma(W_{bs}) = G_1(W_{bs}) + G_2(W_{bs}) .... G_{10}(W_{bs}) \; [g]$$

3 ***Content Cassia occidentalis in percent***

$$\text{Percent Cassia occidentalis} = \frac{\Sigma(W_{co})\,[g] \times 100}{100\,[g]} \; [\%]$$

4 ***Content foreign matter in percent***

$$\text{Percent Foreign Matter} = \frac{\Sigma(W_{fm})\,[g] \times 100}{100\,[g]} \; [\%]$$

5 ***Content black seeds in percent***

$$\text{Percent black seeds} = \frac{\Sigma(W_{bs})\,[g] \times 100}{100\,[g]} \; [\%]$$

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6 ***Requirements:*** see specification

TESTING STANDARD

CASSIA TORA/OBTUSIFOLIA SEEDS	Quality assurance Test.No.: NC 02602 Version: 980501 Author: Dr.Kt Issued: 20.05.98
CONTAMINANT: CASSIA OCCIDENTALIS	**page 6 of 6**

VII Documentation

1 document results in lab journal

2 put segregated categories (c. occidentalis/foreign matter/black seeds) of all ten groups together and seal them in plastic bags

TAB 2

SOP-IPXX 003

SOP

GENERAL WORKING ORDER

Title:	Raw Material Handling
Valid since:	01.10.1997
This SOP replaces:	
Policy:	To describe the general process of the receipt of raw material, sampling, storage.
Responsibility:	General Manager, Production Manager, Senior Officer Quality Control
Changes:	
distribution:	General Manager Production Office

1. Policy:

This SOP describes the steps to be followed at the receipt of raw material in regard of handling, taking samples, and storage.

2. Area and Responsibility:

Loading platform, warehouse, laboratory

Responsible: General Manager
Production Manager
Administration Manager
Lab in charge

3. Regulations

3.1. Handling of the delivering truck

3.1.1. The truck has to be positioned at the forward platform, the canvas to be opened only under the roof during monsoon time to prevent rain water wetting of the gunny bags.

3.1.2. The freight bills have to be checked by the office to ascertain the conformity of the delivery with the raw material orders.

3.1.3. The delivery is labeled with a unique batch number as per usual standard to allow the verification of the material throughout the whole production.

3.1.4. The warehouse officer allots a suitable warehouse space and takes care of orderly storage, labeling, etc. during unloading.

3.1.5. The samples for quality control are taken by the lab in charge during unloading by statistical method, it is advised to take samples of the square root of bag numbers plus 1.
(That is for example at 180 bags $(|\sqrt{180}| + 1) = 14 + 1 = 15$, so samples from 15 bags, e.g. every 12th bag.
This samples are mixed, to get one statistic sample of the whole delivery.

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For the analysis methods applicable refer to :

Quality Control Manual, Analysis Method NC 02602

3.2. Documentation

3.2.1. The delivery is entered into the stock register as per customs requirements.

3.2.2. The batch is also entered into the production stock register with all quality parameters for the purpose of production planning, blending of different qualities, etc.

The production stock register is to be seen also as the data source of suppliers quality supervision.

This register has to be kept on absolutely actual condition on a daily basis for incoming and outgoing quantities and qualities.

3.3. Raw Material Release

According to the content of Cassia Occidentalis, foreign matter and chrysophanic acid, the quality is classified into three different groups (see raw material specifications):

3.3.1. **Super Grade:**

Raw material of this quality will be manufactured for use in food products.

3.3.2. **First Grade:**

Raw material of this quality will be manufactured for use in pet food products.

3.3.3. **Technical Grade:**

Raw material of this quality will be manufactured for use in different technical applications, i.e. textile and paper industry.

TAB 3

Report on the Production of Food Grade Cassia Splits;
Chart of Year 2000 anthraquinone levels in batches of cassia splits

Dr. Ferdinand Utz
Technical Manager

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noveon™

Memo

Date: August 5, 2001

To: Documentation Files

From: Ferdinand Utz

Subject: **Report on the Production of Food Grade Cassia Splits**

Date: July 21, 2001 – July 24, 2001

Location: Bardoli Agro Industries Pvt. Ltd.
Surat Dhulia Road
Ten Naka
Bardoli 391601
Gujarat, INDIA

Summary

Four different master batches with different batch sizes (Batch no. FTR 1 through FTR 4) were produced starting on July 21, 2001. In addition to the regular petfood grade split production, the food grade splits were cleaned twice. The final qualities in all batches met the analytical requirements of food grade cassia split.

Definition: Cassia split = purified endosperm of cassia tora and obtusifolia seed. The split mainly contains polysaccharides, which are the energy reservoir of the seed. As well husk and germ are removed from the seed.

1. Manufacturing procedure

The production of Cassia tora/obtusifolia split follows a procedure as shown in appendix 1.
The seeds came from the warehouse Dal Godwin No 2 (fresh crop from the seed collection season 2000/2001) and were packed in jute sacks.

The seed was cleaned in a five step purification process in order to remove foreign material like sand, small stones, litter, and seeds with a size different to Cassia tora and obtusifolia. The cleaned seeds were temporarily stored in open silos for further processing.

The splitting process started with roasting of the cleaned seed at a roasting temperature of 200 - 300°C. The temperature of the roasted seed is documented in a batch report and 50 g samples of the cleaned seed were gathered hourly from the pipe to the roasting oven. Both batch report and seed samples are part of each delivery to Indiamalt Pvt. Ldt.. The temperature of the product maintained a temperature of more than 100°C during all further processing steps.

The dehusking and cleaning process starts with two dehusking steps (DH-1/DH-3, resp. DH-2/DH-4), followed by a round sieve (RS-6) and a separator (SE-5). After conveying to a different hall, the product passes a round sieve (RS-5) again, followed by two dehusking mashines (DH5 and DH6). The split is purified by round sieving (RS-4), a separator (SE-6), two stone polishers (P1 and P2), and a brusher (B1).

For the current production the splits, after four times of dehusking and cleaning were packed into bags directly after the brusher B1, the production was interrupted, and the purification starting with round sieve RS-5 was repeated.

The final split was conveyed by air into a separate building, where the split was allowed to cool down over night for final packing into jute bags with PE inliner.

It was not possible to determine the yield from seed to split.

Batch		FTR 1	FTR 2	FTR 3	FTR 4
Seed feeding rate to the roaster		380 kg/h	380 kg/h	320 kg/h	350 kg/h
Split Quantity	4-times dehusking	3417	6097	3834	5245
[kg]	6-times dehusking	2600	4817	2990	4225
	Difference	-24%	-21%	-22%	-19%

2. Quality

As to an agreement in between BAPL and Indiamalt the final quality control is exclusively carried out at Indiamalt. However, BAPL carries out IPCs by visual judgement of the split in terms of the split color and completeness of dehusking.

2.1 Seeds

As per appendix 2 as well the cassia occidentalis content and the ratio in between tora and obtusifolia is not evenly distributed in the product

Batch No BAPL	Average Seeds size [mg]	Cassia Tora [%]	Cassia Obtusifolia [%]	Cassia Occidentalis [%]	Other Seeds [%]	Foreign Matter [%]
FTR 1	15,40	79,93	20,07	0,025	0,050	0,732
FTR 2	15,33	83,06	16,94	0,175	0,030	0,933
FTR 3	15,60	73,67	26,33	0,192	0,050	1,115
FTR 4	15,09	78,17	21,83	0,057	0,070	0,960
Average of all seed collection 2000/2001	19.58	74,6	25,4	0,040	0,090	3,50

The share of Cassia tora in the seed used for the master batches was relatively high and the seed size through the high content of Cassia tora lower than average.

2.2 Split

Contrary to regular split production the split was cleaned again through passing the equipment a second time starting with elevator E 16. The efficiency of the additional cleaning is shown below:

Samples for analysis were taken directly from the production and differ therefore to some extent from the final split analysis:

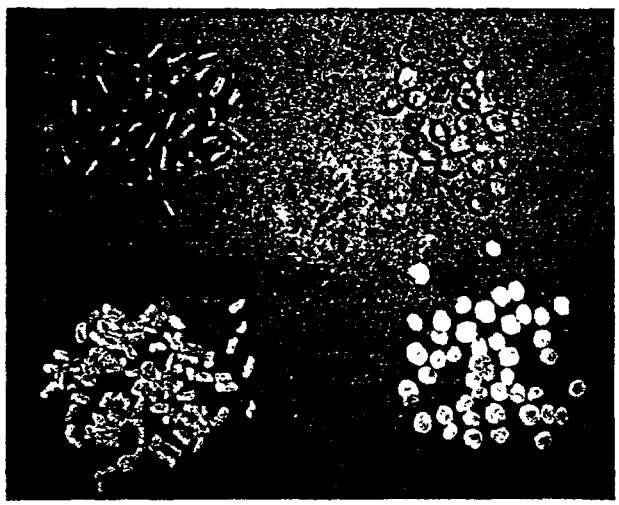
Batch		FTR 1	FTR 2	FTR 3	FTR 4	Average
Colour Value (anthraquinones) [ppm]	4-times dehusking	156	145	156	191	
	6-times dehusking	122	129	115	149	
	Difference	-22%	-11%	-26%	-22%	-20,3%
Chrysophanic acid [ppm]	4-times dehusking	4,0	4,0	3,2	3,7	
	6-times dehusking	3,4	3,1	2,2	2,7	
	Difference	-15%	-23%	-31%	-27%	-23,9%
Gel strength [g]	4-times dehusking	161	172	166	173	
	6-times dehusking	167	172	169	173	
	Difference	4%	0%	2%	0%	1,4%

2.3 Cassia tora/obtusifolia and Cassia occidentalis

It was observed through comparison of the seed analysis with the split produced out of these seeds, that nearly all types of foreign seeds were destroyed and milled into powder during the roasting and splitting process. The powder is

separated along with husk and germ powder of Cassia Tora / Obtusifolia into the so called "Cassia Meal" (Appendix 3). Cassia meal from roasted seeds is traditionally used in the India as an additive to Cattle Feed in concentrations up to 4 %.

Cassia occidentalis seeds survive to some extent the roasting and splitting process and are splitted themselves. As well the different types of Cassia Seeds and the splits of Cassia Tora / Obtusifolia and Cassia occidentalis are easy to distinguish.



Picture 1:

Cassia tora/ Obtusifolia seeds

C. occidentalis seeds

C. tora/obtusifolia Splits

C. occidentalis Splits

It was found that the percentage (w/w) of Cassia Occidentalis in the split samples was much lower than the results from the seed used for the split production.

While in 12 different seed samples of FTR 2 an average of 0.175% (w/w) Cassia occidentalis was found (Appendix 2), the average share of Cassia occidentalis splits, analyzed as an IPC from 10 different bags of the same batch after 4 times of dehusking, was much smaller:

Number	split weight [g]	occidentalis weight [g]	percentage
1	20,221	0,029	0,143%
2	34,240	0,028	0,082%
3	42,220	0,024	0,057%
4	19,720	0,012	0,061%
5	57,750	0,037	0,064%
6	40,830	0,041	0,100%
7	10,710	0,011	0,103%
8	49,030	0,025	0,051%
9	45,850	0,037	0,081%
10	21,950	0,004	0,018%
Total	**342,521**	**0,248**	**0,072%**

Table 3: Content of Cassia occidentalis split in FTR 2:

3. Food safety statement

Bardoli Agro Industries Pvt Ldt is a company specialized on the production of Cassia split since more than 20 years. A food safety statement edited by K.C. Joshi, the owner of the splitting plant, is enclosed (Appendix 4).

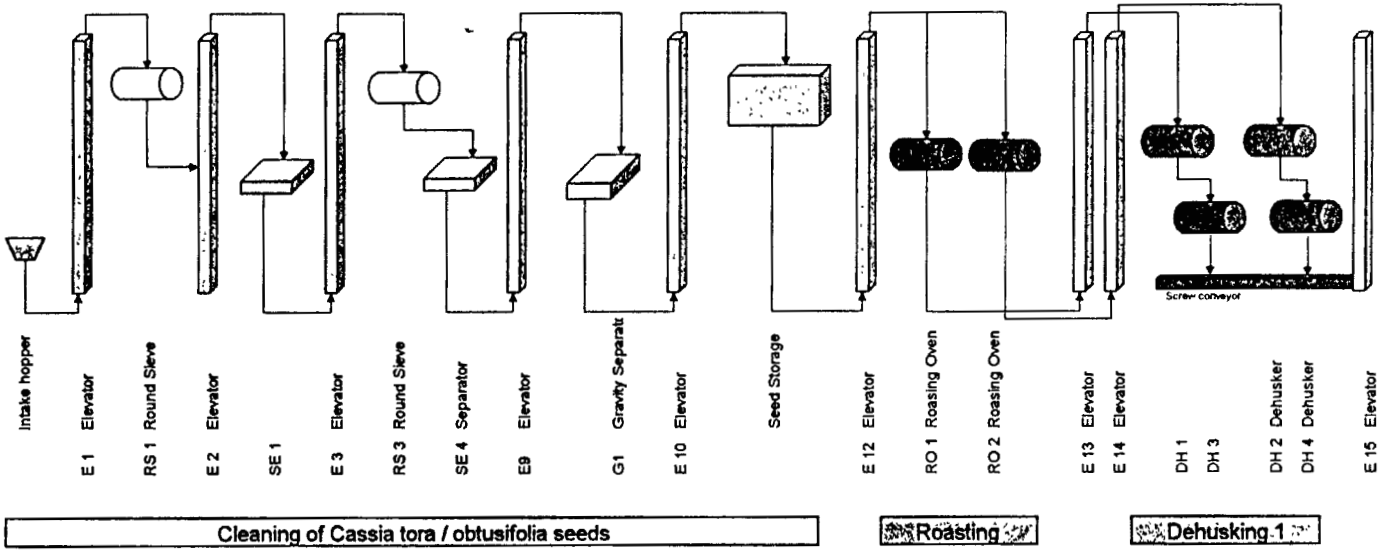
4. Conclusion

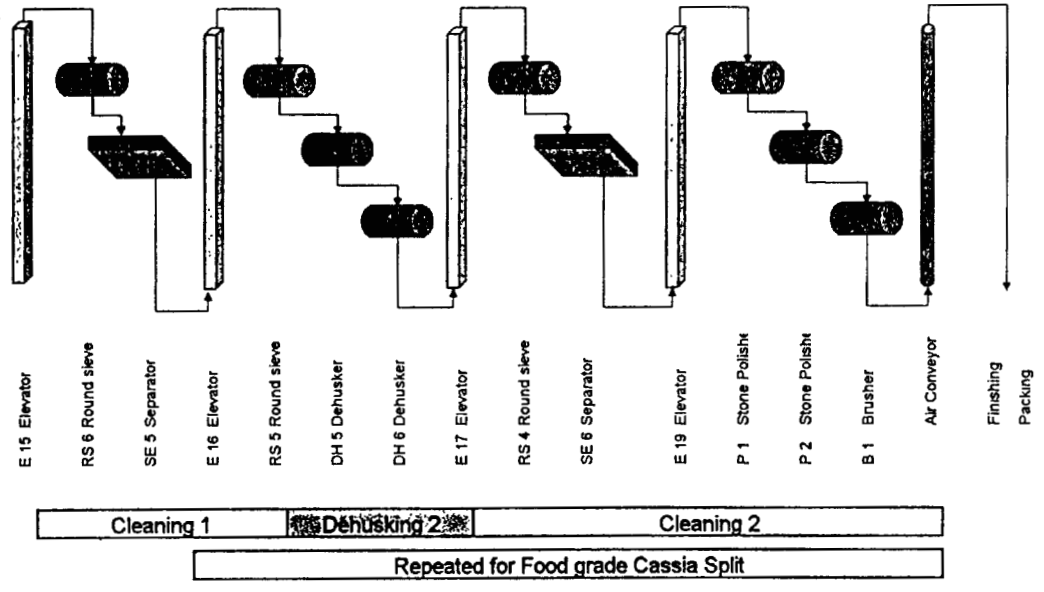
The Cassia split batches FTR-1 through FTR 4 produced at BAPL for the master batches of food grade Cassia gum resulted in material suitable as a raw material for the Indiamalt production.

As a result of our investigations regarding foreign seeds and splits, we will add an additional test to the food grade split analysis: the share of Cassia occidentalis split. The limit will be 0.1% (w/w) of the total weight of the split.
Other split different to Cassia tora/obtusifolia and Cassia occidentalis was nowhere to be found.

August 5, 2001
Ferdinand Utz

Appendix 1





Production Flow Chart

Cassia Split

Bardoli Agro Industries Pvt. Ltd.

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Appendix 2

Analysis of seed samples

DATE	SEEDS TAKEN FROM (TYPE)	PL.NO. OR INWARD NOS.	AVERAGE SEED SIZE (mg)	LOD (%)	GOOD SEEDS % (A) C.TORA	GOOD SEEDS % (B) C OBTUSIFOLIA	INSIDE SEED REPORT A & B % NORMAL YELLOW	% BROWN YELLOW	% DARK BROWN	CASSIA OCCIDENTALIS %	THER FOREIG MATTER SAND / SILICA %	REMARK % Extra Seeds
20.07.01	C-19	PL-FTR-1	16,30	4,4	78,92	21,08	90	10	0	0,000	0,500	0,10
20.07.01	C-20	PL-FTR-1	14,80	4,4	77,71	22,29	85	15	0	0,000	1,057	0,00
20.07.01	C-21	PL-FTR-1	15,80	4,7	78,92	21,08	60	40	0	0,000	0,903	0,20
20.07.01	C-22	PL-FTR-1	15,60	4,2	79,00	21,00	90	10	0	0,000	0,996	0,00
20.07.01	C-23	PL-FTR-1	14,70	4,6	86,00	14,00	70	30	0	0,000	0,799	0,00
21.07.01	C-1	PL-FTR-1	15,70	4,7	88,38	11,62	85	15	0	0,000	0,700	0,10
21.07.01	C-2	PL-FTR-1	15,60	4,3	74,75	25,25	95	5	0	0,000	0,501	0,00
21 07 01	C-24	PL-FTR-1	14,70	4,7	75,80	24,20	90	10	0	0,200	0,401	0,00
		AVERAGE	15,40	4,5	79,93	20,07	83	16,875	0	0,025	0,732	0,05
-	C-14	PL-FTR-2	14,50	5,0	80,00	20,00	90	10	0	0,398	0,498	0,00
21.07.01	C-12	PL-FTR-2	16,40	4,8	79,80	20,20	70	30	0	0,000	1,000	0,00
21.07.01	C-13	PL-FTR-2	15,30	4,8	72,40	27,60	80	20	0	0,500	1,401	0,00
21.07.01	C-16	PL-FTR-2	17,10	4,4	79,40	20,60	70	30	0	0,398	2,492	0,00
21.07 01	C-17	PL-FTR-2	14,80	4,8	79,00	21,00	70	30	0	0,200	0,400	0,00
21.07.01	C-18	PL-FTR-2	14,60	5,0	92,22	7,78	95	5	0	0,000	0,500	0,30
21.07.01	C-19	PL-FTR-2	15,30	4,5	95,79	4,21	75	25	0	0,200	1,200	0,00
21.07.01	C-20	PL-FTR-2	14,50	5,0	85,60	14,40	85	15	0	0,000	0,900	0,00
21.07.01	C-21	PL-FTR-2	14,00	4,9	88,42	11,58	80	20	0	0,300	0,903	0,10
21.07.01	C-22	PL-FTR-2	15,80	4,4	89,20	10,80	80	20	0	0,000	0,599	0,00
21.07.01	C-23	PL-FTR-2	16,10	4,3	77,40	22,60	90	10	0	0,100	0,600	0,00
21.07.01	C-24	PL-FTR-2	15,50	4,0	77,51	22,49	85	15	0	0,000	0,700	0,00
		AVERAGE	15,33	4,7	83,06	16,94	80,8	19,2	0	0,175	0,933	0,03
22.07.01	C-15	PL-FTR-3	18,00	4,3	58,75	41,25	65	35	0	0,000	0,300	0,00
22.07.01	C-16	PL-FTR-3	14,40	4,6	70,22	29,78	90	10	0	0,900	0,700	0,00
22.07.01	C-17	PL-FTR-3	14,20	4,5	78,40	21,60	90	10	0	0,000	0,801	0,00
23.07.01	C-1	PL-FTR-3	15,60	4,6	70,26	29,74	85	15	0	0,100	1,601	0,00
23.07.01	C-2	PL-FTR-3	17,00	4,1	59,68	40,32	85	15	0	0,199	0,299	0,00
23.07.01	C-3	PL-FTR-3	15,90	4,3	76,66	23,34	75	25	0	0,000	0,599	0,30
22.07.01	C-18	PL-FTR-3	18,00	4,3	69,08	30,92	70	30	0	0,300	2,400	0,00
22.07.01	C-19	PL-FTR-3	15,70	4,6	67,27	32,73	80	20	0	0,899	1,298	0,00
22.07.01	C-20	PL-FTR-3	15,40	4,8	80,72	19,28	90	10	0	0,000	1,200	0,00
22.07.01	C-21	PL-FTR-3	14,70	4,8	83,10	16,90	80	20	0	0,000	0,796	0,00
22.07.01	C-22	PL-FTR-3	13,30	4,5	80,64	19,36	95	5	0	0,100	2,102	0,00
22.07.01	C-23	PL-FTR-3	15,10	4,7	87,78	12,22	80	20	0	0,000	1,301	0,00
23.07.01	C-13	PL-FTR-3	16,10	4,2	75,15	24,85	90	10	0	0,000	1,100	0,30
		AVERAGE	15,60	4,5	73,67	26,33	82,7	17,3	0	0,192	1,115	0,05
24.07.01	C-1	PL-FTR-4	16,60	4,2	75,25	24,75	65	35	0	0,000	0,300	0,00
24.07.01	C-2	PL-FTR-4	14,20	4,7	76,55	23,45	80	20	0	0,000	1,698	0,00
23.07.01	C-12	PL-FTR-4	15,80	4,5	69,00	31,00	85	15	0	0,299	0,000	0,00
23.07.01	C-13	PL-FTR-3	16,10	4,2	75,15	24,85	90	10	0	0,000	1,100	0,30
23.07.01	C-14	PL-FTR-4	15,30	4,7	81,04	18,96	55	45	0	0,000	0,901	0,00
23.07.01	C-15	PL-FTR-4	14,70	4,6	88,80	11,20	80	20	0	0,000	1,701	0,00
23.07.01	C-16	PL-FTR-4	15,70	4,4	73,65	26,35	75	25	0	0,000	0,500	0,00
23.07.01	C-17	PL-FTR-4	15,80	4,5	77,30	22,70	75	85	15	0,000	0,000	0,00
23.07.01	C-18	PL-FTR-4	15,10	4,2	85,80	14,20	85	15	0	0,300	0,500	0,70
23.07.01	C-19	PL-FTR-4	15,30	4,7	86,03	13,97	70	30	0	0,040	1,200	0,00
23.07.01	C-20	PL-FTR-4	14,90	4,7	81,16	18,84	90	10	0	0,120	0,603	0,00
23 07.01	C-21	PL-FTR-4	14,80	4,2	85,34	14,66	90	10	0	0,040	1,696	0,00
23.07.01	C-22	PL-FTR-4	14,10	4,5	75,70	24,30	95	5	0	0,000	1,501	0,00
23.07.01	C-23	PL-FTR-4	14,00	4,5	71,54	28,46	85	15	0	0,053	1,996	0,00
23.07.01	C-24	PL-FTR-4	13,90	4,3	70,28	29,72	85	15	0	0,000	0,699	0,10
		AVERAGE	15,09	4,5	78,17	21,83	80,33	23,67	1,00	0,057	0,960	0,07

Appendix 3

TEST ON TEPHROSIA PURPUREA SEEDS

* Tephrosia purpurea seeds were sorted from Cassia seeds at Bardolli Agro Industries and sent to Diamalt for tests.

* 5 gm. of Tephrosia purpurea seeds were ground in an electric coffee mill for two minutes and the ground material sieved through a 1 mm mesh sieve (this is finer than the mesh used at Bardolli which is 1.5 mm)

* 5 gm. of mixed Cassia tora/obtusifolia seeds were also processed in the same way.

* The two minutes grinding produced Cassia splits which were partly dehusked (i.e. less dehusking than the normal process at Bardolli in India)

* Comparison of the two seed types showed that while Cassia splits were retained on the sieve only a very small amount of Tephrosia remained; about 99% went through.

* The experiment was repeated with both lots of seeds 'roasted' at 150'C for 2 minutes (oil bath) before grinding (this was to simulate the process at Bardolli)

* The results were similar with the Cassia seeds being more efficiently dehusked.

* The conclusion from this work is that because the Tephrosia seed is softer and more fragile than Cassia it is ground to a meal in the dehusking process and rejected to cattle feed at one of the sieves.If any did survive it would be present in the finished Diagum CS in quantities << 0.04 % (very much less than the level found in the seed mix in India prior to roasting)

Appendix 4

From: "kcco" <kcco@vsnl.com>
To: "Dr.Ferdinand Utz" <ferdinand.utz@noveoninc.com>
Date: 8/3/01 6:20am
Subject: FOOD SAFETY PROGRAM AT BARDOLI

Dr. Ferdinand Utz,
NOVEON INC
Georg Reismuller Strasse 32
D 80999 Munchen
Germany

Dear Dr. Utz,

FOOD SAFETY PROGRAM AT BARDOLI

Further to your letter of July 10th and your visit to Bardoli and further to our personal discussions on the above I am happy to convey to you about Bardoli's status on the above as was discussed enumerated below :

In our staff we have eight supervisors and one Quality Consultant Head who have been assigned responsibilities of the above. Out of above eight supervisors, one on each shift is on duty round the clock and others being given various other responsibilities.

CLEANING : We have twice a day cleaning procedure of the equipment and the factory floor. The equipment cleaning relates to cleaning the wiremeshes of different sieves to check any blockages and to ensure no holes have taken place in the wire meshes.

MAINTENANCE : Other than emergency maintenance there is a detailed weekly regular maintenance and preventive maintenance of all the machinery and post cleaning evaluation of this maintenance is reported in writing by those who have carried our the maintenance.

INSPECTION OF INCOMING MATERIAL TO WAREHOUSES :
Each truck bringing in raw material is given an Inward Lot Number. The trucks are weighed and then taken to the warehouse where they are to be unloaded. At the time of unloading two supervisors are on duty and a sampler gadget is pierced at two places in each bag as it gets unloaded and seed samples drawn to firstly visually check that it is only the Cassia

Seeds. Then after visual inspection the sample is put in a metal trough. At the end of unloading the samples of all bags which are in the trough are mixed and after mixing two 250 gms homogeneous samples are drawn and packed. One being kept in Bardoli factory sample room and one is sent to Indiamalt. The supervisors make out a detailed report of each Inward Lot No. In the report they put their visual perception estimating percentage of sand/silica/earth also percentage of small unusuable seeds, percentage of black seeds and percentage of other seeds if any. The most serious effort is to check percentage of black seeds and whether inside of the same is also black or yellow/orange. Also we seriously look for presence if any of Cassia Occidentalis.

PRODUCTION LOT : Each batch of 154 bags splits of 65 kgs each i.e. a ten tons lot is given a running production lot number. There is a report card for each production lot number. On this report card each hour drawing of sample of seeds going into Roaster is indicated. Also indicated on the report card are temperatures of seeds coming out of a roaster before going in for dehusking and temperature of finished splits coming out of the final brusher machine.

OUR CHECK ON CASSIA OCCIDENTALIS : To eliminate and identify such hazards as presence if any of Cassia Occidentalis, our supervisor on duty every hour round the clock draws a sample from the seeds which enter the roaster. Two ten grams samples are drawn. One sample kept in our sample room and second sample put in production lot box. When the production lot of ten tons split - 154 bags splits is completed all the hourly seed samples drawn during the production of that lot which are already properly marked with P/L No. and the hour of drawing of sample and date and are taken out of production lot box and is packed and made into a parcel and marked with P/L No. (i.e.batch number)

Each lot of production i.e each batch of production is of 154 bags splits whenever any lot of splits is despatched from Bardoli to Indiamalt the parcel containing hourly samples of seeds drawn which went into production of this lot of splits (this batch of splits) and detailed written report showing drawing of hourly samples of seeds coming out of roaster and hourly temperature of dehusked splits coming out of the brushing machine which is the final production machine are all despatched alongwith that truck.

To eliminate and identify hazards every hour of production samples of clean seeds entering production are taken as mentioned above and with each truck load of splits the seeds which went into production of these splits are sent with the truck for prevention and detection of any Cassia Qccidentalis in that lot of splits. The seed samples received by Indiamalt with the truck are to be immediately inspected to detect presence if any of Occidentalis and if sometimes there is any it is generally miniscule and within the tolerance limit and if by any remote chance if it is above the tolerance limit the splits of that truck would not be taken into production of food grade. Thus there is a full proof control in force to prevent by accident entry of Cassia Occidentalis into production.

PROCESSING RECORDS : The processing records from seed to split and the by-product meal are being maintained at factory for entire production on daily basis.

PACKING : The seeds come from sellers packed in secondhand gunny bags but supplier are prohibited from using improper bags.

STORAGE : Normal practice is to store Cassia Seeds year wise in different warehouses. None of these warehouses are located in areas where toxic chemicals or other contaiments are stored. The warehouses where we store Cassia Seeds are exclusively for Cassia Seeds only.

USAGE OF SEEDS : Normal usage of seeds as raw material is FIFO basis

except during the four month period of new crop arrivals as whatever can be processed directly of new crop is processed as it gives a much better qualtity without much efforts. Further in special circumstances where out of 26 warehouses we have to emplty any warehouse early as required by Warehouse owner or due any damage to a warehouse or where Indiamalt needs production of particular year seeds other than FIFO basis.

OPERATIONAL PRACTICES : During the production to avoid inclusion of metal, magnets are provided at several places of production.
In the production of splits there is no hand contact.

The finished product is packed and given production lot number and all bags of that number are marked and stored batchwise.

We produce only one product which is Cassia Splits in our factory and therefore adlulteration which other products is unlikely but all the same care is taken for this also.

RECORDS : For all above points we have proper records.

PERSONNEL PRACTICES : Responsibility for assuring compliance by supervisory personnel to the above policies is properly assigned.

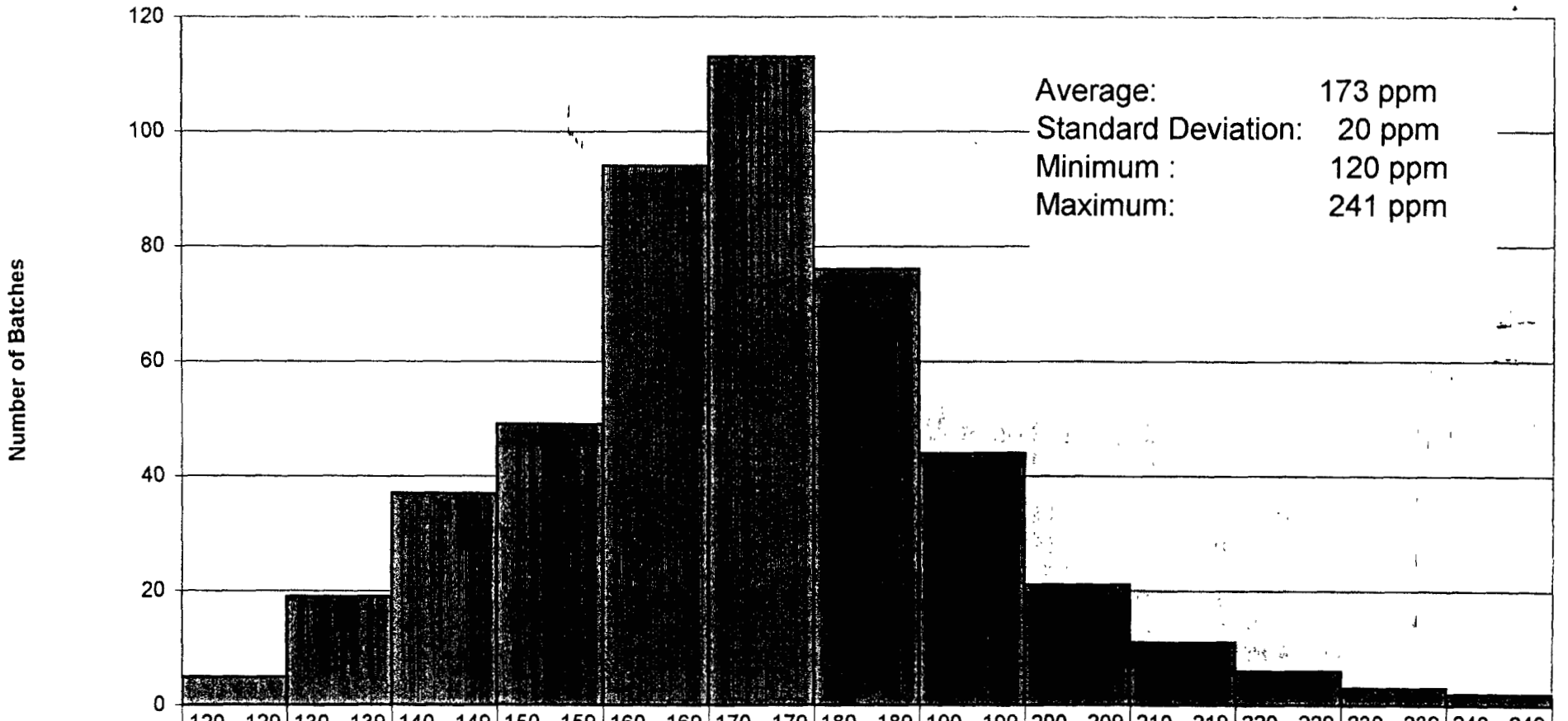
Trust the above is in order.

Best Regards

For Bardoli Agro Industries Pvt Ltd.,

(Kamlakant Joshi)
Director

Year 2000: Total Anthraquinone Content (=Color value) in Cassia splits



	120 - 129	130 - 139	140 - 149	150 - 159	160 - 169	170 - 179	180 - 189	190 - 199	200 - 209	210 - 219	220 - 229	230 - 239	240 - 249
Number of Batches	5	19	37	49	94	113	76	44	21	11	6	3	2

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TAB 4

Report on the Production of Diagum CS;
includes SOP XX004 and Certificates of Analysis for 4 Master Batches of Diagum CS

Dr. Ferdinand Utz
Technical Manager

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Memo

Date: August 5, 2001

To: Documentation File

From: Ferdinand Utz

Subject: Report on the production of food grade Diagum CS

Date : July 26 – 28, 2001

1. Introduction :

Indiamalt Private Limited is a food processing company engaged in the manufacture of edible gum powders. The Company was built in 1996 and came on stream in mid 1997. Indiamalt is a ISO 9002 certified Company (Certificate No. 041009202) and follows a strict quality policy (Appendix 1 : Quality Policy) in order to meet food safety requirements, and to maintain the best levels of customers satisfaction.

From July 26, through July 28, 2001 four master batches of food grade Cassia Gum (Diagum CS) were produced. For this purpose the regular production of Diagum CS was interrupted, all equipment was emptied and checked.

2. Raw Material :

The raw material in the production of Diagum CS (Cassia Gum) is the split (=endosperm) of Cassia tora and/or Cassia sbtusifolia seeds. All splits are exclusively produced by Bardoli Agro Industries Pvt Ltd. Incoming split deliveries, sampling and unloading are subject to SOP-IP XX 003 (Appendix 2). Along with each split delivery we get since April 2001 a record on the split production and samples of the seed used for the actual split lot. 50 g seed samples are taken hourly at BAPL from the roaster feeding equipment.

Each split batch was analyzed along with the cleaned seeds before use in the production of Diagum CS.

Table 1 : Cassia Seeds

Batch No BAPL	Average Seeds size [mg]	Cassia Tora [%]	Cassia Obtusifolia [%]	Cassia Occidentalis [%]	Other Seeds [%]	Foreign Matter [%]
FTR 1	15,40	79,93	20,07	0,025	0,050	0,732
FTR 2	15,33	83,06	16,94	0,175	0,030	0,933
FTR 3	15,60	73,67	26,33	0,192	0,050	1,115
FTR 4	15,09	78,17	21,83	0,057	0,070	0,960
Average of all seed collection 2000/2001	19.58	74,6	25,4	0,040	0,090	3,50

Table 2 : Cassia Splits

Split Lot BAPL	**FTR-1**	**FTR-2**	**FTR-3**	**FTR-4**
Corresp. Split Lot Indiamalt	**240701-1**	**240701-2**	**260701-2**	**260701-1**
Quantity	2600 kg	4800 kg	2290 kg	4225 kg
Gel Strength	173 g	175 g	170 g	171 g
Viscosity 1%	385	405	365	385
Loss on drying	5.3	5.1	5.2	4.9
Colour Value (anthraquinones)	122 ppm	131 ppm	145 ppm	149 ppm
Chrysophanic acid	2.5 ppm	3.5 ppm	3.7 ppm	5.6 ppm
Good split [%]	98.25	97.44	97.89	98.11
Bad Split (having husk) [%]	1.27	2.27	2.11	1.83
Black or brown split [%]	0.48	0.29	0.00	0.05
C. occidentalis split [%]	0.064	0.050	0.060	0.044
Classification	Super Grade	Super Grade	Super Grade	Super Grade

The seeds used for the split trials had an above average content of Cassia tora and a below average seed size. Since we found in addition small quantities of Cassia occidentalis in the seeds, the trials for the production of food grade split were an acid test for the production of food grade Cassia splits

3. Production of Diagum CS

The production process of Diagum CS is described in SOP-IP XX 004 (Appendix 3). In addition to this process, dark splits were removed by a Sortex equipment. The process can be subdivided in four different sections.

- Final cleaning of the splits
- Washing, soaking, squeezing and drying of the flakes
- Milling
- Blending of a batch and final pack

Samples of each side product in the split cleaning procedure were taken and analyzed on hazards (total authraquinone content, chrysophanic acid).

Table 4 : Mass Balance

Split Batch BAPL	FTR 2	FTR 1	FTR 3	FTR 4
Total splits charged [kg]	4810	2600	2990	4225
Split Lot No. Indiamalt	240701-2	240701-1	260701-2	260701-1
Material rejected at M-2 [kg]	4.90	2.93	3.35	4.95
Rejected at Sortex [kg]	40.4	21.8	23.9	33.8
Rejected at Destoner [kg]	6.65	1.34	2.74	2.80
Washing water discharged (l)	5200	2800	3200	4600
Diagum CS Batch No	01072611	01072612	01072711	01072712
Batch size [MT]	4.325	1.900	2.700	3.900

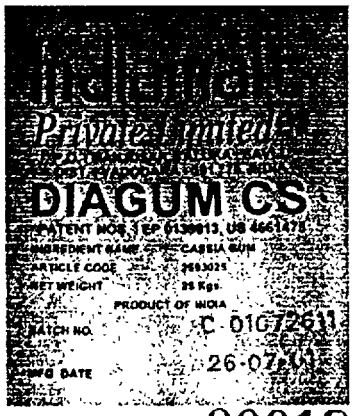
The milling of Cassia gum is a continuous process. In order to ovoid overlaps in the batches, we removed at the beginning and in between the different batches a few hundred kgs each. This material will be blended later on in pet food grade Diagum CS

Table 5 : Color Values of rejected material

	Color value	Rejected	Loss on color value
Rotary shifter	153 ppm	16.1 kg	- 0.1 %
Destoner	308 ppm	13.5 kg	- 0.2 %
Sortex	186 ppm	120 kg	- 1.2 %
Waste water	7.02 ppm	15,800 kg	- 6.1 %
Average Diagum CS value	141.2 ppm	12850 kg	

Table 5 demonstrates that washing of the split and using a color sorting equipment (Sortex) has a positive effect on the quality of the final gum. Previous investigations with less purified split qualities (First grade split) showed a higher percentage of the cleaning effect.

Samples of the final product are withdrawn in the packing station according to the instructions of the Quality Manual. The product is packed in 25 kg paper bags with LDPE inliner labled as shown below.



Diagum CS is stored batch wise on the pallets in the warehouse before further shipped to customers.

4. Quality of the Food Grade Diagum CS Master Batches

Sampling of Diagum CS and final analysis of the finished product is regulated by our ISO manual.

Lot no Diagum CS	01072611	01072612	01072711	01072712
Split Lot BAPL	FTR-2	FTR-1	FTR-3	FTR-4
Quantity	4325 kg	1900 kg	2700 kg	3925 kg
Gel Strength	152	148	146	152
Break Force Index (MARS)	1.10	0.99	1.03	1.05
Break strength Index (FRISKIES)	1.32	1.26	1.20	1.30
Slope Index (FRISKIES)	1.14	1.07	1.09	1.10
Viscosity 1%	230	205	190	220
Loss on drying	12.0	12.0	12.0	10.7
Colour Value (anthraquinones)	145 ppm	145 ppm	140 ppm	136 ppm
Chrysophanic acid	3.5 ppm	2.4 ppm	3.7 ppm	3.6 ppm
PH-Value	7.69	7.71	7.76	7.91
Protein content [%]	5.31	5.43	6.69	5.81
Fat content [%]	0.29	0.35	0.45	0.50
Sieve less than 150 µm [%]	83.65	86.35	86.45	81.50
Sieve less than 180 µm [%]	99.70	98.75	98.70	98.35
Sieve less than 250 µm [%]	99.85	99.75	99.60	99.65
Total aerobic microbial count (CFU/g)	2500	2000	1500	2000
Total combined yeast and mold (CFU/g)	< 50	<50	<50	<50
Test for absence E. Coli (-ve in 1.0 g)	Complies	Complies	Complies	Complies
Test for absence E. Coli (-ve in 1.0 g)	Complies	Complies	Complies	Complies
Heavy metals ≤ 0.002%	Complies	Complies	Complies	Complies
Lead ≤ 5 mg Pb/kg	Complies	Complies	Complies	Complies
Arsenic ≤ 3 mg As/kg	Complies	Complies	Complies	Complies

All analytical testing standards are based on a gum powder with a particle size less than 250 µm. The problem by analyzing split is, that splits can't be totally pulverized with laboratory methods to a fine powder. After repeated milling with a stone mill the yield of a 250 µm powder is only up to 60 %. As hazards (anthroquinones, chrysophanic acid) and soluble gum are not evenly distributed in the split, the analytical results differ to some extent from the results in Diagum CS. However, the data resulting from the split analysis allow a classification into different grades of split qualities. Decisive is the final result in Diagum CS.

5. Food safety requirements

Indiamalt is an ISO certified company. Food safety requirements are included as a main part of our ISO procedure. This includes all parameters from the raw material to the final product, warehousing, a master cleaning schedule, pest control, and so on. Base of our food safety standards is the "AIB Consolidated Standards for Food Safety" published by the American Institute of Baking (ISBN 1-880877-25-2).

August 8, 2001
Dr Ferdinand Utz

Appendix 1

QUALITY POLICY

INDIAMALT PRIVATE LIMITED is a food processing company engaged in the manufacture of edible gum powders. It has an agro-based and environmentally friendly process-operation having undisputed leadership and monopoly in the qualitative and quantitative manufacture of Cassia Gum Powder. INDIAMALT PRIVATE LIMITED has a prominent international reputation and this stature makes it to set its own standards and guidelines for a Quality Policy, which stands compared as among the best internationally.

The Quality Policy of INDIAMALT PRIVATE LIMITED is categorized under 5 heads:

(1) Customer Specific
(2) Product Excellence
(3) Process Efficiency
(4) Employees-result specific
(5) Organization-business oriented

(1) Customer Specific

INDIAMALT PRIVATE LIMITED shall strive to maintain the best levels of customer satisfaction by manufacturing products having qualities as per customer's specifications.

(2) Product Excellence

INDIAMALT PRIVATE LIMITED is committed to manufacture edible gum powders (Cassia) of the best quality and consistent composition to suit customer requirements.

(3) Process Efficiency

INDIAMALT PRIVATE LIMITED uses state of art technology, which is the best available in the industry and proves to be most cost effective and economical. It maintains manufacturing standards comparable to the European food industry.

(4) Employees-result specific

INDIAMALT PRIVATE LIMITED employs people who are professionally qualified and have reasonable relevant past experience. In-house training and orientation has inculcated a result-oriented mind-set.

(5) Organization-business oriented

INDIAMALT PRIVATE LIMITED aims to preserve and maintain the market leadership achieved by its customer specific and product excellence approach. It employs sound and healthy business practices to maximize value addition.

DATE: 01-12-1998 MANAGING DIRECTOR

Appendix

SOP

GENERAL WORKING ORDER

Title: Raw Material Handling

Valid since: 01.10.1997

This SOP replaces:

Policy: To describe the general process of the receipt of raw material, sampling, storage.

Responsibility: General Manager, Production Manager, Senior Officer Quality Control

Changes:

distribution: General Manager

Production Office

written by:	reviewed by:	approved by:
Technical consulting	Quality Control	Managing Director

1. Policy:

This SOP describes the steps to be followed at the receipt of raw material in regard of handling, taking samples, and storage.

2. Area and Responsibility:

Loading platform, warehouse, laboratory

Responsible: General Manager
Production Manager
Administration Manager
Lab in charge

3. Regulations

3.1. Handling of the delivering truck

3.1.1. The truck has to be positioned at the forward platform, the canvas to be opened only under the roof during monsoon time to prevent rain water wetting of the gunny bags.

3.1.2. The freight bills have to be checked by the office to ascertain the conformity of the delivery with the raw material orders.

3.1.3. The delivery is labeled with a unique batch number as per usual standard to allow the verification of the material throughout the whole production.

3.1.4. The warehouse officer allots a suitable warehouse space and takes care of orderly storage, labeling, etc. during unloading.

3.1.5. The samples for quality control are taken by the lab in charge during unloading by statistical method, it is advised to take samples of the square root of bag numbers plus 1.
(That is for example at 180 bags ($|\sqrt{180}| + 1) = 14 + 1 = 15$, so samples from 15 bags, e.g. every 12th bag.
This samples are mixed, to get one statistic sample of the whole delivery.

For the analysis methods applicable refer to :

Quality Control Manual, Analysis Method NC 02602

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3.2. Documentation

3.2.1. The delivery is entered into the stock register as per customs requirements

3.2.2. The batch is also entered into the production stock register with all quality parameters for the purpose of production planning, blending of different qualities, etc.

The production stock register is to be seen also as the data source of suppliers quality supervision.

This register has to be kept on absolutely actual condition on a daily basis for incoming and outgoing quantities and qualities.

3.3. Raw Material Release

According to the content of Cassia Occidentalis, foreign matter and chrysophanic acid, the quality is classified into three different groups (see raw material specifications):

3.3.1. **Super Grade Splits:**

Raw material of this quality , spec nr. 0150383, is used for the production of food and pet food grade DIAGUM CS.

3.3.2. **First Grade Splits:**

Raw material of this quality, spec. nr. 0150478, is used for the production of Cassia Powder.
This Cassia Powder can be released as food or pet food grade DIAGUM CS (sampling and analytical testing see SOP IP XX 004)..if the analytical results are in accordance with the specified values of the DIAGUM CS specification.
If the analytical results are not in accordance with the DIAGUM CS specification, the Cassia Powder is determined for use in technical applications, i.e. textile and paper industry.

3.3.3. **Technical Grade Splits:**

Raw material of this quality will be used for production of Cassia Powder for technical applications, i.e. textile printing and seizing,only.

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Appendix 3

SOP

GENERAL WORKING ORDER

Title:	Production Process Cassia Gum
Valid since:	01.01.1998
This SOP replaces:	
Policy:	This SOP describes the manufacturing process for Cassia Gum Powder
Responsibility:	General Manager, Production Manager, Senior Officer Quality Control
Changes:	
distribution:	General Manager Production Office

000188

written by: Technical Consulting	reviewed by [redacted] Quality Control	approved by: Managing Director

1. Policy:

This SOP describes the basic steps of the process which have to be followed.
It does not contain varaiable and/or secret process paramenters, they are prescribed as required on the daily production orders.

2. Area and Responsibility:

Production Area

Responsible: General Manager
Production Manager
Shift in Charge

3. Regulations

3.1. Raw material supply

The raw material (Cassia Splits) are filled from the gunny bags into the feeding bin/ elevator at the ground floor.

Batch/Quality and Quantity: refer to actual production order

Handling: Take care of the correct setting (mark)of the elevator inlet valve to avoid overloading.

Do not throw threads or bag pieces into the bin.

While floor cleaning, take care that the bin is covered to avoid dirt beeing brushed into the feeding bin.

3.2. Pre - Cleaning of splits

Before reaching the silo cell, the splits pass a round sieve "REIMELT" to remove threads, twigs, stalks, rests of paper or plasik bags etc.

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For inspection and cleaning, refer to SOP IP_Hg 002.

In case of bypassing the sieve due to maintenance etc., the responsible shift in charge has to take care of intensive supervision of the raw mat. filling to exclude foreign bodies in the splits.
The bypassing has to be documented on the production protocol.

3.3. De-stoning, Dust removal

The Stoner (BÜHLER) has to be controlled in his function one time per shift during operation,
This has to be done by hand samples and visual control of the cleaned splits, if they are free of stones, other grains, non-dehusked seeds etc.
Further the waste has to be observed on its content of good splits.

Changes in settings of the aspiration valve, slope and vibration amplitude only by qualified operators.

There are no specific settings prescribed, because they depend on the composition of the splits with foreign materials, the flow rate, etc. and are to be tuned on the spot and by actual requirements.

For basic settings, after repair etc., refer to process manual.

The waste has to be disposed off as fertiliser on the company land, the quantity is to be weighed and documented in the customs stock register.

3.4. Dosage

The dumping balance determines the batch size with the size and number of dumpings.
The settings are to be controlled by the shift in charge and documented in the production protocol.

Changes in settings by qualified personnel only on direct order of the production manager and under his supervision.

For basic settings refer to process manual.

3.5. Elevators

The three elevators have to be controlled weekly on slippage and parallel running of the belts.
(Responsible: Maintenance Chief)
Deviations have to be reported immediately to the production manager, who is responsible for repair to avoid frictions, possibly causing dust explosions.

3.6. Washing machines

The performance of the washing machines have to be controlled by the operators continuously.

Points of control:

- Water pressure: Refer to production order
- rpm: Refer to production order
- function: Visual control during every washing process:
  - turbidity of waste water
  - content of splits in waste water
  - material flow (clogging)

One time per shift the sieve has to be changed, the used one cleaned by high pressure lancet.

3.7. Double Cone Mixers

Runtime of the mixers is essential for continuous product quality.

For settings refer to process manual.

Change of settings by production manager only.

One sample of every batch has to be analysed on humidity by laboratory.
For the analysis method refer to the quality control manual.

3.8. Lump brakers

The operators have to take care of complete emtying of the hopper and needle screw after every batch to avoid resting humid material developing grouth of bacteria and mould.

3.9. Hydraulic Flaker

The Hydraulic Flaker has to be run on pressure only when the feeding roll is delivering material to the rolls.

It is compulsory to turn pressure to zero and separate the rolls when the material feeding stops out of any reason.

000191

For the following settings refer to process manual:

- Setting of slide valve of feeding roll
- Hydraulic pressure of flaker rolls
- rpm and ratio of flaker rolls

3.10. Manual Flakers

The pressure settings of the manual flakers have to be put to zero on stop of material flow equal to the hydraulic flaker.

Because it is impossible to measure the force and balance of pressure at this devices, the settings have to be done by well trained personnel only.

Important for the settings is the visual judgement of flake quality, which requires long term practice and education, and is depending also on the humidity of the splits and the provenience.

For rpm and ratio of the rolls refer to the process manual.

3.11. Dryer

The operation of the dryer requires attention on the following:

- Starting of the fan against closed slide valve only
- Inlet air temperature refer to process manual
- Thermic fluid temperature refer to process manual
- Stopping of dryer:
  Close Oil valve or shut down boiler, run dryer fan for 15 minutes without heating to prevent local overheating of exchangers or piping.

Regular In Process Control of dryed material humidity is compulsory:

For frequency refer to process manual
For Outlet-Humidity refer to process manual

000192

3.11. Milling

The milling is a continuous manufacturing step, including repeated grinding on a series of roller mills and separation of certain particle sizes with plansifters.

For the following refer to process manual:

- Groove specification for every roller mill
- rpm and ratio of rollers
- mesh sizes of every plansifter department (in microns)

Regular In Process Control of particle size of the finished material (sample from the collecting pipe) is compulsory.

For analysis method of particle size refer to quality control manual.

Oversized particles exceeding the limit proof torn sieves, in this case samples from every sifter department have to be checked to locate the damage.

In case of torn sieves, the production has to be stopped immediately and the damaged sieves be changed.

The already produced finished goods in the blender have to be packed and analysed on oversize particles.

The concerned batch of finished goods has to be put on quarantine, for further treatment refer to quality control manual.

Restart production with repaired sieves with new batch number.

3.12. Packing

The finished powder is collected in the blending silo of the concerned production line.

For packing, the blender has to be chosen on the control panel, which controls the direction of the discharging screw.

The product is conveyed over a control round sieve to eliminate potential foreign materials before packing.

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The weighing scale has to be checked on correct settings daily, deviations are to be reported immediately to the production manager, who is responsible for initiating maintenance.

The packed paper bags have to be statistically controlled for their weight on the electronic balance to ensure the proper performance of the weighing scale.

The bags have to be sewed, marked with the batch number of the production order, then palletized and stored in the finished goods warehouse.

While packing, around 50% of the batch, one sample has to be taken from the operators and send to the laboratory for further processing.

- For the following refer to the process manual:
- Settings of the weighing scale
- Regular check of the control sieve
- How to sew the paper bags with the polyethylene-inliner

For the following refer to the quality control manual:

- How to take the sample of finished goods

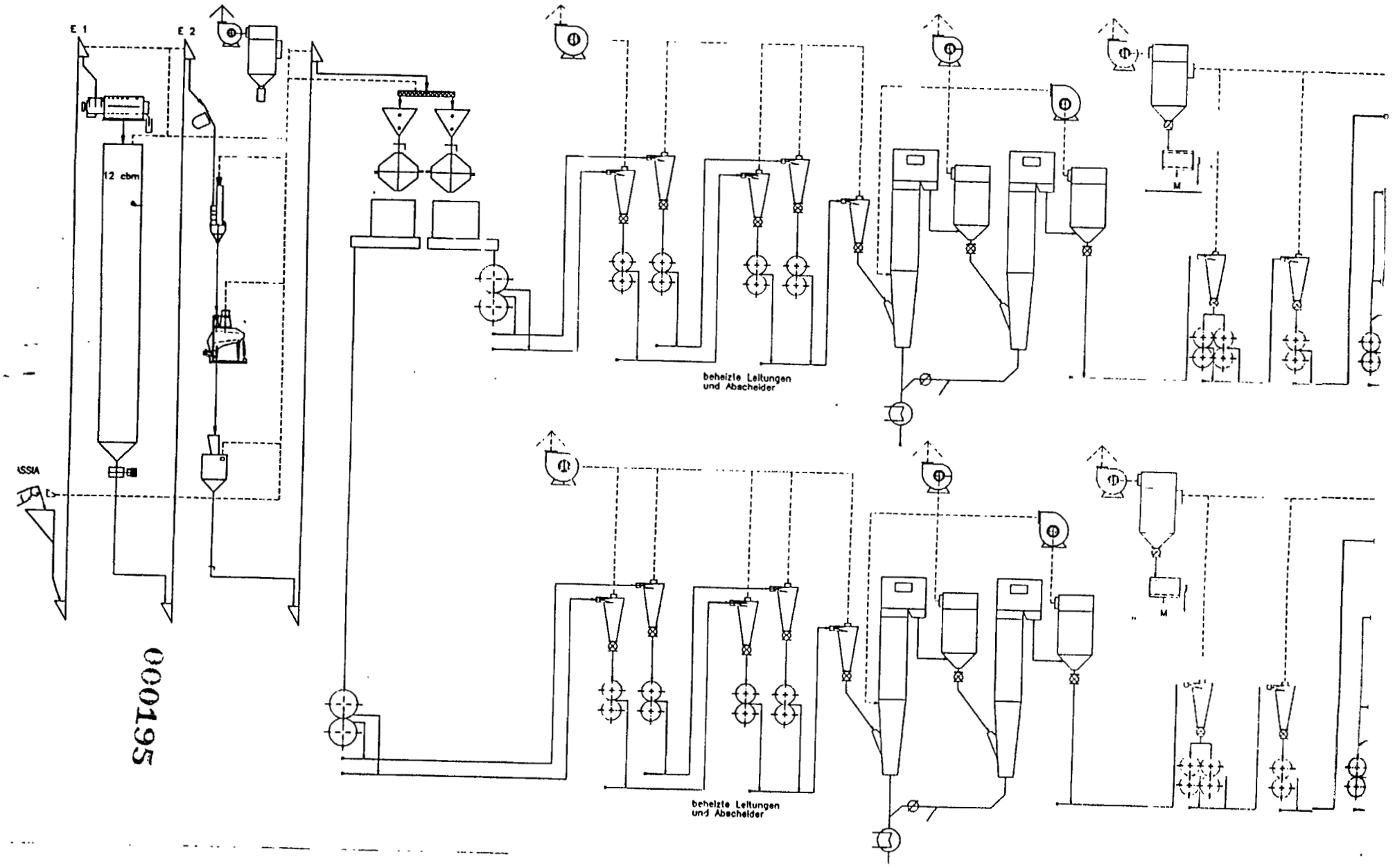
3.13. Documentation

The production process has to be documented by the shift in charge on the production order which is issued for every batch.

Form and contents of production order refer to SOP - IP XXX (not released)

The material movements from and to the stocks have to be entered immediately after return f the production order form to the office into the customs stock register.

The stock entrys of finished goods have to be reported daily in the prescribed form to BF Goodrich Diamalt, Munich, Head of Logistics, Mr. Haitel, to ensure on-time delivery to customers.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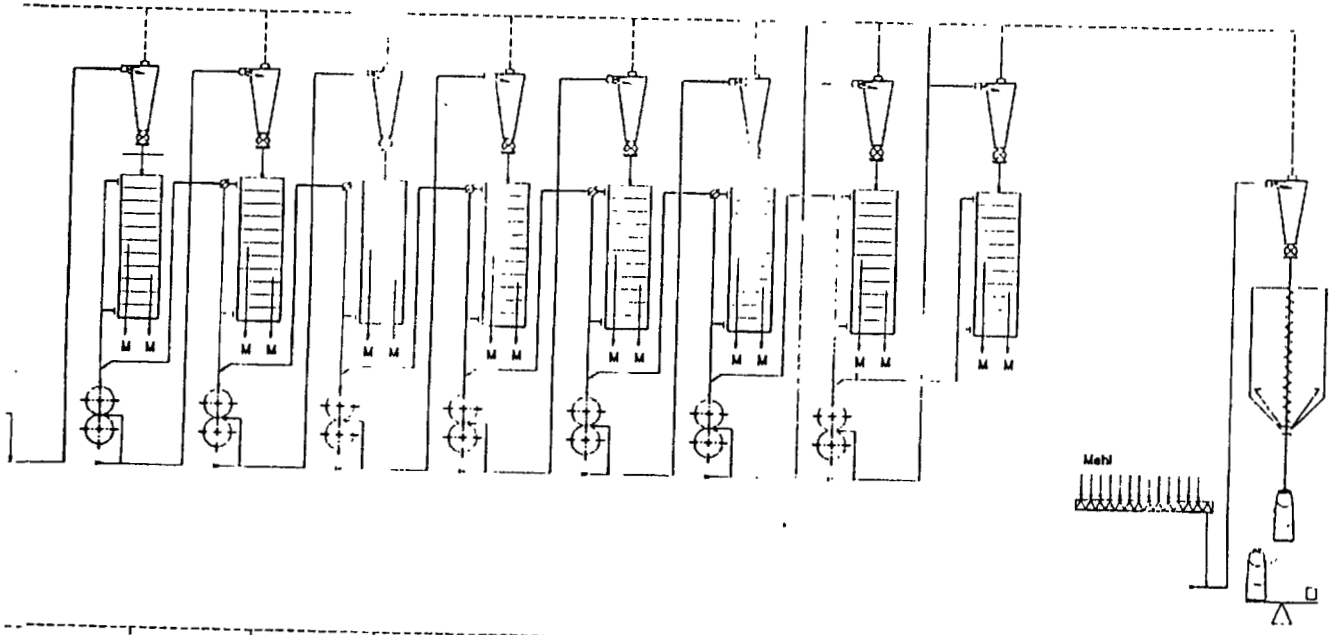

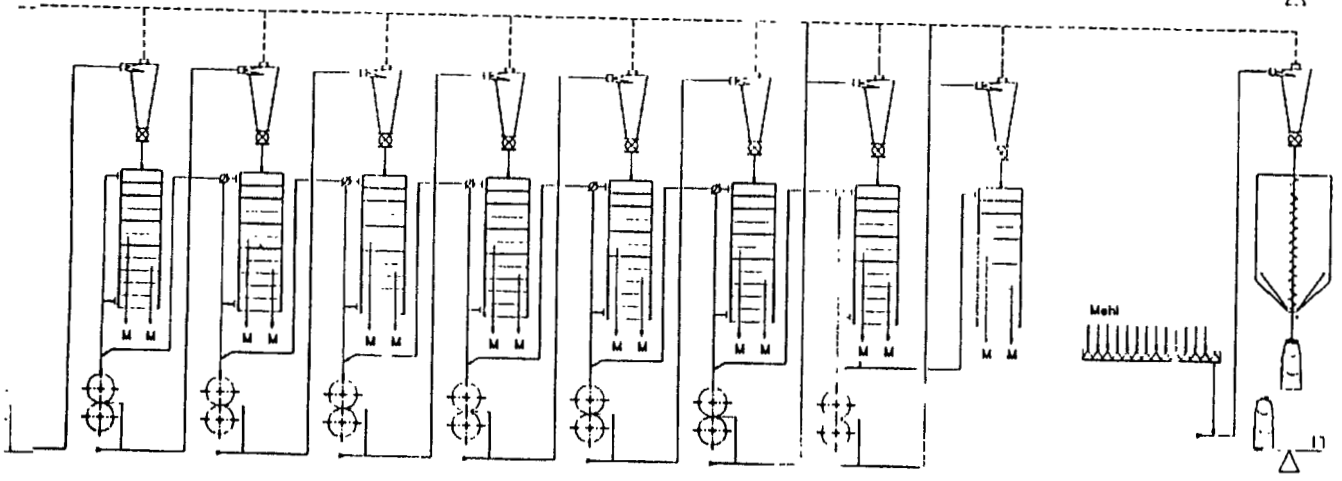

KASTENMÜLLER GmbH
Fraunhoferstraße 7 - Post Planegg
D - 82152 MARTINSRIED
Tel. 089/8573001, FAX 089/8577606

20.3.95 TB/Eb

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BFGoodrich
Performance Materials

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number **C- 01072611**

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	Fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	Properties		
Loss on drying	Max. 12 %	12 %	FCC III
pH-Value	5,5 - 8,0	7,69	FCC III
Gel-strength	Min. 140 g	152 g	NC 02101
Particle size <250µ	Min. 99 %	99,85%	NC 01201
Colour Value*)	Max. 150 ppm	145 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	3.5 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

000197

BFGoodrich
Performance Materials

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number C- 01072612

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	Fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	Properties		
Loss on drying	Max. 12 %	12 %	FCC III
pH-Value	5,5 - 8,0	7,71	FCC III
Gel-strength	Min. 140 g	148 g	NC 02101
Particle size <250µ	Min. 99 %	99,75%	NC 01201
Colour Value*)	Max. 150 ppm	145 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	2.4 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

BFGoodrich
Performance Materials

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number **C- 01072711**

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	Fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	Properties		
Loss on drying	Max. 12 %	12 %	FCC III
pH-Value	5,5 - 8,0	7,76	FCC III
Gel-strength	Min. 140 g	146 g	NC 02101
Particle size <250µ	Min. 99 %	99,60%	NC 01201
Colour Value*)	Max. 150 ppm	140 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	3.7 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

000199

BFGoodrich
Performance Materials

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number C- 01072712

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	Fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	Properties		
Loss on drying	Max. 12 %	11 %	FCC III
pH-Value	5,5 - 8,0	7,91	FCC III
Gel-strength	Min. 140 g	152 g	NC 02101
Particle size <250µ	Min. 99 %	99,65%	NC 01201
Colour Value*)	Max. 150 ppm	136 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	3.6 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

TAB 5

Certificates of analysis, Diagum CS test methods for total anthraquinones (color value) chrysophanic acid, gel-strength and other specifications; Koshioka and Takino (1978) (color value method)

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Certificate of Analysis

DIAGUM CS

Batch Number **C- 01010911**

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	Fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	Properties		
Loss on drying	Max. 12 %	9,7 %	FCC III
pH-Value	5,5 - 8,0	7,63	FCC III
Gel-strength	Min. 140 g	155 g	NC 02101
Particle size <250µ	Min. 99 %	99,9%	NC 01201
Colour Value*)	Max. 150 ppm	133 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	4,4 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

000202

BFGoodrich
Performance Materials

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number **C- 2000040521**

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	properties		
Loss on drying	Max. 12 %	9,6 %	FCC III
pH-Value	5,5 - 8,0	7,2	FCC III
Gel-strength	Min. 140 g	142 g	NC 02101
Particle size <250µ	Min. 99 %	99,9%	NC 01201
Colour Value*)	Max. 150 ppm	133 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	4,8 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

000203

BFGoodrich
Performance Materials

BFGoodrich Diamalt GmbH

Certificate of Analysis

DIAGUM CS

Batch Number **C- 01010912**

TEST	*SPECIFICATION*	*TEST Result*	*TEST-No*
2. Material Description			
Appearance	fine powder	conforms	NC 00201
Consistency	dry,free flowing,with no lumps	conforms	NC 00202
Colour	yellowish to reddish	conforms	NC 00301
Odour	Fruity	conforms	NC 00302
Taste	almost neutral	Conforms	NC 00303
3. Solubilty	gum hydrates colloidal in hot water	Conforms	NC 04201
4. Chem. and phys.	Properties		
Loss on drying	Max. 12 %	9,7 %	FCC III
pH-Value	5,5 - 8,0	7,52	FCC III
Gel-strength	Min. 140 g	154 g	NC 02101
Particle size <250µ	Min. 99 %	99,85%	NC 01201
Colour Value*)	Max. 150 ppm	145 ppm	NC 02501
Chrysophanic Acid	Max. 5 ppm	4,3 ppm	NC 02401
5.FCD Patents	EP 0139913	US 4661475	

QA / QC:

*) total anthraquinone content

Dr. Ferdinand Utz

000204

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 002 ff Version: 970901 Author: Dr.Kt Issued: 16.02.99
APPEARANCE AND CONSISTENCY	page 1 of 3

CONTENTS

I Principle

II Definition

III Equipment

IV Procedure

V Visual designation
a) appearance (NC 00201)
b) consistency (NC 00202)

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 002 ff Version: 970901 Author: Dr.Kt Issued: 16.02.99
APPEARANCE AND CONSISTENCY	**page 2 of 3**

I PRINCIPLE

Visual method

II DEFINITION

A visual designation is descriptive only and is not to be regarded as a standard of purity for a particular lot of an article.

III EQUIPMENT

1	white paper sheet	e.g. DIN A 3
2	spatula	e.g. broad spatula
3	graduated cylinder	100 ml e.g. Duran®

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 002 ff Version: 970901 Author: Dr.Kt Issued: 16.02.99
APPEARANCE AND CONSISTENCY	**page 3 of 3**

IV PROCEDURE

Note:
use a good vented hood to avoid dust

1	substance to be tested / 100 ml	pour onto	white paper sheet
2		spread / plane surface	broad spatula
3		test / structure / flowing / lumps / mechanical impurities	

V VISUAL DESIGNATION

a) Appearance (NC 00201)

1 ***Test result:***
fine powder,without lumps and mechanical impurities

b) Consistency (NC 00202)

1 ***Test result:***
dry,free flowing

Requirement see specification

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 003 ff Version: 970901 Author: Dr.Kt Issued: 16.02.99
COLOUR,ODOUR AND TASTE	page 1 of 3

CONTENTS

I Principle

II Definition

III Equipment

IV Procedure

V Sensory designation
a) colour (NC 00301)
b) odour (NC 00302)
c) taste (NC 00303)

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 003 ff Version: 970901 Author: Dr.Kt Issued: 16.02.99
COLOUR,ODOUR AND TASTE	page 2 of 3

I PRINCIPLE

Sensory method

II DEFINITION

A sensory designation is descriptive only and is not to be regarded as a standard of purity for a particular lot of an article.

III EQUIPMENT

1	open glass dish	e.g. (Kristallisierschale) Duran ®; ∅ 95mm;height: 55 mm
2	analytical balance	e.g. Mettler PM 4000, readings 0,01g

IV PROCEDURE

1 substance to be tested / 25 g
pour into open glass dish

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 003 ff Version: 970901 Author: Dr.Kt Issued: 16.02.99
COLOUR,ODOUR AND TASTE	page 3 of 3

V SENSORY DESIGNATION

a) Colour (NC 00301)

1 ***Test result:***
yellowish to reddish

Note:
colour may darken with age;
yellowish colour changes to reddish when exposed to light

b) Taste (NC 00302)

1 ***Test result:***
almost neutral

b) Odour (NC 00303)

1 ***Test result:***
fruity

1 ***Note:***
terms such as "fruity odour" apply to examination,after exposure to the air for 15 min,of a freshly opened package (see also USP XXII,page 7)

Requirement see specification

000210

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 04201 Version: 970901 Author: Dr.Kt Issued: 16.02.99
SOLUBILITY	**page 1 of 8**

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I Principle

II Definition

III Equipment

IV Reagents

V Test solution

VI Determination of viscosity
a) qualitative
b) viscometer
c) calculation

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 04201 Version: 970901 Author: Dr.Kt Issued: 16.02.99
SOLUBILITY	**page 2 of 8**

I PRINCIPLE

The solubility of Cassia gum is tested by dispersing the gum in hot water. After cooling an appreciable increase in viscosity is produced.

The increase in viscosity after boiling is due to the complete hydration and solubilisation of the high molecular Cassia hydrocolloid.

II DEFINITION

The solubility is expressed by the increase in viscosity.

The viscosity can be determined:
qualitative: visual
or quantitative: with a viscometer.

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 04201 Version: 970901 Author: Dr.Kt Issued: 16.02.99
SOLUBILITY	**page 3 of 8**

III EQUIPMENT

1	Porcelain beaker	e.g. 500 ml / (Haldenwanger Berlin / type no 42H-Oa top-Ø:100 mm / bottom-Ø: 75 mm / height: 130 mm wall thickness: 5 mm
2	stirring rod	e.g. V2A-steel / IKA; type R1363 / rod-Ø: 8 mm / rod-length: 320 mm / stirrer (cage-type)-Ø: 40 mm
3	high shear mixer	e.g. Heidolph-Rührwerk RZR 1 / 280-2200 rpm / 77W
4	balance	e.g. Mettler PC 4400 , readings 0.01 g
5	heating bath (150°C)	e.g. Universal-Heizbad "UH-EL" / Nr 2025-3101 / temperature range up to 250°C
6	cooling water bath (20°C)	e.g. type Kühlmobil® / Fa. Peter v.d.Heijden, Dörentrop
7	timer	e.g type Junghans
8	wood spatula	e.g. 5 x 25 x 50 mm
9	plastic weighing dish	e.g flexible material / 80 x 80 x 20 mm
10	viscometer	Brookfield RVT, Brookfield - Stoughton (Mass.) USA

IV	**REAGENTS**		
1	tap water	10-15 ° dH (degree German hardness)	
V	**TEST SOLUTION (5%)**		
1		place beaker on balance	
2		put stirring rod into beaker	
3		note tara weight of beaker with stirrer	
4	tap water / 285,0 g	weigh	beaker
5	substance to be tested / 15,00 g	weigh	weighing dish
	SET UP OIL BATH		
1		check level of oil,so that beaker will be immersed 4 cm in the oil	
2		heat oil bath up to 140 °C	
3		adjust high shear mixer / rpm-position No 4	

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 04201 Version: 970901 Author: Dr.Kt Issued: 16.02.99
SOLUBILITY	**page 5 of 8**

6	place beaker into oil bath
7	start stirrer
	NOTE: the stirrer is placed 1 cm off-center and 1 cm above bottom of beaker to obtain maximum turbulance of the lower portion of vortex
8	add substance to be tested
	NOTE: the thickener should be sifted carefully onto the upper wall of the vortex to avoid lumps.The addition of powder should be completed in about 15 sec
9	heat up to boiling
	NOTE: it will take about 15 to 20 min until test solution starts to boil
10	boil for 5 min
11	remove beaker with stirring rod
12	place beaker with stirring rod on balance
13	replace water lost by evaporation
14	stirr and homogenize
15	place in water bath (20°C)

16	allow to stand for 2 hours
	NOTE: while cooling stirr in between to avoid skim forming on the surface of the solution
VI	**VISCOSITY**
a)	**Qualitative**
1	stirr colloidal solution by hand
2	***Measuring result:*** *solution is very viscous*
	Requirements: solubility = solution is very viscous
b)	**Viscometer** (only in the case viscosity has to be determined)
	NOTE: set up Brookfield viscometer RVT according to manual / adjust speed control knob to 20 rpm / check bubble level / use appropiate spindle
1	Test solution (5 %) / 20° remove from water bath
2	stirr with wood spatula 5-10 times
3	wait for 1-2 minutes
4	insert spindle in test solution to groove cut

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 04201 Version: 970901 Author: Dr.Kt Issued: 16.02.99
SOLUBILITY	**page 7 of 8**

5	start Brookfield viscometer RVT with 20 rpm
6	wait 5-10 revolution of dial or until pointer has stabilized
7	note pointer's position on dial (between 0 and 100% full scale)
8	***First measuring result:*** dial readings S_1
9	stop viscometer / wait 1- 2 min / repeat viscosity measurement from pos. 2
10	***Second measuring result:*** dial readings S_2
11	***Partial measuring result:*** *Average value V:* $V = (S_1 + S_2) / 2$

c) **Calculation of viscosity**

1 ***Measuring result:***
Viscosity η = V x F [mPas]
V = average value of two dial readings
F = factor for appropiate spindle-# at 20 rpm)
= 2000 for spindle-# 7 at 20 rpm:

Requirements: see specification

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02401 Version: 970901 Author: Dr.Kt Issued: 16.02.99
CHRYSOPHANIC ACID	page 1 of 12

CONTENTS

I Principle

II Definition

III Equipment

IV Reagents

V Sample Preparation
- a Hydrolysis
- b Extraction
- c Clean-up

VI Calibration solution

VII HPLC
- a Eluent
- b Chromatographic conditions
- c Calibration run

VIII Determination of chrysophanic acid

IX Requirements

X Chromatograms
- a Calibration
- b Test solution

I PRINCIPLE

The viscous galactomannan-matrix is hydrolysed with sulfuric acid and the anthraquinone-glycosides are cleaved into aglycones and monosacchrides. The aglycones are extracted with n-hexane and chrysophanic acid is cleaned-up with Extrelute® NT 1 (diatomaceous earth) at pH 9.The purified eluate is chromatographed by HPLC on ODS with 1,8-dihydroxyanthraquinone as internal standard.

II DEFINITION

The chrysophanic acid is measured in mg / kg (= ppm).

III EQUIPMENT

1	analytical balance	e.g. Mettler AT 20; readings 0,000001 g
2	analytical balance	e.g. Mettler AE 200; readings 0,0001 g
3	weighing glass	e.g. glass plate 15x15 mm
4	glass beads	
5	hot plate with sand or metallic bath	e.g. Gestigkeit SG5, 220V , 2500 Watt
6	250 ml round bottom flask with ground joint	e.g. Duran®,
7	reflux condenser	e.g.Dimroth condenser, Duran®, length: 500 mm
8	pipettes	1 ml 100 ml
9	lead rings	e.g. ∅ 60-80 mm
10	water bath 20°C	
11	HPLC-system	requirements: isocratic pump/ UV-detector 254 nm / syringe injection / integrator / degasser unit

IV REAGENTS

1	distilled water	HPLC grade
2	10% sulfuric acid	p.a. grade;
4	32% hydrochloric acid	p.a. grade;e.g. Merck 1.00319.1000
3	1,8-dihydroxy-anthraquinone (99%)	e.g. EGA D10,810-3 (m.p. 191-193°C)
4	chrysophanic acid	HPLC-grade, e.g. Roth 7442
5	methanol	HPLC-grade; e.g. Merck LiChrosolv 1.06007.2500
6	n-hexane	HPLC-grade; e.g. Merck LiChrosolv 1.04391.2500
7	Extrelut® NT 1	prepacked column; Merck 1.15094.0001 with cannula 1.15373,vials 1.15622 and rack 1.15623
8	buffer solution pH=9	e.g. Metrohm 62307020
9	HPLC column	250 x 4,6 ID,Shandon Hypersil ODS,5µm

V SAMPLE PREPARATION

a Hydrolysis

1	1,8-dihydroxyanthraquinone (= internal standard) / 0,20 mg	weigh	glass plate (15x15 mm)
2		put glass plate with 1,8-dihydroxyanthraquinone into 250 ml round botttom flask	
3	glass beads	add	
4	sample to be tested / 5,0000 g	add	
5	10% sulfuric acid (p.a. grade) / 100 ml	add	
6		connect reflux condenser	
7		reflux for 3 hours	hot plate / sand bath
8		replace reflux condenser by plastic stopper / place lead ring on flask / cool to 20°C in water bath	

b **Extraction**

1 n-hexane (HPLC grade) / 100 ml
add to hydrolysate

2 shake for 10 min

3 allow to stand for at least 2 hours
for phase separation

> ***note:***
> There is an emulsion layer between the aqueous and hexane-layer.
> For faster phase separation: centrifugation at 2000 rpm / 2 min

4 use hexane-phase for clean-up procedure

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02401 Version: 970901 Author: Dr.Kt Issued: 16.02.99
CHRYSOPHANIC ACID	**page 7 of 12**

c **Clean up**

1 buffer solution pH= 9 / 1 ml
place onto prepacked Extrelut® NT 1-column
(discard effluent)

2 wait for 5 min

3 hexane phase (see V / b4) / 1ml
apply to Extrelut® NT 1-column
(discard effluent)

4 wait for 5 min

5 dist. water / 1 ml
place onto Extrelut® NT 1-column
(to elute hexane)

6 collect hexane eluate in vial
(= test solution for HPLC)

note:
collect only hexane phase;avoid any aqueous interference

VI CALIBRATION SOLUTION

1	1,8-dihydroxyanthraquinone (= internal standard) / 0,20 mg	weigh	glass plate (15x15 mm)
2	chrysophanic acid (HPLC-grade) / 0,20 mg	add	
3		put glass plate with 1,8-dihydroxy-anthraquinone and chrysophanic acid into 250 ml round botttom flask	
4	glass beads	add	
5	10% sulfuric acid (p.a. grade) / 100 ml	add	
6		connect reflux condenser	
7		reflux for 3 hours	hot plate/ sand bath
8		replace reflux condenser by plastic stopper / place lead ring on flask / cool to 20°C in water bath	
9	n-hexane (HPLC grade) / 100 ml	add	
10		shake for 10 min	

11 allow to stand for phase separation

note:
hexane layer must be yellow and aqueous layer must be completely colourless

12 separate hexane-layer and store for calibration

note:
calibration solution:
40 ppm chrysophanic acid in respect to 5,000 g subtance to be tested
40 ppm internal standard in respect to 5,000 g subtance to be tested

note:
calibration solution is stable for at least 3 month when stored in a cool and dark place.

VII HPLC

a Eluent

1 methanol (HPLC grade) / 850 ml
place into 1000 ml bottle

2 water (HPLC-grade) / 150 ml
add

3 hydrochloric acid 32% (p.a.) / 3 ml
add

4 degas eluent before use

Note
the addition of a few ml of hydrochloric acid prevents tailing of peaks

b Chromatographic conditions

1	program	isocratic
2	flow	0,5 ml / min
3	typical pressure	500 - 1000 psi
4	temperature	room temperature
5	UV-detector	254 nm

6	typical injection volume	20-50 µl
7	typical retention times	15 - 20 min
8	integration	internal standard method / peak area

c Calibration run

1	calibration solution (see VI / 12) / 20-50µl	inject
2		make a HPLC run
3		calibrate peak area internal standard: 40 ppm chrysophanic acid: 40 ppm
4		repeat calibration run: maximal deviation of chrysophanic acid: 40±2 ppm

VIII CHRYSOPHANIC ACID DETERMINATION

1	test solution (see V c/6) / 20-50µl	inject
2		carry out a HPLC run
3		contents of chrysophanic acid:

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TESTING STANDARD

	DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02401 Version: 970901 Author: Dr.Kt Issued: 16.02.99
	CHRYSOPHANIC ACID	**page 12 of 12**
	read integrator report	
IX	**Requirements** see specification	
Xa	**Chromatogram of calibration solution**	
Xb	**Chromatogram of test solution**	

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02501 Version: 970901 Author: Dr.Kt Issued: 16.02.99
TOTAL COLOUR VALUE (520 nm)	**page 1 of 9**

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III Equipment

IV Reagents

V Extraction solution

VI Test solution

VII Photometric determination

VIII Calculation

IX Photometer

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 0250 Version: 970901 Author: Dr.Kt Issued: 16.02.99
TOTAL COLOUR VALUE (520 nm)	**page 2 of 9**

I PRINCIPLE

The colour of Cassia gum is determined by alkaline extraction.The gum matrix is precipitated with methanol and removed by filtration,and the remaining red-coloured filtrate is measured photometrically at 520 nm.

II DEFINITION

The total colour value (= anthraquinone content) of cassia gum is standardized to 1,8-dihydroxyanthraquinone.The unit for colour value is ppm.

TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02501 Version: 970901 Author: Dr.Kt Issued: 16.02.99
TOTAL COLOUR VALUE (520 nm)	**page 3 of 9**

III EQUIPMENT

1	analytical balance	e.g. Mettler PE 360 , readings 0,001 g
2	volumetric flask	1000 ml, with ring mark and ground in stopper; e.g. Duran®; class A ; DIN 12664;20°C
3	volumetric flask	10 ml, with ring mark and ground in stopper; e.g. Duran®; class A ; DIN 12664;20°C
4	NS-Erlenmeyer flask (*brown glass)*	250 ml,graduated, *brown glass* with ground joint; e.g. Duran®
5	reagent bottle (*brown glass)*	Steilbrustflasche 50 ml, *brown glass*; wide neck ; with ground joint ;e.g. Duran®;
6	volumetric pipette	50 ml;e.g. with ring mark;. class AS Ex+15 sec;20°C
7	volumetric pipette	5 ml;e.g. with ring mark;. class AS Ex+15 sec;20°C
8	pipetting ball (Peleus ball)	or suitable pipetting aid e.g. Hirschmamm Pipetus ®
9	magnetic stirring bar	e.g. PTFE coated ,length 50 mm
10	magnetic stirrer	e.g. IKAMAG REO; 0... 1100 rpm (without heater)
11	plastic funnel	suitable for Ederol®-filter, e.g (Ø 50 mm ;length about 80mm)
12	filter	folded filter Ederol® (Ø110 mm;quality 13)

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TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02501 Version: 970901 Author: Dr.Kt Issued: 16.02.99
TOTAL COLOUR VALUE (520 nm)	**page 4 of 9**

13	photometer	vis-range ; 520 nm e.g. Zeiss
14	cuvette	suitable for photometer; e.g Zeiss Quartzküvette S; 1 cm ; No 558503
15	calculatur	e.g. electronic pocket calculator
16	glass beaker	500 ml; e.g. graduated ; Duran®;
17	stopp watch	e.g. Junghans table stop watch 312/4810
IV	**REAGENTS**	
1	distilled water	photometrical pure
2	sodium hydroxide	photometrical pure; e.g. Merck 106498
3	ammonia solution 25%	photometrical pure; e.g Merck 105432
4	methanol	photometrical pure; e.g Merck 106009
5	1,8-dihydroxy-anthraquinone (99%)	e.g. EGA D10,810-3 (m.p. 191-193°C)

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02501 Version: 970901 Author: Dr.Kt Issued: 16.02.99
TOTAL COLOUR VALUE (520 nm)	**page 5 of 9**

V EXTRACTION SOLUTION

1	sodium hydroxide (solid) / 60,0 g	weigh	1000 ml volumetic flask
2	dist. water / 400 ml	add / dissolve	
3		cool	
4	ammonia solution (25%) / 200 ml	dilute with 200 ml dist. water	500 ml glass beaker
5		combine diluted ammonia solution with sodium hydroxide solution	

Note
when adding ammonia solution avoid evolving of NH_3-vapour / cool at first and stir very carefully / use a good ventilated hood

6	dist. water	add,fill up to ring mark	

the extraction solution can be stored in a stoppered bottle at room temperature for 2 month

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TOTAL COLOUR VALUE (520 nm)	**page 6 of 9**

VI TEST SOLUTION

1 substance to be tested / 1,000 g
weigh — 250 ml Erlenmeyer flask ***brown glass !***

2 add stirring bar to flask/
place on magnetic stirrer/
start stirrer with appropiate rpm

3 extraction solution / 50 ml
add while stirring

4 start stopp watch

> ***note:***
> after the extraction solution is completely added,start immediately stopp watch (time t_0 = 0 min)

5 stirr 5 min

> ***note***
> avoid formation of lumps;if there are lumps,discard solution and start over again

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6 methanol / 50 ml
add while stirring (time t_1 = 5 min)

7 stirr 5 min

8 prepare funnel with folded filter (time t_2 = 10 min)

9 start filtration /
use brown glass for recipient (50 ml reagent bottle)

Note
filtration must be finished within total time of 20 min (time t_3 = 20 min), when stopp watch was startet;
filtration volume : not more than 15 ml are necessary

VII PHOTOMETRIC DETERMINATON

Note
set up photometer according to instruction manual;turn power on;allow 15 min warm-up;use absorbance-mode

Prepare blank

1	extraktion solution / 5 ml	pipette	10 ml volumetric flask
2	methanol / 5 ml	add / mix / cool (20°C)	

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Note
because of volume contraction when methanol is added to aqueous

systems, the final volume is less than 10 ml

3 transfer into cuvette

---

1 set wave length control at 520 nm photometer

2 insert cuvette with blank into sample compartment,close cover,and adjust "zero control" until meter reads zero

3 remove blank

Note
use the same cuvette for both sample and blank measurement;rinse the cuvette 2 times with testing solution.

4 insert cuvette with testing solution into sample compartment after exactly 20 minutes the stopp watch was startet

5 read absorbance and document value

6 ***Partial measuring result:***
readings of absorbance in [au]; [au] = absorbance units

VIII CALCULATION

1 *Test result:*

$$\text{total colour value}_{(520\ nm)}\ \text{in [ppm]}^{1)} = \frac{(au - b) \times 1000}{s}$$

[1)] ppm = s x [au] + b
(b = intercept; s = slope; [au] = absorbance units)
for Zeiss photometer: (b = 0,005 ; s = 0,435)

2 **Requirement** see specification

IX ZEISS PHOTOMETER

TESTING STANDARD

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TESTING STANDARD

DIAGUM CS (= Cassia Gum)	Quality assurance Test.No.: NC 02101 Version: 970901 Author: Dr.Kt Issued: 16.02.99
GEL STRENGTH	Page 2 of 10

I PRINCIPLE

Cassia gum is forming a gel with carrageenan in a phasphate buffer in the presence of potassium chloride.The resistance of this gel to rupture is measured on the FIRA jelly tester by an immersed paddle which is rotated by 30°.

II DEFINITION

The gel strength is defined as the weight of grams of water required to give a 30° deflection on the FIRA jelly tester.

III EQUIPMENT

1	analytical balance	e.g. Mettler PM 4600 , readings 0.01 g
2	plastic weighing dish	e.g. flexible material / 80 x 80 x 20 mm
3	glass beaker,low form	1000 ml , graduated; e.g. Duran®,
4	magnetic stirring bar	e.g. PTFE coated ,length 50 mm
5	magnetic stirrer with heater	e.g. IKAMAG RET; RT...+300°C; 0...1100 rpm
6	magnetic stirrer without heater	e.g. IKAMAG REO; 0... 1100 rpm
7	hair dryer	e.g. neoLab® hot air blower

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8	thermostatic chamber (or water bath)	e.g. precision incubator Memmert BKE 30; 0...+60°C ;thermostability: 20±0,1 °C
9	pH-meter glass electrode calibration buffer	e.g. Metrohm pH-meter 632 e.g. Metrohm 6.203.100 pH = 7: e.g. Metrohm 6.2307.010 pH = 9: e.g: Metrohm 6.2307.020
10	2" square jelly box	plexiglass (wall-thickness: 4mm ;internal dimensions 50x50x60 mm customer made by: Clark & Smith Manufacturing Melbourne House,Melbourne Road, Wallington,Surrey SM6 8SD,UK Tel. 0181 669 4411
11	FIRA-jelly tester equipped with flow meter	H.A. Gaydon &Co Ltd Clyde Works;Clyde Road, Wallington, Surrey SM6 8PZ / England e.g.Fischer & Porter , type D10A3137N (with needle valve)
12	measuring flask	1000 ml ,with ring mark (class a); grounded joint and polystopper; e.g. Duran® (DIN 12664 T1)
13	laboratory timer	
14	thermometer	20°C,accuracy 0,1°C

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IV REAGENTS

1. distilled/de-ionised water
2. sodium dihydrogen phosphate dihydrate ($NaH_2PO_4x2H_2O$); e.g. Merck 106345 (reinst DAB;BP;USP; / 98,0-100,5 %)
3. anhydrous disodium hydrogen phosphate (Na_2HPO_4); e.g. Merck 106586 (p.a. / min. 99 %)
4. anhydrous potassium chloride (KCl); e.g. Merck 104936 (p.a. / min. 99,5%)
5. standard semi refined carrageenan; Danagel PF 8263
   FMC GmbH,Food Ingredients Division,
   Hahnstr. 38
   60528 Frankfurt
6. 5 M sodium hydroxide solution

TESTING STANDARD

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V BUFFER SOLUTION (pH = 6,60)

1 sodium dihydrogen phosphate dihydrate ($NaH_2PO_4x2H_2O$) / 8,00 g
weigh into 1000 ml measuring flask

2 anhydrous disodium hydrogen phosphate (Na_2HPO_4)/ 5,00 g
add

3 anhydrous potassium chloride (KCl) / 3,00 g
add

4 dist./de-ionised water
add,dissolve,fill up to ringmark

6 check pH pH-meter
pH = 6,60 ± 0,05

NOTE: if pH is outside range,adjust using 5 M sodium hydroxide;if adjusting is not possible,the buffer must be discarded and a fresh batch made up

VI TESTING CONDITIONS

1	0,08 M phoshate buffer with 0,04 M KCL:	pH= 6,6
2	total gel concentration (cassia gum = 0,3 % ; carrageenan = 0,3%)	0,6 %
3	boiling time (95-100°C)	5 min
4	gel setting time (room temperature :4 hours ; 20°C: 1 hour)	min. 5 hours
5	gel strength determination	FIRA jelly tester

VII MAKING THE JELLY SOLUTION

NOTE: all safety procedures associated with the apparatus and operations described below are observed on every occasion; use safety goggles and gloves when working with hot solutions.

1 buffer solution (pH=6,60) / 497,00 g
weigh into 1000 ml beaker

2 place beaker on magnetic stirrer and add magnetic stirring bar

3 sample to be tested (Diagum CS) / 1,50 g
weigh into plastic weighing dish

4 standard carrageenan / 1,50 g
add plastic weighing dish

5 switch on stirrer and add slowly gel powder from weighing dish into cold buffer solution

NOTE: add the powder slowly enough to eliminate the possible formation of lumps in the solution

6 determine total weight of [beaker +magnetic stirring-bar+ buffer solution + gel powder]

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7 ***Partial measuring result:***
$m_{total} = m_{beaker} + m_{magnetic\ stirring\ bar} + m_{buffer} + m_{gel\ powder}$ in [gram]

8	switch on heater and bring solution to boil	stirrer with heating unit

NOTE: when boiling a sudden froth might be formed on the surface; to avoid a boiling over,blow gentle with a hot air dryer on the surface of the solution;this will keep down the froth.

9	when temperatur has risen to boiling (95-100°C) start timer and boil for 5 min
10	remove beaker from heating unit and place on cold stirrer
11	stir for 5 min at room temperature
12	place beaker on balance and fill up to total weight with cold buffer solution to compensate for loss on evaparotion
13	stir test solution
14	fill up 3 jelly boxes with test solution while still hot
15	allow the jelly boxes to stand undisturbed for at least 4 hours at room temperature (room temperatur must be below 30°C)
16	place jelly boxes in an incubator

	at 20±0,1°C for another 1 hour
17	gel ready for gel-strength measurement

VIII GEL TESTING

NOTE: set up FIRA jelly tester acc. to manual
- allow water to flow through FIRA jelly tester for 5 min before testing
- run water through the tester into the bucket for 2 min and discard
- check flow rate: 100±5 ml / min

1	place FIRA jelly tester bucket on balance and re-set the tara weight;
2	attach bucket on FIRA jelly tester and couterpoise
3	Set scale to zero with the damping brake on SET ZERO
4	place jelly box on the FIRA jelly tester
5	raise platform until paddle penetrates the jelly as far as the lower mark on the shaft of the paddle

NOTE: in penetrating the jelly the paddle might turn slightly and cause a change in zero setting of scale,but this is unimportant.The initial scale reading is noted.

6	release damping brake from SET ZERO to TEST

7 depress water valve key and allow water to flow into bucket

8 stop water flow into bucket immediately scale passes the 30° deflection

9 lock damping brake

10 detach bucket from tester and place on balance

11 note weight of water

12 ***Measuring result:***
gel strength = weight of water in gram

IX CALCULATION

1 Calculate the average value for gel strength for the 3jelly boxes.
If the range exceeds 5 g,repeat the test.

X FIRA JELLY TESTER

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NA- Not applicable