GREEN

Galactomannans

CIR EXPERT PANEL MEETING

DECEMBER 12-13, 2011

Cosmetic Ingredient Review
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Novemmber 21, 2011

Memorandum

To: CIR Expert Panel

From: Wilbur Johnson, Jr.
Manager/Lead Specialist

Subject: Draft Report on Galactomannans

A Scientific Literature Review (SLR) on this group of ingredients was announced on September 7, 2011. Unpublished data received during the 60-day comment period are summarized in the draft report.

A copy of the draft report on these ingredients is included along with the CIR report history, Literature search strategy, Ingredient Data profile, Personal Care Products Council (Council) comments on the Galactomannans SLR (pcpc 1, pdf file), Council memorandum on Trigonella foenum-graecum seed extract raw material from one supplier (pcpc 2, pdf file), and Council memorandum on Trigonella foenum-graecum seed extract raw material from another supplier (pcpc 3, pdf file). Comments received from the Council will be addressed prior to the Panel meeting. The unpublished data included with this report are:

1. Guar hydroxypropyltrimonium chloride trade name material data profile submitted on 9-28-2011 (data 1 pdf file);
2. Guar hydroxypropyltrimonium chloride trade name material (different material) data profile submitted on 9-28-2011(data 1 pdf file);
3. Acute oral toxicity study on guar hydroxypropyltrimonium chloride trade name material submitted on 9-28-2011 (data 1, pdf file);
4. Genotoxicity study on guar hydroxypropyltrimonium chloride trade name material submitted on 9-28-2011 (data 1 pdf);
5. Trigonella foenum-graecum seed extract data sheet submitted on 8-16-2011 (data 2, pdf file); and
6. Use concentration data on galactomannans from Council survey submitted on 10-26-2011 (data 3, pdf file)

After reviewing the draft report, the Panel needs to determine whether additional data are needed or whether the available data are sufficient for arriving at a conclusion on the safety of galactomannans in cosmetic products.

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Galactomannans (Guar Gum)

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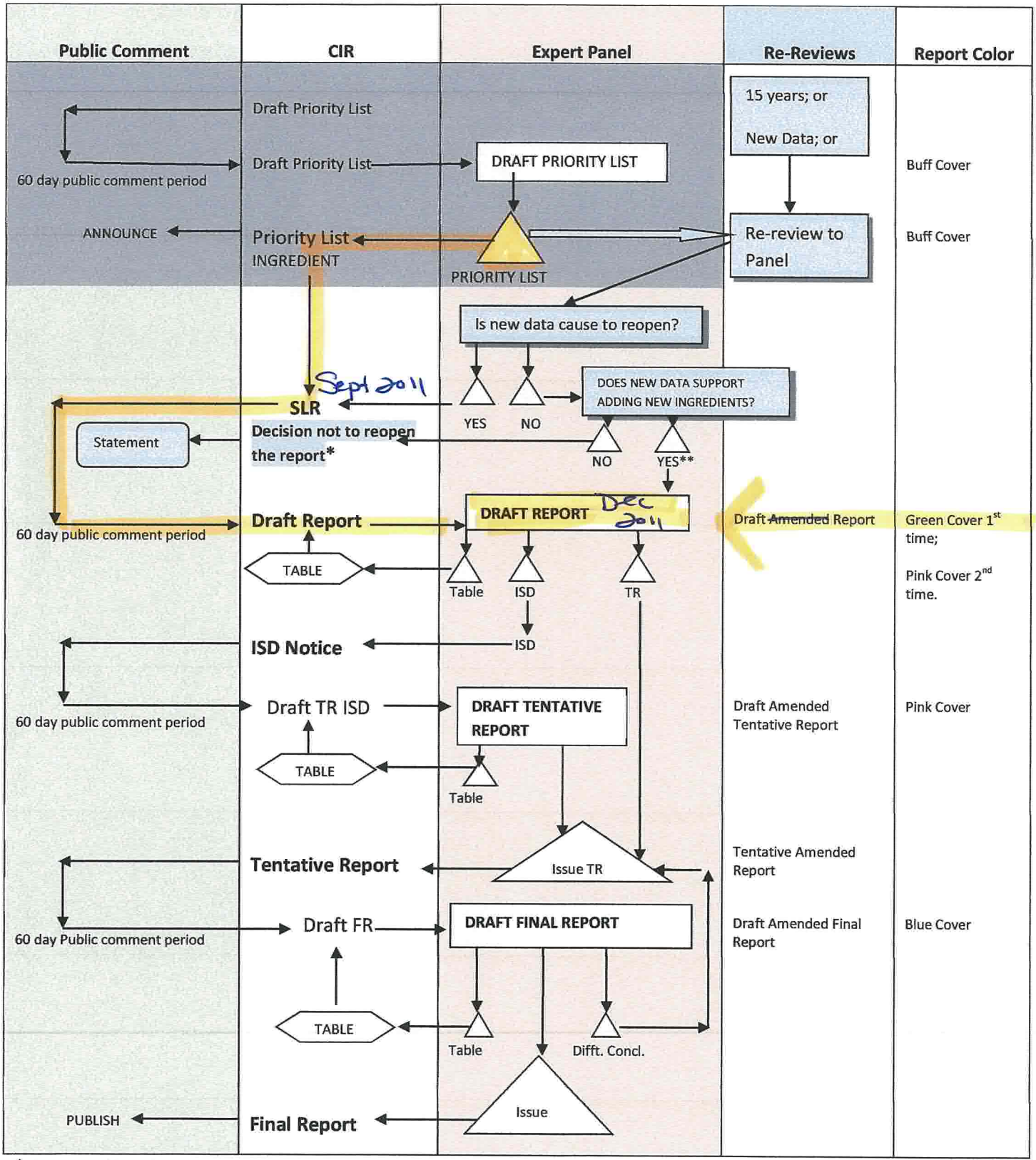
SAFETY ASSESSMENT FLOW CHART

Dec 2011



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

△ Expert Panel Decision

▭ Document for Panel Review

⇨ Option for Re-review

CIR History of:

Galactomannans

A Scientific Literature Review (SLR) was announced on September 7, 2011, and unpublished data from the Personal Care Products Council (Council) were received during the 60-day comment period.

Draft Report, Belsito and Marks Teams/Panel: December 12-13, 2011

The SLR (now a draft report) was revised to include the following unpublished data received from the Council:

1. Guar hydroxypropyltrimonium chloride trade name material data profile submitted on 9-28-2011
2. Guar hydroxypropyltrimonium chloride trade name material (different material) data profile submitted on 9-28-2011
3. Acute oral toxicity study on guar hydroxypropyltrimonium chloride trade name material submitted on 9-28-2011
4. Genotoxicity study on guar hydroxypropyltrimonium chloride trade name material submitted on 9-28-2011
5. Trigonella foenum-graecum seed extract data sheet submitted on 8-16-2011 (data 2, pdf file); and
6. Use concentration data on galactomannans from Council survey submitted on 10-26-2011
7. Council memorandum - information on Trigonella foenum-graecum seed extract raw material from one supplier – dated 9-9-2011

Council comments on the SLR (dated 10-3-2011) were also received, but have not been addressed in this draft report.

Galactomannans Check List for December, 2011. Writer – Wilbur Johnson																				
			Acute toxicity					Repeated dose toxicity				Irritation			Sensitization					
	Skin Penetration	Penetration Enhancement	ADME	Oral	Parenteral	Dermal	Inhale	Oral	Parenteral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
GG			X	X				X				X	X		X	X	X	X	X	
HG																		X		
HHG																				
GHC																				
HGH																				
CHG																				
HZG				X				X										X		
CSG			X	X				X				X	X			X	X	X	X	
LBH																				
HCS																				
CSP			X					X				X	X				X	X	X	
CSH																				
HCSG																				
TFS				X					X							X	X	X		
TFH																				
HTF																				
CG			X	X				X				X	X				X	X		
CHC																				

INGREDIENTS

<u>Guar Gums</u>
Cyamopsis Tetragonoloba (Guar) Gum **(GG)**

Hydroxypropyl Guar **(HG)**

C18-22 Hydroxyalkyl Hydroxypropyl Guar **(HHG)**

Guar Hydroxypropyltrimonium Chloride **(GHC)**

Hydroxypropyl Guar Hydroxypropyltrimonium Chloride **(HGH)**

Carboxymethyl Hydroxypropyl Guar **(CHG)**

Hydrolyzed Guar **(HZG)**

<u>Locust Bean Gums (Carob Gums)</u>
Ceratonia Siliqua Gum **(CSG)**

Locust Bean Hydroxypropyltrimonium Chloride **(LBH)**

Hydrolyzed Ceratonia Siliqua Gum Extract **(HCS)**

<u>Tara Gums</u>
Caesalpinia Spinosa Gum **(CSP)**

Caesalpinia Spinosa Hydroxypropyltrimonium Chloride **(CSH)**

Hydrolyzed Caesalpinia Spinosa Gum **(HCSG)**

<u>Fenugreek Gums</u>
Trigonella Foenum-Graecum Seed Extract **(TFS)**

Trigonella Foenum-Graecum Hydroxypropyltrimonium Chloride **(TFH)**

Hydrolyzed Trigonella Foenum-Graecum Seed Extract **(HTF)**

<u>Cassia Gums</u>
Cassia Gum **(CG)**

Cassia Hydroxypropyltrimonium Chloride **(CHC)**

Literature Search on Guar Gum and Related Ingredients*

Ingre-dients	Toxline &PubMed	ChemIDplus	Multidatabase (See legend*)	DART	Household Products	Beilstein	Registry	Kosmet	Napralert	RTECS	CAplus
GG	489	1	3	12							
HG	5	1	0	0							
HHG	0	0	0	0							
GHC	6	1	0	0							
HGH	1	1	0	0							
CHG	0	1	0	0							
HZG	12	1	0	0							
CSG	93	1	2	2							
LBH	0	0	0	0							
HCS	0	0	0	0							
CSP	14	1	2	1							
CSH	0	0	0	0							
CSG	0	0	0	0							
TFS	58	1	0	0							
TFH	0	0	0	0							
HTF	1	0	0	0							
CG	11	0	0	0							
CHC	0	0	0	0							
GLM	142	1	0	3							

*Data in Table: Publications found; Multidatabase = HSDB, CCRIS, ITER, IRIS, Gene-Tox, and LacMed;

Searches Performed on4/25-26/2011
Search updated on 11/3/2011 using PubMed, Toxline, and SciFinder

INGREDIENTS

Guar Gums

Cyamopsis Tetragonoloba (Guar) Gum **(GG)**
9000-30-0
Guar
Guar Gum

Hydroxypropyl Guar **(HG)**
39421-75-5

C18-22 Hydroxyalkyl Hydroxypropyl Guar **(HHG)**

Guar Hydroxypropyltrimonium Chloride **(GHC)**
65497-29-2

Hydroxypropyl Guar Hydroxypropyltrimonium Chloride **(HGH)**
71329-50-5

Carboxymethyl Hydroxypropyl Guar **(CHG)**
68130-15-4

Hydrolyzed Guar **(HZG)**
70892-12-5

Locust Bean Gums (Carob Gums)

Ceratonia Siliqua Gum **(CSG)**
9000-40-2
Carob Bean Gum
Locust Bean Gum

Locust Bean Hydroxypropyltrimonium Chloride **(LBH)**

Hydrolyzed Ceratonia Siliqua Gum Extract **(HCS)**

Tara Gums

Caesalpinia Spinosa Gum **(CSP)**
39300-88-4
Tara Gum

Caesalpinia Spinosa Hydroxypropyltrimonium Chloride **(CSH)**
742071-24-5

Hydrolyzed Caesalpinia Spinosa Gum **(CSG)**

Fenugreek Gums

Trigonella Foenum-Graecum Seed Extract **(TFS)**
73613-05-5
Fenugreek Seed Extract

Trigonella Foenum-Graecum Hydroxypropyltrimonium Chloride **(TFH)**
742071-25-6

Hydrolyzed Trigonella Foenum-Graecum Seed Extract **(HTF)**

Cassia Gums

Cassia Gum **(CG)**
No CAS No.

Cassia Tora Gum
Cassia Obtusifolia Gum

Galactomannan **(GLM)**
11078-30-1

Cassia Hydroxypropyltrimonium Chloride **(CHC)**

Search Strings

Guar Gum OR Guar OR Cyamopsis Tetragonoloba Gum OR 9000-30-0 OR 39421-75-5 OR 65497-29-2 OR 71329-50-5 OR 68130-15-4 OR 70892-12-5 OR Ceratonia Siliqua Gum OR 9000-40-2 OR Carob Bean Gum OR Locust Bean Gum OR Locust Bean OR Carob Bean OR Caesalpinia Spinosa Gum OR 39300-88-4 Tara Gum OR Caesalpinia Spinosa OR Trigonella Foenum-Graecum Seed Extract OR 73613-05-5 OR Fenugreek Seed Extract OR 742071-25-6 OR Trigonella Foenum-Graecum OR Cassia Gum OR Cassia Tora Gum OR Cassia Obtusifolia Gum OR Cassia OR 11078-30-1

9000-30-0 OR 39421-75-5 OR Guar OR 65497-29-2 OR 71329-50-5 OR 68130-15-4 OR 70892-12-5 OR 9000-40-2 OR Locust Bean OR Ceratonia Siliqua Gum Extract OR 39300-88-4 OR 742071-24-5 OR Caesalpinia Spinosa Gum OR 73613-05-5 OR 742071-25-6 OR Cassia Gum OR Cassia Tora Gum OR Cassia Obtusifolia Gum OR Cassia OR 11078-30-1

Report

Draft Safety Assessment

Galactomannans as Used in Cosmetics

December 13, 2011

All interested persons are provided 60 days from the above date to comment on this Draft Safety Assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.

The 2011 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Wilbur Johnson, Manager/Lead Specialist.

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TABLE OF CONTENTS

INTRODUCTION 1
CHEMISTRY 1
Definition and Structure 1
Properties 1
Method of Manufacture 3
Composition/Impurities 4
USE 6
Cosmetic 6
Noncosmetic 6
TOXICOKINETICS 7
Absorption, Distribution, Metabolism, and Excretion 7
TOXICOLOGY 8
Acute Oral Toxicity 8
Repeated Dose Toxicity – Oral Studies 9
Repeated Dose Toxicity – Parenteral Study 12
Ocular Irritation 13
Skin Irritation 13
Allergenicity 13
Case Reports 13
Clinical Testing 15
Animal Testing 15
Hyperplastic Effect 16
Insecticidal/Antifungal Activity 16
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY 16
Oral Studies 16
Dermal Study 20
GENOTOXICITY 20
CARCINOGENICITY 24
Co-Carcinogenicity 25
Antitumorigenicity 25
Anticarcinogenicity 25
SUMMARY 26

INTRODUCTION

Available data relevant to the safety of the following legume polysaccharides, commonly called galactomannans, as used in cosmetics are reviewed in this draft report:

- Cyamopsis Tetragonoloba (Guar) Gum
- Hydroxypropyl Guar
- C18-22 Hydroxyalkyl Hydroxypropyl Guar
- Guar Hydroxypropyltrimonium Chloride
- Hydroxypropyl Guar Hydroxypropyltrimonium Chloride
- Carboxymethyl Hydroxypropyl Guar
- Hydrolyzed Guar
- Ceratonia Siliqua Gum
- Locust Bean Hydroxypropyltrimonium Chloride
- Hydrolyzed Ceratonia Siliqua Gum Extract
- Caesalpinia Spinosa Gum
- Caesalpinia Spinosa Hydroxypropyltrimonium Chloride
- Hydrolyzed Caesalpinia Spinosa Gum
- Trigonella Foenum-Graecum Seed Extract
- Trigonella Foenum-Graecum Hydroxypropyltrimonium Chloride
- Hydrolyzed Trigonella Foenum-Graecum Seed Extract
- Cassia Gum
- Cassia Hydroxypropyltrimonium Chloride

These ingredients function mostly as hair/skin conditioning agents and viscosity increasing agents in cosmetic products.[1]

Gum guar (oxidized 2-hydroxypropyl 3-hydroxy-3-(trimethylammonio)propyl ether, chloride – also known as cationic guar), while not a cosmetic ingredient, is similar to guar hydroxypropyltrimonium chloride, and acute oral toxicity data on cationic guar are being considered along with the limited acute oral toxicity data on guar hydroxypropyltrimonium chloride. Similarly, acute oral toxicity data on carboxymethyl guar (not a cosmetic ingredient) are being considered in the absence of acute oral toxicity data on carboxymethyl hydroxypropyl guar. Clinical allergenicity data on trigonella foenum-graecum seed powder are being considered in the absence of these data on trignella foenum-graecum seed extract.

CHEMISTRY

Definition and Structure

Definitions of the ingredients reviewed in this safety assessment along with their chemical structures are found in Table 1.[1]

Properties

Seed-bearing plants deposit energy-containing reserves to support the growth of the embryo within the seed. Seed reserves can be of protein, lipid or polysaccharide character. Although the most ubiquitous reserve polysaccharide is starch, the reserve polysaccharides, or gums, of the plant family Leguminoseae, are comprised of polysaccharides that consist of a poly-mannose backbone, with galactose pendent groups. Because of the mannose/galactose chemical make-up of these legume polysaccharides, they are commonly called galactomannans. Unlike starch, which is stored in amyloplasts in the cytoplasm, non-starch reserve polysaccharides, such as galactomannans, are deposited in the cell walls of the seeds.

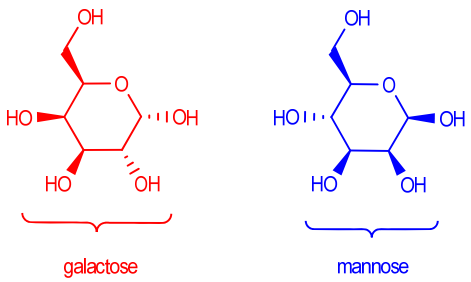


Figure 1. Galactose and Mannose, the building blocks of the Galactomannans

The five primary galactomannan gums are guar gum from the seeds of *Cyamopsis tetragonoloba,* locust bean gum from the seeds of *Ceratonia siliqua* (carob tree), tara gum from seeds of *Cesalpinia spinosa,* fenugreek gum from the seeds of *Trigonella foenum-graecum,* and cassia gum from seeds of *Cassia obtusifolia* or *tora*. While all seed galactomannans possess the same basic structure of a β(1→4)linked polymannose backbone with α(1→6)linked galactose pendent residues, the ratio of galactose to mannose is species specific, and is roughly a constant average within each species. In other words, guar gums average a mannose:galactose ratio of 2:1, locust bean gums are 4:1, tara gums are 3:1, fenugreek gums are 1:1 and cassia gums are 5:1, roughly. The distribution of galactose pendent groups along the polymannose backbone, in each group, is not uniform but more closely resembles a random block, co-polymer. In other words, within one polysaccharide chain there are sections that are highly substituted with galactose pendent groups and there are sections that are more sparsely substituted with galactose pendent groups. Yet, the overall average ratio is maintained within the gum of a species (Figure 2).

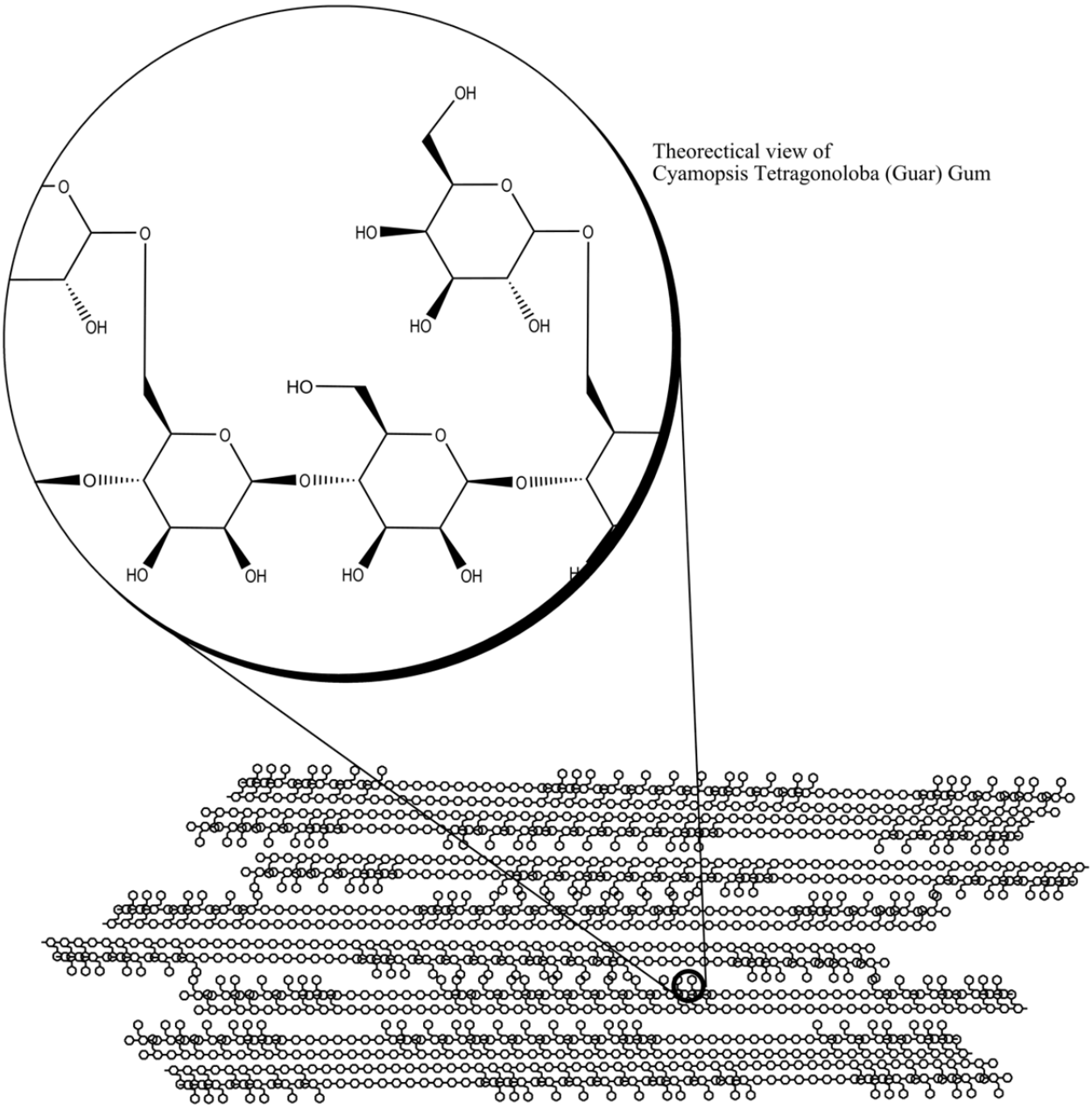


Figure 2. Packing and structure of Cyamposis Tetragonoloba (Guar) Gum

While the ratio of mannose to galactose is 2:1, in guar gums, and the above exploded theoretical structure depicts a galactose pendent group on every other mannose, the true structure is an assortment of pendent placements. These natural gums are typically of a molecular weight greater than 50,000 grams per mole, and often exceed 2,000,000 grams per mole.

In addition to these underivatized, natural gums, are those ingredients which have been etherified at the C6 position of galactose and/or mannose, with various substituents (Figure 3). While the most likely point of derivatization is at the C6 positions of a galactose pendant groups and the polymannose backbone, all of the free hydroxyl groups are potentially susceptible substrates for condensation. That being said, reported degrees of substitution are related as saturated (i.e. equal to 1.0) when there is one stoichiometric equivalent of substituent per one potentially free C6 hydroxyl group (Figure 3). The average degree of substitution, for these types of derivatized gums, is reportedly 0.7 or less. Accordingly, these ingredients have less than one substituent (e.g., hydroxypropyltrimonium chloride) per mannose or galactosyl-mannose. For example, a hydroxypropyl trimonium derivative of guar gum (Guar Hydroxypropyltrimonium Chloride) would be substituted at some available C6 hydroxyls, but might not be at all (Figure 3).

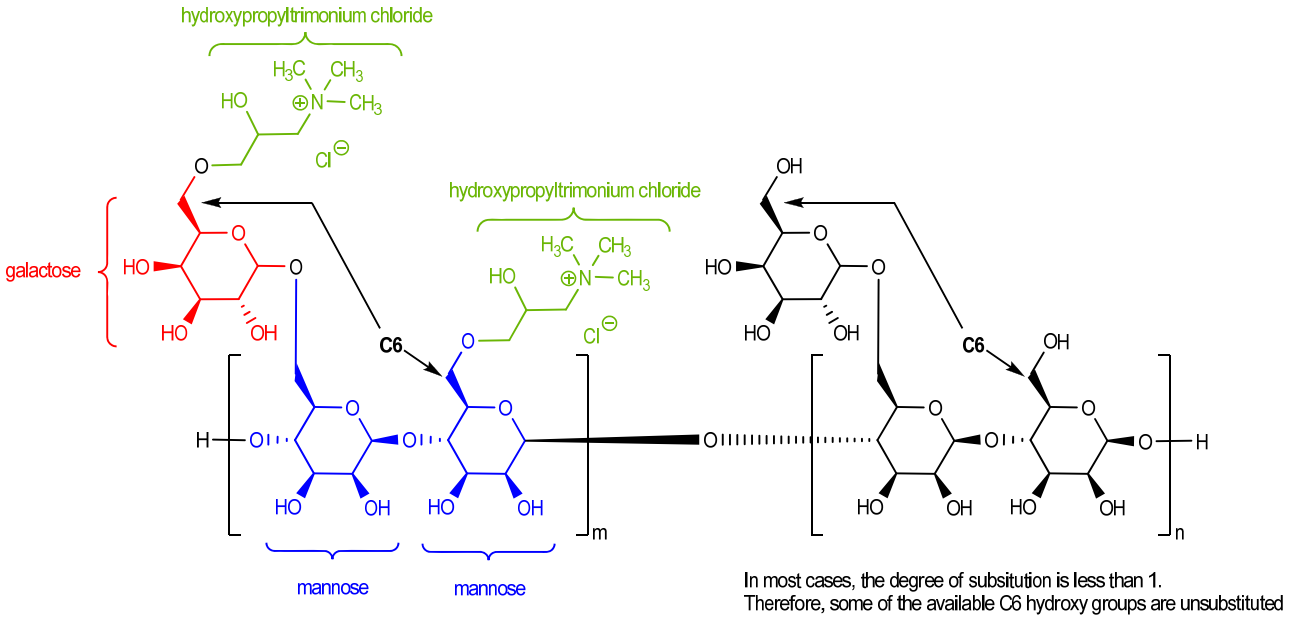


Figure 3. Idealized structure of Guar Hydroxypropyltrimonium Chloride.

Those gums listed in this report as "hydrolyzed" are really ***partially*** hydrolyzed polysaccharides (e.g., hydrolyzed guar gum = guar oligomers). For example, Hydrolyzed Guar is a mixture of guar gum fragments that have been broken down at the 1→6 and 1→4 linkages (via acidic, enzymatic or other methods of hydrolysis) into shorter, lower molecular weight oligosaccharides.

According to the *Food Chemicals Codex*, both cyampopsis tetragonoloba (guar) gum and ceratonia siliqua gum occur as a white to yellow-white powder.[2] Each gum is dispersible in either hot or cold water, forming a sol (pH between 5.4 and 7.0) that may be converted to a gel by addition of small amounts of sodium borate.

Method of Manufacture

Production of natural gums consists of various techniques for the milling of seeds, followed by simple purification steps such as dissolving in hot water, filtering and precipitation with isopropanol. Typical production of the derivatized gums in this report involves the reaction of the natural gum with the appropriate epoxide. Additional information relating to the production of cyamopsis tetragonoloba (guar) gum, hydroxypropyl guar, carboxymethyl hydroxypropyl guar, ceratonia siliqua gum, caesalpinia spinosa gum, and cassia gum, and is included below.

Cyamopsis Tetragonoloba (Guar) Gum

Cyamopsis tetragonoloba (guar) gum is a gum obtained from the ground endosperms of *Cyamopsis tetragonolobus* (Linné) Taub.[3]

Hydroxypropyl Guar

Hydroxypropyl guar gum can be prepared by alkaline etherification of guar gum with propylene oxide.

Guar Hydroxypropyltrimonium Chloride

One method of production of a Guar Hydroxypropyltrimonium Chloride trade name material involves the conversion of guar with 3-chloro-2 hydroxypropyl trimethyl ammonium chloride.[4] Data on the properties/composition of this trade name material are found in Table 4.

Carboxymethyl Hydroxypropyl Guar

Carboxymethylation and hydroxypropylation of cyamopsis tetragonoloba (guar) gum are carried out simultaneously using monochloroacetic acid and propylene oxide, in the presence of a hydrophilic solvent (i .e., 2-propanol) with an alkaline pH.[5] These simultaneous reactions result in the formation of carboxymethyl hydroxypropyl guar gum.

Ceratonia Siliqua Gum

Ceratonia siliqua gum is a gum obtained from the ground endosperms of *Ceratonia siliqua* Linné Taub. (Fam. Leguminosae).[3]

Caesalpinia Spinosa Gum

Commercial production of caesalpinia spinosa gum involves the incomplete separation of the endosperm from the germ and husk. Thus, commercial products may contain small amounts of husk as well as varying amounts of protein and fat from unsepaerated germ. Consequently, commercially available caesalpinia spinosa gum may contain larger percentages of ash and acid insoluble than are present in the hand-dissected endosperm.[6]

Cassia Gum

The production method for cassia gum includes cleaning of the source material, de-husking, and de-germing by thermal and mechanical treatment.[7] These steps are followed by milling and screening of the endosperm, which is further purified by extraction with isopropanol. Semi-refined cassia gum is produced in a similar manner, with the exception of an additional isopropanol step to significantly reduce the level of anthraquinones in the latter.

Composition/Impurities

Specifications on the following gums relating to their use in foods/drugs in the United States are included in Table 2: cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, caesalpinia spinosa gum, and cassia gum.

Cyamopsis Tetragonoloba (Guar) Gum

Cyamopsis tetragonoloba (guar) gum typically consists of the following: galactomannan (80%), water (12%), protein (5%), acid insoluble matter (2%), and fat (1%).[8] Available data on 11 bulk commercial samples of this gum indicate that glycine, glutamic acid, aspartic acid, serine, and alanine are the most abundant amino acids and that their relative proportions vary considerably. Proportions of the following amino acids remain remarkably constant: histidine, isoleucine, phenylalanine, threonine, tyrosine, and valine.[9]

Commercial samples of cyamopsis tetragonoloba (guar) gum, purified and unpurified, were analyzed in this study.[10] The gum was purified using the following 4 methods: (1) treatment with proteolytic enzyme (porcine pancreatin), (2) successive steps of dissolution, centrifugation and precipitation with acetone and ethanol, (3) Fehling solution used as precipitation agent, and (4) application of the second method, followed by the third one. In all samples (purified and unpurified), mannose and galactose were the major consituents, and glucose and arabinose (2 monosaccharide contaminants) were also present. Uronic acid content ranged from 3.28 to 4.17%. The unpurified sample had a protein content of 3.6%. All purification methods reduced the protein and mono/oligo/polysaccharide contaminants. Method 4 resulted in total elimination of protein. However, the 2 methods (methods 3 and 4) that used Fehling solution contaminated the gum with small amounts of Cu(II), 0.079% and 0.044%, respectively. Methods 2 and 4 resulted in purer (i.e., small amount of protein) cyamopsis tetragonoloba (guar) gum, with method 4 resulting in the total elimination of protein.

The following main components in the lipid fraction of cyamopsis tetragonoloba (guar) gum were identified using GLC-MS: palmitate (hexadecanoate), oleate (9-octadecenaoate), and linoleate (9,12-octadecadienoate).[11]

Using mass spectrometry, very low fluoroacetate concentrations (0.07 -1.42 µg/g, 10 samples) have been detected in cyamopsis tetragonoloba (guar) gum used as a raw material for a guar gum powder. A sample of a guar gum pharmaceutical formulation contained 0.08 ppm fluoroacetate.[12]

The noncatalytic hydrolysis of cyamopsis tetragonoloba (guar) gum under hydrothermal conditions (temperature range: 180 to 240ºC) resulted in the following major products: oligosaccharides with degrees of polymerization up to ~ 20, monosaccharides containing mannose and galactose, and 5-hydroxymethyl-2-furaldehyde.[13]

In 2007, the Rapid Alert System for Food and Feed (RASFF) received a notification from Switzerland concerning a finding of serious contamination of cyamopsis tetragonoloba (guar) gum, originating from India, with dioxins and pentachlorophenol (PCP).[14] The levels of these contaminants in certain batches of cyamopsis tetragonoloba (guar) gum were approximately 1000 times the level of what can be considered as normal background contamination. According to a European Commission decision in 2008, all consignments of cyamopsis tetragonoloba (guar) gum or products containing cyamopsis tetragonoloba (guar) gum at significant amounts originating in or consigned from India and imported into the Community intended for human or animal consumption shall be accompanied by an analytical report, endorsed by the competent authority from the country where the laboratory that has performed the analysis is located. With the 2008 decision in effect, it was determined that there had been no improvement in the control system and no significant reduction in the associated risks. Therefore, in accordance with a 2010 European Commission decision (to replace the 2008 decision), additional measures were taken, requiring official sampling , analysis, and certification by competent authorities of India of all consignments of cyamopsis tetragonoloba (guar) gum intended for export to the European Union.

Guar Hydroxypropyltrimonium Chloride

The ash content of a Guar Hydroxypropyltrimonium Chloride trade name material is no more than 8.7%.[4] This information as well as additional data on the properties/composition of this trade name material are found in Table 3.

Ceratonia Siliqua Gum

Ceratonia siliqua gum has been known to contain tannins and trypsin inhibitors.[15]

Cassia Gum

Cassia gum is composed of at least 75% high molecular mass (~ 200,000 to 300,000) polysaccharide, which consists primarily of a linear chain of 1,4-β-D-mannopyranose units with 1,6-linked α-D-galactopyranose units.[7] The saccharides have the following composition: mannose (77.2 to 78.9%), galactose (15.7 to 14.7%), and glucose 6.3 to 7.1%). The ratio of mannose to galactose is 5:1. *Cassia occidentalis* is a naturally occurring contaminant of cassia gum. In the process of cleaning the source material in the manufacture of this gum, the content of *Cassia occidentalis* is reduced to < 0.05%. The concentration of anthraquinones in cassia gum is < 0.5 mg/kg detection limit. Semi-refined cassia gum contains ~70 mg total anthraquinones/kg. Purified semi-refined cassia gum can contain 8.6 mg total anthraquinones/kg.[7,16]

Trigonella Foenum-Graecum Seed Extract

Composition/properties data on a Trigonella Foenum-Graecum Seed Extract trade name material are included in Table 4.[17] Additionally, according to a 2011 memorandum from the Personal Care Products Council, one supplier (anonymous) indicated that Trigonella Foenum-Graecum Seed Extract produced by that company is a supercritical CO_2 extract. It was also indicated that galactomannans (polysaccharides) are not extractable with supercritical CO_2, and that only fat soluble components, such as essential oils and fatty oils, are extractable using supercritical CO_2.[18] Another 2011 memorandum from the Personal Care Products Council indicates that, based on a GC analysis from another supplier (anonymous), the total polysaccharides in Trigonella Foenum-Graecum Seed Extract are < 0.1 mg/g.[19]

USE

Cosmetic

The ingredients reviewed in this safety assessment function mostly as hair/skin conditioning agents and viscosity increasing agents in cosmetic products. These and additional functions are included in Table 1.

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2011, the following 10 ingredients were being used in cosmetic products: cyamopsis tetragonoloba (guar) gum, hydroxypropyl guar, guar hydroxypropyltrimonium chloride, hydroxypropyl guar hydroxypropyltrimonium chloride, hydrolyzed guar, ceratonia siliqua gum, caesalpinia spinosa gum, hydrolyzed caesalpinia spinosa gum, trigonella foenum-graecum seed extract, and cassia hydroxypropyltrimonium chloride.[20] These data are summarized in Table 5. Results from a survey of ingredient use concentrations conducted by the Personal Care Products Council (also included in Table 5) in 2011 indicate that locust bean hydroxypropyltrimonium chloride and hydrolyzed trigonella foenum-graecum seed extract were also being used in cosmetics. Additionally, results from this survey indicate that galactomannans were being used at concentrations up to 93% (hydroxypropyl guar in a rinse-off product) in cosmetic products. Of the ingredients included in Table 5, the lowest use concentration is reported for trigonella foenum-graecum seed extract (0.00001% in a leave-on product).

Cosmetic products containing the ingredients reported as being used may be applied to the skin and hair, or, incidentally, may come in contact with the eyes and mucous membranes. Products containing these ingredients may be applied as frequently as several times per day and may come in contact with the skin or hair for variable periods following application. Daily or occasional use may extend over many years.

Hydroxypropyl guar, guar hydroxypropyltrimonium chloride, and trigonella foenum-graecum seed extract are used in hair sprays, and ceratonia siliqua gum is used in face powders. Therefore, effects on the lungs that may be induced by powders or aerosolized products containing these ingredients are of concern. The particle size of aerosol hair sprays (~ 38 μm) and pump hair sprays (≥ 80 μm) is large when compared to respirable particle sizes (≤ 10 μm). Therefore, because of their size, most aerosol particles are deposited in the nasopharyngeal region and are not respirable.[21,22,23,24]

Noncosmetic

The following ingredients reviewed in this safety assessment are approved for use as direct food additives by the U.S. Food and Drug Administration (FDA): cyamopsis tetragonoloba (guar) gum (21 CFR 133.124)[25] ; ceratonia siliqua gum and trigonella foenum-graecum seed extract (21CFR 182.20)[26] ; and cyamopsis tetragonoloba (guar) gum and ceratonia siliqua gum (21 CFR 133.178; 133.179; 150.141; 150.161)[27,28,29,30]. Additionally, cyamopsis tetragonoloba (guar) gum and ceratonia siliqua gum are direct food additives that are considered generally recognized as safe (21 CFR 184.1339; 184.1343)[31,32]. Hydroxypropyl guar is approved for use as an indirect food additive by FDA (21 CFR 176.170)[33]. An evaluation of the health aspects of ceratonia siliqua gum as a food, prepared for FDA, is available.[15]

Cyamopsis Tetragonoloba (Guar) Gum

Noncosmetic uses of cyamopsis tetragonoloba (guar) gum include:[34] in paper sizing; as a protective colloid, stabilizer, thickening and film forming agent for cheese, salad dressings, ice cream, soups; as a binding and disintegrating agent in tablet formulations; in pharmaceutical jelly formulations; in suspensions, emulsions, lotions, creams, toothpastes; in the mining industry as a flocculant, as a filtering agent; and in water treatment as a coagulant aid. It also functions as an emulsifier.[2]

The distribution of Cal-Ban 3000 diet tablets and capsules was halted by the Food and Drug Administration because it was determined that they may cause esophageal, gastric, and intestinal obstruction .[35] Cyamopsis tetragonoloba (guar) gum, was listed as the main ingredient of Cal-Ban 3000. This gum is a complex sugar that swells when it becomes wet and can create a sense of fullness when ingested.

Cyamopsis tetragonoloba (guar) gum has been approved for use as a food ingredient by the Joint FAO/WHO Expert Committee on Food Additives, and this committee assigned a “not specified” value for the acceptable daily intake (ADI) by man.[36] This means that use of cyamopsis tetragonoloba (guar) gum as a food substance does not represent a human health hazard, and, therefore, the establishment of an ADI in mg/kg body weight was not deemed necessary.

Hydroxypropyl Guar

Hydroxypropyl guar is used as a gelling polymer in an artificial tear solution. It is believed to mimic the mucin layer of the tear film.[37]

Ceratonia Siliqua Gum

Ceratonia siliqua gum has been approved for use as a food ingredient by the Joint FAO/WHO Expert Committee on Food Additives, and this committee assigned a "not specified" value for the acceptable daily intake (ADI) by man.[36] This means that use of ceratonia siliqua gum as a food substance does not represent a human health hazard, and, therefore, the establishment of an ADI in mg/kg body weight was not deemed necessary. Ceratonia siliqua gum functions as a stabilizer and thickener in food.[2]

Caesalpinia Spinosa Gum

Caesalpinia spinosa gum has also been approved for use as a food ingredient by the Joint FAO/WHO Expert Committee on Food Additives, and this committee assigned a "not specified" value for the acceptable daily intake (ADI) by man.[38] This means that use of cyamopsis tetragonoloba (guar) gum as a food substance does not represent a human health hazard, and, therefore, the establishment of an ADI in mg/kg body weight was not deemed necessary.

Cassia Gum

Noncosmetic uses of cassia gum include: thickener, emulsifier, foam stabilizer, moisture retention agent and/or texturizing agent in processed cheese, frozen dairy desserts and mixes, meat products, and poultry products. After considering data supporting the low oral toxicity and negative genotoxicity of cassia gum (included in current CIR report), the Joint FAO/WHO Expert Committee on Food Additives allocated an average daily intake (ADI) " not specified" for cassia gum, when used in the applications specified and in accordance with Good Manufacturing Practice. [7]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

The only available data for these ingredients that relate to absorption, distribution, metabolism, and excretion were found in dietary studies.

Cyamopsis Tetragonoloba (Guar) Gum

When a basal diet containing cyamopsis tetragonoloba (guar) gum (dose = 100 g/kg) was fed to 5 rats, the galactomannan of the gum was fermented almost quantitatively. Thus, approximately 1% of the mannose and 4% of the galactose were excreted in the feces.[39] The minor components (i.e., arabinose, glucose, and uronic acids) were also fermented almost completely.

Caesalpinia Spinosa Gum

Two groups of 20 Wistar rats were fed a semisynthetic diet containing caesalpinia spinosa gum (73.9% galactomannan) at galactomannan dietary concentrations of 2% and 5%, respectively, for 11 days.[40] At the end of this feeding period, 12 rats from each dietary group were selected (based on body weight gain, etc.), for an experimental feeding period of 10 days. Digestibility was defined as the portion of ingested material that was not excreted in the stool. Digestibility of galactomannan was 97% and 98.1% for the 2% and 5% diets, respectively. In another study, rats were fed a normal diet containing caesalpinia spinosa gum.[41] The composition and dietary concentration of the gum as well as the feeding periods were not stated. Stools were analyzed for mannose obtained by hydrolysis. It was concluded that all of the mannose added to the feed in the form of galactomannan was excreted in the feces.

Ceratonia Siliqua Gum

Groups of Purdue rats (5 males, 5 females per group; ages not stated) were fed 1% ceratonia siliqua gum in the diet for 18 h.[42] Eighty-five to 100% of the mannose fed as 1% ceratonia siliqua gum was excreted in the feces over a 30-h period.

It was noted that some decrease in galactomannan chain length may have occurred. This decrease was belived to have been due to the action of the microflora, because mammals are not known to have the mannosidase enzyme. The liberation of galactose units was not determined.

Cassia Gum

The Joint FAO/WHO Expert Committee on food Additives noted that specific absorption, distribution, metabolism, and excretion data were not available on cassia gum at the time of its evaluation of this food additive.[7] However, based on data on related galactomannans, the Committee concluded that cassia gum will be largely excreted unchanged, though fermentation by gut microflora may occur to some extent. It was noted that if hydrolysis of cassia gum occurs, the resulting oligosaccharides or monosaccharides would be expected to be absorbed and metabolized in normal biochemical pathways.

TOXICOLOGY

Acute Oral Toxicity

Cyamopsis Tetragonoloba (Guar) Gum

The acute oral toxicity of cyamopsis tetragonoloba (guar) gum was evaluated using 10 F344 rats (5 males, 5 females; ages not stated) and ten B6C3F1 mice (5 males, 5 females).[43] Each animal received a single dose (0.42 g/kg) of the gum (in water) by gavage. None of the animals died and no test substance-related effects were observed.

In another acute oral toxicity study, 18 rats (strain not stated) received cyamopsis tetragonoloba (guar) gum (in cocoa butter), at a dietary concentration of 30% for 48 h. No adverse effects were observed.[44]

Guar Hydroxypropyltrimonium Chloride

In an acute oral toxicity study on guar hydroxypropyltrimonium chloride (40% w/v in corn oil), 4 groups of 10 rats (5 males, 5 females) received doses of 7.1,10.0, 14.2, and 20.0 g/kg, respectively, by oral intubation.[45] Dosing was followed by a 14-day observation period, and gross necropsy was performed on all animals that died spontaneously. Clinical signs observed included: ataxia; tremors; nasal and oral discharge; urinary and fecal staining; abdominal griping; soft stool; decreased motor activity; labored breathing; and piloerection. The acute oral LD50 was 12.5 g/kg (95% confidence limits of 10.22 to 14.78 g/kg).

The acute oral toxicity of a guar hydroxypropyltrimonium chloride trade name material (composition data in Table 3) was evaluated using Bor: WISW (SPF) Cpb rats (5 males, 5 females; ~ 10 weeks old).[46] A single oral dose of the test substance (in arachidis oil, 2,000 mg/kg) was administered to each animal by gavage. Dosing was followed by a 14-day observation period. Animals that survived were killed and subjected to post-mortem examination. None of the animals died and there were no macroscopic findings at necropsy. An LD50 of > 2,000 mg/kg was reported.

Gum guar, oxidized 2-hydroxypropyl 3-hydroxy-3-(trimethylammonio)propyl ether, chloride (a.ka. (Cationic Guar and Guar Hydroxypropyltrimonium Chloride)

The acute oral toxicity of guar hydroxypropyltrimonium chloride (40% w/v solution in corn oil) was evaluated using 3 groups of 10 Sprague-Dawley albino rats (5 males, 5 females/group) receiving doses of 10.0, 14.2, and 20.0 g/kg, respectively.[47] Dosing was followed by a 14-day observation period, and gross necropsy was performed on animals that died spontaneously. In-life observation included: ataxia; nasal, oral, and ocular discharge; urinary and fecal staining; abdominal griping; decreased motor activity; irritability; and piloerection. Convulsions, tremors, hyopothermia, and prostration were observed only at the highest dose (20.0 g/kg), which yielded 90% mortality. The acute oral LD50 was 15 g/kg (95% confidence limits of 13 to 17 g/kg).

Hydroxypropyl Guar Hydroxypropyltrimonium Chloride

In an acute oral toxicity study on hydroxypropyl guar hydroxypropyltrimonium chloride (40% w/v solution in corn oil), 4 groups of 10 Sprague-Dawley albino rats (5 males, 5 females/group) received doses of 7.1,10.0, 14.2, and 20.0 g/kg, respectively, by oral intubation.[48] Dosing was followed by a 14-day observation period, and gross necropsy was performed on all animals. Clinical signs observed included: ataxia; fine and coarse tremors; nasal, oral, and ocular discharge; decreased

respiratory rate and motor activity; abdominal griping; piloerection; irritability; and generally poor condition. The acute oral LD50 was 12.0 g/kg (95% confidence limits of 9.2 to 14.8 g/kg).

Carboxymethyl Guar

The acute oral toxicity of carboxymethyl guar (40% w/v solution in corn oil) was evaluated using 5 groups of 10 Sprague-Dawley albino rats (5 males, 5 females/group) receiving doses of 5.0, 7.1, 10.0, 14.2, and 20.0 g/kg, respectively, by oral intubation.[49] Dosing was followed by a 14-day observation period, and gross necropsy was performed on all animals that died spontaneously. Clinical signs observed included: ataxia; tremors; nasal, oral, and ocular discharge; labored breathing; piloerection; increased/decreased activity; hypothermia; generally poor condition; urinary and fecal staining; and irritability. The acute oral LD50 was 17.8 g/kg (95% confidence limits of 13.1 to 22.5 g/kg).

Hydrolyzed Guar

An acute oral toxicity study on partially hydrolyzed guar was performed using groups of 16 (8 males, 8 females per group) 4-week-old Jcl:ICR mice and Jcl:SD rats.[50] Partially hydrolyzed guar was administered by gavage at a concentration of 30% in distilled water (dose = 6000 mg/kg body weight; dose volume = 20 ml/kg) to one group per species. The control group was dosed orally with distilled water. Dosing was followed by a 14-day observation period, after which all animals were killed and examined macroscopically. Soft feces were reported for male and female mice, but no abnormal signs were reported for rats. There were no test substance-related effects on body weight (rats and mice), food consumption (rats), or necropsy findings (mice and rats). None of the animals died, and the LD50 was > 6,000 mg/kg in both species.

Ceratonia Siliqua Gum

The acute oral toxicity of ceratonia siliqua gum (in corn oil; dose = 10 g/kg body weight) was evaluated using 5 male Sprague-Dawley rats.[51] None of the animals died, and transient depression was the only sign observed in animals tested. In other studies involving rats (number and strain not stated), acute oral LD50s of 5000 mg/kg body weight and 13,100 mg/kg body weight were reported. Details relating to the test protocol and study results were not included.[52]

In an acute oral toxicity studies involving mice, rabbits, and hamsters (number and strain not stated for each), the following LD50's were reported: 13,100 mg/kg body weight (mice), 9,100 mg/kg (rabbits), and 10,300 mg/kg (hamsters). In each of the 3 studies, details relating to the test protocol and study results were not included.[52]

Cassia Gum

The acute oral toxicity of semi-refined cassia gum was evaluated using 5 male Wistar-Han-Schering rats. The animals received 2 oral doses of 5,000 mg/kg body weight at a 2-h interval. An oral median lethal dose (LD50 value) of > 5,000 mtg/kg body weight was reported.[7,53] In a similar test, 10 male and 10 female KM mice received 4 oral doses of cassia gum (gavage, 10,000 mg/kg body weight) over a 24-h period. The acute oral LD50 was > 10,000 mg/kg body weight.[7,53] Additional details relating to study results (both studies) were not provided.

Trigonella Foenum-Graecum Seed Extract

In an acute oral toxicity study involving male Balb-C mice (ages not stated), an LD50 of > 1 g/kg was reported. Details relating to the test procedure were not included.[54]

Repeated Dose Toxicity – Oral Studies

Cyamopsis Tetragonoloba (Guar) Gum

Five male Sprague-Dawley rats received oral doses of cyamopsis tetragonoloba (guar) gum (in corn oil; dose = 5 g/kg) daily for 5 days.[55] No unusual or adverse effects were observed. The feeding of 27% cyamopsis tetragonoloba (guar) gum to rats for 7 days resulted in the death of 7 of 10 animals. The deaths were probably due to intestinal blockage.[56]

Groups of rats (strain not stated) were fed cyamopsis tetragonoloba (guar) gum in the diet at concentrations of 0, 1, 2, and 5%, respectively, for 90 days.[57] Dosing with this gum did not affect general behavior, appearance, or survival. Growth was described as relatively low in males fed the 2% and 5% cyamopsis tetragonoloba (guar) gum diets. Dietary feeding also had no effect on the following: hematology, urinalysis, serum enzyme activities, or blood sugar levels.

However, blood urea nitrogen values were slightly increased in males of the 5% dietary group. The relative weight of the thyroid was increased only in males of the 2% and 5% dietary groups. The results of gross and histopathological examinations did not reveal any changes that were attributable to gum ingestion.

The following dietary levels of cyamopsis tetragonoloba (guar) gum were fed to 6 groups of 130 male and female Osborne-Mendel rats (~ 4 wk. old; 65 males, 65 females/group) for 91 days: 0, 1.0, 2.0, 4.0, 7.5, and 15.0% in diet.[58] A significant reduction in body weight was noted for female rats of all dietary groups and in males of the 7.5% and 15% dietary groups. There were no deaths related to dietary administration of the gum. Hematocrit values for males were less than control values in all dietary groups; however, this decrease was of borderline significance. Male hemoglobin levels and erythrocyte and leucocyte counts were all within control ranges. In females, hemoglobin levels and erythrocyte counts were significantly decreased only in the 4% dietary group. Compared to control males, liver weights in all dietary groups were significantly less. Kidney weights were significantly less in 7.5% and 15.0% dietary groups, and were also decreased (borderline significance) in the 4.0% dietary group. Except for the bone marrow of male rats in the 15.0% dietary group, no tissue examined had consistent histopathological alterations attributed to ingestion of cyamopsis tetragonoloba (guar) gum. Though there was a suggestion of regressive changes in the bone marrow (moderate bone marrow cellularity) at this dietary level, this finding was within normal limits. The fact that results for several rats fed 15.0% cyamopsis tetragonoloba (guar) gum were at the lower end of the normal range suggested a subtle or borderline effect.

A long-term toxicity study on cyamopsis tetragonoloba (guar) gum was performed using 2 groups of rats (15 males, 15 females/group; ages and strain not stated). One of the groups was fed 5% cyamopsis tetragonoloba (guar) gum in the diet, and the other group (control) was fed diet only. Seven males and 8 females in each group survived and were monitored for 24 months. Of these, 1 test animal died after 12, 18, 19, and 22 months, and the last survivor was killed after 24 months. Three control animals survived to 24 months. All animals appeared in good health and had similar body weights.

Cyamopsis tetragonoloba (guar) gum (1 g in diet) was fed daily to 2 monkeys (ages not stated).[44] After 16 months of feeding, one of the monkeys died. The other monkey was killed at 24 months. Well-being, growth, and hematology (red blood cells, white blood cells, hemoglobin, and urea) were considered normal. The results of gross and histopathological examinations did not indicate any abnormalities. At microscopic examination, there were no abnormalities in any of the following organs/tissues: liver, kidney, spleen, gut, and bone marrow.

Cyamopsis Tetragonoloba (Guar) Gum and Hydrolyzed Guar

Diets containing 5% cyamopsis tetragonoloba (guar) gum and 5% hydrolyzed guar (partially hydrolyzed) were fed to 2 groups of 5 Sprague-Dawley rats (8 months old), respectively, for 3 weeks.[59] Another group of rats was fed a diet containing 5% cellulose. A significant decrease in food intake and weight gain as well as a significant increase in liver weight were reported after feeding with 5% cyamopsis tetragonoloba (guar) gum. The serum IgG level of rats fed 5% cyamopsis tetragonoloba (guar) gum was significantly lower when compared to that of rats fed 5% cellulose. Also, the IgA, IgG, and IgM productivity in mesenteric lymph node (MLN) lymphocytes was significantly higher in rats fed 5% cyamopsis tetragonoloba (guar) gum, compared to rats fed 5% cellulose. The effect of 5% cyamopsis tetragonoloba (guar) gum on IgA, IgG, and IgM productivity in spleen cells was not as marked. The epididymal adipose tissue weight in rats fed 5% hydrolyzed guar was significantly higher than that reported for rats fed 5% cellulose. The results of this study suggest that the enhancement of immune function by cyamopsis tetragonoloba (guar) gum is expressed mainly in the gut immune system.

Hydrolyzed Guar

In a 28-day oral feeding study, 2 groups of 10 rats (5 males, 5 females/group) were fed partially hydrolyzed cyamopsis tetragonolobus (guar) gum in the diet (500 and 2,500 mg/kg doses, respectively) daily.[60] Body weights and food consumption were measured, and gross and microscopic pathology were evaluated. No adverse effects were observed at either administered dose.

A repeated dose oral toxicity study was performed using 7-week-old male and female Jcl:SD rats. The 2 groups of 20 rats per sex were control (diet without partially hydrolyzed guar) and 5.0% partially hydrolyzed guar dietary groups, respectively.[50] The remaining 2 groups of 10 rats per sex received dietary concentrations of 0.2 and 1.0 %. All groups were fed daily for 13 weeks. Ten rats per sex per group were then randomly selected from control and 5.0% dietary groups and maintained on the control diet for an additional 4 weeks (recovery period). Recovery animals were used to investigate the reversibility of possible partially hydrolyzed guar toxicity. All surviving animals (recovery animals included) were killed and subjected to macroscopic and microscopic examination. There were no deaths during dosing or recovery periods, or test substance-related effects on body weight, or food/water consumption. Additionally, there were no test substance-related

changes relative to the following: opthalmoscopic examination, urinalysis, hematological examination, blood biochemical examination, necropsy, organ weights, or histopathological examination.

Cyamopsis Tetragonoloba (Guar) and Ceratonia Siliqua Gum

A pre-cooked mixture of cyamopsis tetragonoloba (guar) and ceratonia siliqua gum (proportions not stated) was fed to groups of 5 female beagle dogs (5 males, 5 females per group) for 30 weeks.[61] The gum was fed at dietary concentrations of 0, 1, 5, and 10%. Hypermotility was observed at the highest dietary concentration. There was no evidence of adverse hematological, urinary, gross/histopathological, or ophthalmological findings.

Ceratonia Siliqua Gum

In a short-term oral toxicity study, 18 rats (strain not stated) received cyamopsis tetragonoloba (guar) gum (in cocoa butter), at a dietary concentration of 30% for 48 h.[56] There was no evidence of adverse effects. Five male Sprague-Dawley rats (ages not stated) received oral doses of ceratonia siliqua gum (in corn oil; dose = 5 g/kg) daily for 5 days.[51] No unusual or adverse effects were observed. The feeding of 10 rats with 27% cyamopsis tetragonoloba (guar) gum for 7 days resulted in death of 7 animals, possibly due to intestinal blockage.[56]

Three groups of 8 rats (ages and strain not stated) were fed a stock diet, a stock diet with 1% cholesterol, and a stock diet with 1% cholesterol and 10% ceratonia siliqua gum, respectively for 28 days.[62] There were no significant differences in weight gain between the 3 groups, and no adverse effects were reported. A soybean-corn meal diet containing 2% ceratonia siliqua gum was fed to groups of newly weaned Sprague-Dawley rats (10 rats per group, ages not stated) for 36 days.[63] There were no significant effects on growth.

Ceratonia siliqua gum was fed, in the diet, to groups of rats (strain not stated) at concentrations of 0, 1, 2, and 5%, respectively, daily for 90 days.[57] Except for increased blood glucose in the 5% dietary group, there were no treatment-related differences between test and control groups regarding the following parameters: general behavior, survival, growth, food intake, hematology, blood biochemistry, and urinalysis. Neither gross nor microscopic examination results indicated any pathological changes that were due to ingestion of the gum.

Caesalpinia Spinosa Gum

In a 90-day oral feeding study, 50 rats (strain and ages not stated) of each sex were fed diets containing 0, 1, 2, or 5% caesalpinia spinosa gum.[64] At the 5% dietary level, body weight gains were depressed in both sexes, and possibly, in males at the 2% dietary level. Compared to the other dietary groups, food intake in the 5% dietary group was less. There were no effects on hematology or urinalysis parameters in any of the groups tested. A statistically significant increase in blood urea nitrogen was noted in rats fed 5% caesalpinia spinosa gum in the diet; however, blood levels were within the normal range of values for rats. There were no treatment-related differences in other clinical chemistry parameters between the control group and any group fed caesalpinia spinosa gum in the diet. Increased male kidney-body weight ratios (5% group) and increased relative weights of the thyroids and testes (2% and 5% groups) were observed. Results of gross and microscopic examination of tissues, including those associated with increased relative organ weight, were not indicative of changes related to caesalpinia spinosa gum in the diet.

Groups of 10 F344 rats and 10 B6C3F1 mice of each sex (ages of animals not stated) were fed diets containing 0, 0.31, 0.63, 1.25, 2.50, or 5.0% caesalpinia spinosa gum for 13 weeks.[65] The gum supplied contained 86.2% galactomannan. The animals were killed at the end of the study and microscopic examination was performed on control animals and those fed 5% caesalpinia spinosa gum in the diet. None of the mice or rats in any of the groups died during the feeding period. Compared to controls, male rats experienced small decreases in body weight gain, while weight increases were reported for female rats. Opposite effects on body weight gain were reported for mice. Changes in body weight gain were not dose-related. The only changes related to feeding with caesalpinia spinosa gum included fewer mature spermatozoa in 4 of 10 male rats in the 5% dietary group.

Purebred beagle dogs (3 per sex) were fed experimental diets containing 1% or 5% caesalpinia spinosa gum for 90 days and the control group was fed α-cellulose.[66] For male dogs fed the 5% diet, feed intake values were 14% below those reported for the control group. No behavioral changes were reported, and hematological, urinalysis, and clinical chemistry results were unremarkable. There also were no gross or microscopic findings that were related to feeding with diets containing caesalpinia spinosa gum.

Cassia Gum

Groups of 5 male and 5 female crl:CD (SD)BR Sprague-Dawley rats (5 to 6 weeks old) were fed semi-refined cassia gum daily at the following dietary concentrations in a 28-day study: 0; 2,500; 10,000; 25,000; or 50,000 mg/kg (= 0; 250; 1,030; 2,590; and 4,960 mg/kg body weight [males] and 0; 230; 1,110; 2,360; and 4,590 mg/kg body weight [females]).[7,67] An additional group of rats received the test substance (in distilled water; dose = 1,000 mg/kg body weight) by gavage twice daily. Histopathologic examination was performed only on the major organs of animals from the following groups: control; 50,000 mg/kg dose (in feed) group, and the group dosed (1,000 mg/kg body weight) via gavage twice daily. There were no treatment-related deaths or clinical changes in any of the groups. Statistically significant reductions in body weight were noted in males (50,000 mg/kg dose group) and females (10,000 and 25,000 mg/kg dose groups); however, these changes were considered related to the viscous nature of cassia gum and toxicologically irrelevant.

The statistically significant hematological and clinical chemistry findings were not dose-related or occurred in one sex only. These values were within the normal range for this species, however, historical control data were not provided. The only changes outside of the historical control range that could have been treatment-related were increased mean concentrations of glucose and triglyceride in the 10,000 mg/kg and 25,000 mg/kg, but not the 50,000 mg/kg, dose groups. The statistically significant reductions in kidney weights (inconsistent pattern in 1,000; 10,000; and 50,000 mg/kg dose groups) were not considered treatment-related. At necropsy or during microscopic examination of major organs (kidneys included), no treatment-related effects were observed. It was concluded that the effects observed in this study were not of toxicological significance. The no-observed-adverse-effect level was 50,000 mg/kg feed (= 4590 mg/kg body weight per day). [7,67]

In a 30-day study, groups of Sprague-Dawley rats (10 males,10 females per group) were fed cassia gum in the diet at doses of 0; 250; 500; and 1,000 mg/kg body weight per day There were no treatment-related effects on the following: mortality, body weight gain, food consumption, food utilization, hematological parameters, or various biochemical parameters (e.g., albumin, cholesterol, aspartate aminotransferase). Gross examination results were negative and there were no treatment-related histopathologic changes or effects on weight in the following organs: liver, kidney, spleen, ovaries, and testes. There also were no histopathologic changes in the stomach or intestines. An NOAEL of 1,000 mg/kg body weight was reported for cassia gum in rats. [7,53]

Semi-refined cassia gum (in dog food) was administered to groups of 4 male and 4 female Beagle dogs at a dietary dose of 7,500 or 25,000 mg/kg body weight (= doses of 980 and 3290 mg/kg body weight per day [males] and 1130 and 3890 mg/kg body weight per day [females]) for 90 days.[7,68] A control group received ceratonia siliqua gum (in dog food) at a daily dose of 2,300 mg/kg body weight per day. The study was performed according to OECD Test Guideline 409. A dose-related increase in water consumption was the only treatment-related effect noted, but was not considered toxicologically significant. Hematological effects and effects on biochemical parameters and organ weight were not considered treatment-related. It was concluded that the NOAEL for cassia gum in dogs was 25,000 mg/kg feed (= 3290 mg/kg body weight per day).

Groups of 5 male and 5 female cats (breed and species not stated) were fed semi-refined cassia gum in the diet at doses of 0; 5,000; or 25,000 mg/kg body weight (= doses of 0; 520; and 2,410 mg/kg body weight per day [males] and 0; 530; and 2,740 mg/kg body weight per day [females]) for 13 weeks.[7,69] The study was conducted according to modifications of OECD Test Guideline 409. Neither adverse effects nor treatment-related effects on the following were reported: mortality, behavior, clinical signs, body weight gain, food and water consumption, hematology, clinical biochemistry, organ weights, macroscopy, or microscopy. A no-observed-effect level (NOEL) of 25,000 mg/kg feed (= 2,410 mg/kg body weight per day) was reported.

Repeated Dose Toxicity – Parenteral Study

Trigonella Foenum-Graecum Seed Extract

When 6 Balb-C male mice (ages not stated) were dosed i.p. with trigonella foenum-graecum seed extract (alcohol extract), 200 mg/kg/day for 7 days, there were no toxic effects relating to changes in body weight or general appearance. Additionally, no pathological changes in vital organs were observed.

Ocular Irritation

**Cyamopsis Tetragonoloba (Guar) Gum,
Ceratonia Siliqua Gum, Caesalpinia Spinosa Gum,
and Cassia Gum**

Cyamopsis tetragonoloba (guar) gum (0.1 g) was instilled into the right eyes of New Zealand white rabbits.[70] The eyes of 3 rabbits were rinsed after instillation, and the eyes of 6 were not rinsed. Ocular irritation was scored according to the Draize scale at 1 h, 24 h, 48 h, and 72 h post-instillation. Cyamopsis tetragonoloba (guar) gum induced minimal ocular irritation in rinsed and unrinsed eyes. Results for other gums (same procedure) were as follows: ceratonia siliqua gum (minimally irritating, rinsed and unrinsed eyes), and cassia gum (non-irritating - rinsed eyes; minimally irritating – unrinsed eyes).

Skin Irritation

**Cyamopsis Tetragonoloba (Guar) Gum
Ceratonia Siliqua Gum, Caesalpinia Spinosa Gum,
and Cassia Gum**

The skin irritation potential of cyamopsis tetragonoloba (guar) gum was evaluated using New Zealand white rabbits (3 males, 3 females).[70] The gum (0.5 g/test site) was moistened with saline and applied to shaved, intact skin. Test sites were covered with a semi-occlusive wrap for 4 h. Reactions were scored according to the Draize scale at 4.5 h, 24 h, 48 h, and 72 h. Cyamopsis tetragonoloba (guar) gum was non-irritating to the skin of rabbits. Results for other gums (same procedure) were as follows: ceratonia siliqua gum (minimally irritating; PII = 0.04/8.0), caesalpinia spinosa gum (non-irritating), and cassia gum (non-irritating).

Allergenicity

Cyamopsis Tetragonoloba (Guar) Gum

The prevalence of occupational asthma and immunologic sensitization to cyamopsis tetragonoloba (guar) gum was evaluated in 162 empolyees of a carpet-manufacturing plant where this gum was used to adhere dye to the fiber.[71] IgE and IgG antibodies to cyamopsis tetragonoloba (guar) gum were measured in 133 of the 162 subjects who agreed to blood tests. Thirty-seven subjects (23%) had a history of occupational asthma and 59 (39%) had a history of occupational rhinitis. Skin prick tests with cyamopsis tetragonoloba (guar) gum (1 mg/ml) were conducted. Immediate skin reactivity to cyamopsis tetragonoloba (guar) gum was observed in 8 subjects and 11 subjects had serum IgE antibodies to cyamopsis tetragonoloba (guar) gum. In the second part of the study (161 subjects), spirometry and assessment of bronchial responsiveness to methacholine were performed during a regular working day at the time of the workshift or in the following 3 to 4 hours. Five subjects had a concentration of methacholine causing a 20% decrease in forced expiratory volume (FEV_1) of < 16 mg/ml (significant bronchial hyperresponsiveness) and positive skin reactions to cyamopsis tetragonoloba (guar) gum. It was concluded that the prevalence of IgE sensitization to guar gum was between 5% (8 of 162 subjects, as assessed by skin tests) and 8.3% (11 of 133 subjects, as assessed by measurement of serum IgE antibodies).

Case Reports

Cyamopsis Tetragonoloba (Guar) Gum

Three male patients (27, 42, and 49 years) developed allergic rhinitis after exposure to cyamopsis tetragonoloba (guar) gum.[72] Two of the subjects developed rhinitis after 2 years of exposure to fine cyamopsis tetragonoloba (guar) gum (insulator in rubber cables) powder when opening cables in a power cable factory. The allergenicity of cyamopsis tetragonoloba (guar) gum in these subjects was confirmed using scratch-chamber, nasal provocation, and radioallergosorbent (RAST) tests, and observations of nasal eosinophilia. A third subject also developed allergic rhinitis after 2 years of exposure to another cyamopsis tetragonoloba (guar) gum product in a paper factory. A positive skin test and nasal provocation test confirmed the gum-induced allergenicity.

Symptoms of rhinitis and asthma were reported for 3 male atopic subjects (29, 30, and 32 years) after exposure to cyamopsis tetragonoloba (guar) gum on the job.[73] An immediate skin reaction to cyamopsis tetragonoloba (guar) gum was observed in skin prick tests, and all 3 had high levels of serum IgE antibodies to the gum. When the subjects were exposed for short intervals (≤ 4 minutes) to cyamopsis tetragonoloba (guar) gum powder, isolated immediate bronchospastic reactions were observed in 2 subjects, and a dual reaction was observed in the remaining subject.

Eight patients (5 men, 3 women; 49.6 ± 3.05 years) with noninsulin-dependent diabetes mellitus consumed at least 30 g of cyamopsis tetragonoloba (guar) gum in the diet for at least 16 weeks.[74] Each subject was able to consume four granola-type bars (6.6 g guar gum/bar) per day. There were no changes in hematologic, hepatic, or renal function. Serologic screening results indicated no changes in lipid, protein, or mineral metabolism, and no changes in electrolyte balance. It was concluded that consumption of 30 g cyamopsis tetragonoloba (guar) gum per day for prolonged periods is without serious consequences.

An allergy prick test was performed on a 38-year-old male employee of a pet food processing company, where he frequently inhaled guar powder.[75] Within 20 minutes of the test, the patient reacted positively to cyamopsis tetragonoloba (guar) gum. A 10 mm wheal with pseudopods and surroundiong flare was observed initially. At 4 h later, the reaction developed into an erythematous swelling (2 cm diameter) that remained for 24 h.

A 52-year-old male subject experienced generalized urticaria and anaphylactic shock after consuming a meal substitute that contained cyamopsis tetragonoloba (guar) gum.[76] Skin and radioallergosorbent (CAP system) tests on this gum as well as ceratonia siliqua gum were performed. Details relating to test procedures were not provided. Results were positive for both gums, with evidence of an IgE-mediated mechanism.

Five subjects ingested cyamopsis tetragonoloba (guar) gum (1 g in capsule) daily for 10 days.[77] No effects were apparent.

Cyamopsis Tetragonoloba (Guar) Gum and Ceratonia Siliqua Gum

A 63-year-old male experienced a life-threatening immediate-type hypersensitivity reaction after mucosal application of a local anesthetic gel that contained cyamopsis tetragonoloba (guar) gum. Severe contact urticaria and dyspnea were reported, and the patient collapsed. [78] A 1-fold positive prick test reaction to cyamopsis tetragonoloba (guar) gum and a 2-fold positive prick test reaction to native guar beans were reported. Prick test results for a highly purified molecular grade ceratonia siliqua gum were negative. Negative IgE assay results for cyamopsis tetragonoloba (guar) gum and ceratonia siliqua gum were reported. A possible explanation for the discrepancy between prick test and IgE assay results for cyamopsis tetragonoloba (guar) gum could be the varying degree of allergen contamination in different guar products, remaining from the germ or hull of the Cyamopsis bean, that are not detected by commercial IgE assays that were probably established with highly purified cyamopsis tetragonoloba (guar) gum.

A 48-year old male complained of work-related rhinitis, irritated eyes, and asthma after exposure to ceratonia siliqua gum and cyamopsis tetragonoloba (guar) gum on the job in a jam factory.[79] Skin prick tests on ceratonina siliqua gum and a blend of cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, and carrageenan were negative. Asthma attacks did not occur after the patient stopped handling the gums. When a single-blind provocation test (manipulation of carob bean flour for 15 min) was performed, a cough, rhinitis, and sneezing developed. Both cyamopsis tetragonoloba (guar) gum and ceratonia siliqua gum were evaluated in the RAST, and results were positive.

A 59-year-old female developed lip edema a few minutes after ingesting a dessert.[80] She also complained of nasal hydrorrhea and sneezing while handling powder to prepare the dessert. Results of skin prick tests indicated a positive reaction to ceratonia siliqua gum in saline (11 mm wheal); the test concentration was not stated. Skin prick test results for ceratonia siliqua gum at concentrations of 5, 15, and 25 mg/ml induced 6 mm, 8 mm, and 9 mm wheals, respectively. Positive skin prick tests were also reported for raw ceratonia siliqua gum (14 mm wheal) and boiled ceratonia siliqua gum (9 mm wheal). Control skin prick test results were negative in 10 nonatopic subjects. The positive test results and high titers of serum specific IgE to ceratonia siliqua gum supported an IgE-mediated mechanism. Skin prick test results for cyamopsis tetragonoloba (guar) gum were negative.

Ceratonia Siliqua Gum

A 30-year old male with allergic rhinitis developed asthma regularly after handling carob bean flour.[81] A prick test for ceratonia siliqua gum and a radioallergosorbent test (RAST) were both positive (U RAST = 8.86, class 2).

Urticaria and vomiting were reported after feeding of an 8-month-old infant with a milk-based antiregurgitation formula containing ceratonia siliqua gum as a thickening agent.[82] Feeding with a milk-based antiregurgitation formula thickened with waxy rice starch was readily accepted.

Trigonella Foenum-Graecum Seed Powder

Cases of immediate allergy following exposure to trigonella foenum-graecum seed powder have been reported.[83] A 36-year-old female with a history of allergy to chickpeas and mild asthma experienced sneezing, rhinorrhea, and excessive tearing after smelling trigonella foenum-graecum seed powder. These signs were followed by persistent coughing, wheezing, and fainting. Scratch testing with 10 µl of the legume extract (contained ~ 22 to 25 µg protein) revealed a severe (4+) reaction. According to another report, a 45-year-old female with a history of allergic rhinitis and asthma developed congestion and hoarness shortly after applying trigonella foenum-graecum seed paste to the scalp. These signs were followed by facial angioedema, wheezing, and numbness of the head. Scratch testing revealed a 3+ reaction to the legume extract.

A 26-year-old female developed systemic itching after eating curry and rice.[84] Laboratory test results indicated a slightly increased eosinophil count of 869/µl and an increased serum total IgE level of 8410 IU/ml. An oral provocation test was performed to confirm the causal relationship between curry and rice and allergic symptoms. Generalized itching was evident 20 minutes after eating curry and rice and persisted for 30 minutes. The forced expiratory volume in one second (FEV_1) decreased to 78% of the value before provocation, and the serum histamine level increased from 1.4 µM before challenge to 58.0 µM 1 h after challenge. Spices known to be used in curry powder were ingested to identify the causative agent in curry powder. After ingestion of trigonella foenum-graecum, it was determined that this spice was a causative allergen. The subject developed wheezes and the FEV1 was decreased by 20%.

The enzyme-linked immunosorbent assay (ELISA) was used to measure allergen-specific serum IgE antibody in this patient. Ten atopic patients who had a positive radioallergosorbet test (RAST) score to house dust and a high serum IgE level served as controls. The patient had a substantially high level of IgE antibody against trigonella foenum-graecum.

Clinical Testing

Cyamopsis Tetragonoloba (Guar) Gum

An experimental trial to evaluate the effect of cyamopsis tetragonoloba (guar) gum on arterial blood pressure was performed using 40 moderately overweight men with mild hypertension.[85] Each participant received 7 g of the gum 3 times daily for 2 weeks. There were no changes in body weight or body composition; however, total cholesterol decreased during the feeding period. Blood pressure was found to decrease by 9.8% (systolic) and 9% (diastolic) after 2 weeks of feeding. Blood pressure readings returned to pre-treatment levels after a 3-week wash-out period. It was concluded that cyamopsis tetragonoloba (guar) gum exerts a lowering effect on high blood pressure in moderately obese men, even in the absence of any change in body weight.

A study was conducted to determine the efficacy of dietary fiber cyamopsis tetragonoloba (guar) gum as a therapeutic option for reducing body weight, by performing a meta-analysis of randomized controlled trials.[86] Of the 34 experimental trials identified, only 11 provided data that were suitable for statistical pooling. Results of the meta-analysis indicated a nonsignificant difference in patients that received cyamopsis tetragonoloba (guar) gum, when compared to patients that received a placebo (weighted mean difference -0.04 kg; 95% confidence interval (CI): -2.2 to 2.1). Furthermore, these findings were corroborated by an analysis of 6 trials with similar methodologic features (weighted mean difference -0.3 kg; 95% CI: -4.0 to 3.5). The more frequently reported adverse events included abdominal pain, flatulence, diarrhea, and cramps. The meta-analysis suggested that cyamopsis tetragonoloba (guar) gum is not efficacious for reducing body weight.

Animal Testing

Ceratonia Siliqua Gum

In a study investigating the effect of various gums on nitrogen balance and dry matter digestibility, a group of 12 weanling Sprague-Dawley rats was fed ceratonia siliqua gum at a concentration of 10% in a casein-saccharose-corn starch diet.[87] Trypsin inhibitory activity of ceratonia siliqua gum in the diet was measured. Only slight enzyme inhibition was associated with this gum.

Trigonella Foenum-Graecum Seed Extract

To evaluate anti-inflammatory activity, groups of 6 Sprague-Dawley albino rats (ages not stated) received i.p. doses of trigonella foenum-graecum seed extract (alcohol extract, 100 and 200 mg/kg doses).[54] A group of control rats was also included. At 30 min post-injection, carrageenan (0.1 mL from 10 mg/mL solution) was injected into the plantar aponeurosis of the right handpaw of treated and control rats. At doses of 100 and 200 mg/kg, 45% ± 11.5% and 62.3% ± 12.9% inhibition of inflammatory swelling, respectively, was reported. Inflammatory swelling (100%) was observed in the control group.

Hyperplastic Effect

The effect of trigonella foenum-graecum seed extract (alcohol extract) on the number of macrophages and peritoneal exudate cells was evaluated using 3 groups of 6 Balb-C male mice.[54] Two groups received i.p. injections of the test substance at doses of 100 and 200 mg/kg, respectively, and the control group received an equivalent volume of normal saline. At 24 h post-injection, intraperitoneal fluid was collected and intraperitoneal cells and macrophages (stained with neutral red) counted. The average number of peritoneal exudate cells per normal mouse was $0.78 \pm 0.12 \times 10^7$. A significant increase ($p < 0.01$) in the number of peritoneal cells was noted at both doses; however, actual numbers were not included. Treatment with trigonella foenum-graecum seed extract also enhanced the number of macrophages (actual number not included).

Insecticidal/Antifungal Activity

An acetone extract of trigonella foenum-graecum seeds was found to have insecticidal activity against *Tribolium castaneum* and *Acanthoscelides obtectus*.[88] The antifungal activity of an aqueous extract of trigonella foenum-graecum seeds against the following has also been reported: *Botrytis cinerea*, *Fusarium graminearum, Alternaria sp*., *Pythium aphanidermatum*, and *Rhizoctinia solani*.[89]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral Studies

Cyamopsis Tetragonoloba (Guar) Gum

Groups of male and female Osborne-Mendel rats (4 weeks old; 34 to 40/group) were fed cyamopsis tetragonoloba (guar) gum in the diet at concentrations of 0, 1, 3, 4, 7.5, or 15% 13 weeks prior to mating, during mating, and throughout gestation.[90] Groups of female rats consumed 0, 0.7, 1.4, 2.7, 5.2, or 11.8 g/kg body weight/day, respectively, during gestation. The animals were killed on gestation day 20. None of the females died during the study. The ingestion of cyamopsis tetragonoloba (guar) gum prior to mating had no effect on fertility. No effects on the number of corpora lutea or implantations were observed in the 1% to 7.5% cyamopsis tetragonoloba (guar) gum dietary groups. When compared to the control group, slightly fewer corpora lutea and implantations were observed in the 15% dietary group; however, there was no effect on implantation efficiency. A slight reduction (not statistically significant) in the number of viable fetuses per litter was also noted in the 15% dietary group. Because the number of resorptions was not affected in this group, this reduction appeared to have been an effect of the decreased number of corpora lutea. It was concluded that cyamopsis tetragonoloba (guar) gum in the diet had no effect on fetal development or sex distribution and was not teratogenic.

In another teratogenicity study on cyamopsis tetragonoloba (guar) gum, groups of virgin adult female albino CD-1 outbred mice (ages not stated) were mated with young adult males, and observation of the vaginal sperm plug was considered day 0 of gestation.[91] The following groups of mated female mice received daily oral doses of cyamopsis tetragonoloba (guar) gum (in corn oil; dose volume = 1 ml/kg body weight) on days 6 through 15 of gestation: 22 mice (8 mg/kg/day), 26 mice (37 mg/kg/day), 25 mice (170 mg/kg/day), and 29 mice (800 mg/kg/day). A sham-treated control group was also included and aspirin served as the positive control. Doses up to 170 mg/kg/day had no clearly discernible effect on nidation or on maternal or fetal survival. Additionally, the number of abnormalities observed in either skeletal or soft tissues of test groups did not differ from the number that occurred spontaneously in sham-treated controls. In the 800 mg/kg/day dose group, a significant number of maternal deaths (6 of 29) was reported. Surviving dams appeared completely normal and the same was true of fetuses. No effects on the rate of nidation or survival of live pups in utero was noted. It was concluded that, under the conditions of this test, cyamopsis tetragonoloba (guar) gum was not teratogenic in mice. The teratogenicity of cyamopsis tetragonoloba (guar) gum in rats and hamsters was also evaluated in this study. Except for the administration of different doses and a shorter dosing period for hamsters, the test procedure was the same. Results are summarized below.

Four groups of 24 mated female rats (ages not stated) received cyamopsis tetragonoloba (guar) gum (in corn oil) at daily oral doses of 9, 42, 200, and 900 mg/kg/day, respectively. Doses up to 900 mg/kg/day had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities observed in either skeletal or soft tissues of test groups did not differ from the number that occurred spontaneously in sham-treated controls. The following groups of mated female hamsters (ages not stated) received daily oral doses of cyamopsis tetragonoloba (guar) gum (in corn oil): 22 hamsters (6 mg/kg/day), 22 hamsters (28 mg/kg/day), 25 hamsters (130 mg/kg/day), and 20 hamsters (600 mg/kg/day) on days 6 through 10 of gestation. Doses up to 600 mg/kg/day had no clearly discernible effect on nidation or on maternal or fetal survival. Furthermore, the number of abnormalities observed in either skeletal or soft tissues of test groups did not differ from the number that occurred spontaneously in sham-treated controls.[91]

Ceratonia Siliqua Gum

A 3-generation reproduction study was performed using groups of Charles River albino rats of the CD strain (10 males, 10 females per group).[92] Two groups were fed chow diet containing 2% and 5% ceratonia siliqua gum, respectively. The control group was fed chow diet containing 5% cellulose. Parental animals in each generation were fed the test diet for 11 weeks prior to mating and through mating, gestation, and weaning. Two or 3 litters were raised per generation, and the second litter was used to produce the F_{3b} generation. Histopathological examination of major organs and tissues was performed on 10 males and 10 females from each treatment group of the F_{3b} generation. All of the other animals were subjected to gross necropsy only. Statistically significant decreases in pre-mating body weight gain (F_0 females fed 2% ceratonia siliqua gum) and final body weight (F_0 females fed 5% ceratonia siliqua gum) were noted. Compared to controls, the following significant differences in organ weight ratios were reported for the F_{3b} group fed 5% ceratonia siliqua gum: smaller spleen-to-body weight and liver-to-body weight ratios, smaller absolute liver weights, and larger brain-to-body weight ratio. These differences were due to the highly variable values for these parameters in young rats and because all of the animals may not have been at the same age when killed. These age differences may have also had an effect on organ weight ratios in young animals. It was concluded that ceratonia siliqua gum did not cause significant treatment-related effects on reproductive indices or gross microscopic pathology.

The teratogenicity of ceratonia siliqua gum (in anhydrous corn oil) was evaluated using the following 6 groups of pregnant adult female albino, CD-1 outbred mice: 13 mg/kg ceratonia siliqua gum (20 mice), 60 mg/kg (20 mice), 280 mg/kg (21 mice), 1,300 mg/kg (21 mice), 0 mg/kg (20 mice, sham treated), and aspirin, 150 mg/kg (21 mice).[93] Doses were administered orally (intubation) on gestation days 6 through 15 (10 days). On day 17, fetuses were removed by Caesarean section and each was examined grossly for the presence of external congenital abnormalities. Detailed visceral examinations were performed on one-third of the fetuses of each litter, and the remaining two-thirds were examined for skeletal defects. There were no clearly discernible effects on nidation or on maternal or fetal survival at doses up to 280 mg/kg. The number of soft or skeletal tissue abnormalities in test groups did not differ from the number that occurred spontaneously in sham-treated controls. A significant number of maternal deaths (5 of 21 females) occurred in the 1300 mg/kg dose group. However, the surviving dams in this group appeared completely normal and delivered normal fetuses, and there were no effects on the rate of nidation or survival of live pups in utero. It was concluded that ceratonia siliqua gum was not teratogenic in mice at the doses administered in this study. The positive control was teratogenic.

In other experiments in the preceding study, the teratogenicity of ceratonia siliqua gum was evaluated in rats, hamsters, and rabbits according to a similar test procedure. The following 6 groups of adult female Wistar albino rats were used: 13 mg/kg ceratonia siliqua gum (23 rats), 60 mg/kg (21 rats), 280 mg/kg (24 rats), 1300 mg/kg (23 rats), 0 mg/kg (23 rats, sham treated), and aspirin (250 mg/kg, 21 rats). On day 20, the fetuses were delivered by Caesarean section. There were no clearly discernible effects on nidation or on maternal or fetal survival at doses up to1300 mg/kg. The number of soft or skeletal tissue abnormalities in test groups did not differ from the number that occurred spontaneously in sham-treated controls. It was concluded that ceratonia siliqua gum was not teratogenic. In the experiment involving pregnant adult female, golden outbred hamsters, the following groups were used: 10 mg/kg ceratonia siliqua gum (21 hamsters), 45 mg/kg (20 hamsters), 220 mg/kg (24 hamsters), 1000 mg/kg (20 hamsters), 0 mg/kg (20 hamsters, sham treated), and aspirin, 250 mg/kg (20 hamsters). Doses were administered orally on gestation days 6 through 10 (5 days). Except for the dosing period, doses administered, and day of Caesarean section (day 14), the protocol was identical to the one used for mice and rats. There were no clearly discernible effects on nidation or on maternal or fetal survival at doses up to1300 mg/kg. The number of soft or skeletal tissue abnormalities in test groups did not differ from the number that occurred spontaneously in sham-treated controls. It was concluded that ceratonia siliqua gum was not teratogenic in hamsters.[93]

The teratogenicity of ceratonia siliqua gum (in anhydrous corn oil) was evaluated using the following 6 groups of pregnant adult Dutch-belted female rabbits: 9 mg/kg ceratonia siliqua gum (11 rabbits), 42 mg/kg (12 rabbits), 196 mg/kg (13 rabbits), 910 mg/kg (12 rabbits), 0 mg/kg (14 rabbits, sham treated), and 6-amino nicotinamide, 2.6 mg/kg (13 mice, on

day 9). Doses were administered orally (intubation) on gestation days 6 through 18 (13 days) of gestation, and the fetuses were delivered by Caesarean section on day 29. Except for the dosing period, doses administered, and day of Caesarean section, the protocol was identical to the one used for mice and rats. Significant maternal toxicity was observed at doses of 910 mg/kg, and, other than hemorrhage of the intestinal mucoasa (small intestine), there were no gross pathological findings at autopsy. The fetuses of the highest dose group were normal. All fetuses were examined grossly for the presence of external congenital abnormalities, and subjected to examination for visceral abnormalities and skeletal defects. There were no discernible effects on nidation or on maternal or fetal survival at doses up to 910 mg/kg. The number of soft or skeletal tissue abnormalities in test groups did not differ from the number that occurred spontaneously in sham-treated controls. It was concluded that ceratonia siliqua gum was not teratogenic in rabbits.[93]

Caesalpinia Spinosa Gum

A multigeneration reproduction study on caesalpinia spinosa gum was performed using groups of Charles River CD albino rats (22 days old).[94] The gum was administered to male and female rats at a dietary level of 5% (50,000 ppm) through 3 successive generations. All matings involved 10 males and 20 females per group, and fertility was described as high. Litters were maintained until the end of lactation, at which time they were at least 21 days old. There were no consistent, statistically significant test substance-related adverse effects on any of the parameters evaluated, including mortality, food consumption, body weight gains, general health, and behavior. Similarly, regarding mating and reproductive performance indices, there were no consistent, statistically significant differences between control and gum-treated groups. These data suggest that caesalpinia spinosa gum had no adverse effect on reproductive performance and in utero development. Data from those progeny selected as parental animals for subsequent generations (F_1 and F_2 parents) indicated that these animals had normal growth patterns and reproductive performance. Gross examination of the parental animals and offspring and microscopic examination of tissues from selected F_{3b} progeny did not identify any abnormalities that were related to administration of the gum. It was concluded that caesalpinia spinosa gum did not have an adverse effect on reproductive performance or development of progeny.

The teratogenicity and embryotoxicity of caesalpinia spinosa gum was evaluated using groups of Wistar/HAN rats. Groups of 25 rats were fed pellet-sized diets containing 0; 1.25% (12,500 ppm); 2.5% (25,000 ppm); or 5% (50,000 ppm) of the gum from days 6 to 16 of gestation. All females were killed on day 21 postcoitum, and fetuses removed by caesarean section. None of the animals died and there were no statistically statistically significant differences in food consumption, body weight gain, general health, or behavior between dams that received control diet and those that received caesalpinia spinosa gum in the diet. Necropsy results did not reveal any abnormalities that were related to gum administration. There was no evidence of test substance-related abnormalities after external, visceral, and skeletal examinations of fetuses were performed. Additionally, there were no differences in the sex ratios of fetuses or statistically significant differences in fetal body weights. It was concluded that caesalpinia spinosa gum did not induce maternal toxicity, embryotoxicity, or teratogenicity. Based on results from this study and the preceding study, the no-observable-adverse-effect-level (NOAEL) was considered to be > 50,000 ppm (5%) in the diet.[94]

Trigonella Foenum-Graecum Seed Extract

The developmental toxicity of trigonella foenum-graecum seed extract (25% aqueous extract solution) was evaluated using male and female Swiss mice (ages not stated).[95] After mating, pregnant mice were divided into 3 groups of 5 mice. The extract solution was administered orally (gavage) to 2 groups at doses of 500 and 1000 mg/kg/day, resepectively, during the gestational period. Distilled water was administered to control mice. Any mortalities, abortions, or premature deliveries were recorded daily from day 0 until delivery. Detection of any signs of abortion and/or fetal expulsion were based on an examination of vaginal bloodstains on day 13 of gestation. None of the animals died and there were no signs of treatment-related abnormal behavior in females. There also were no significant differences in body weight gain between dams in test (both dose groups) and control groups. The gestation period also did not differ between test and control groups ($P = 0.869$). However, compared to the control group, a significant decrease ($P = 0.01$) in the pregnancy index was observed in the higher dose group.

There were no differences in delivery, viability, or weaning index between test (both dose groups) and control mice. However, compared to controls, the live-birth index was significantly reduced ($P < 0.001$) in the higher dose group. A significant reduction in litter size was noted at both doses ($P < 0.001$). Compared to the control group, pup body weights of treated fetuses at birth (500 and 1000 mg/kg doses) were also significantly reduced ($P < P < 0.001$). The following 3 external malformations observed in fetuses of treated dams were described as follows: aplasia of the external ear in 1 fetus (500 mg/kg dose group) and 2 fetuses with bump on the head and median cleft of lower lip, respectively (1000 mg/kg dose group). There were no obvious external malformations in the control group. Compared to controls, the absolute brain weight for fetuses of prenatally treated mice was significantly decreased in both dose groups ($P < 0.001$). Additionally, the brain-to-

body weight ratio was significantly higher ($P < 0.001$) in both dose groups when compared to the control group. It was concluded that trigonella foenum-graecum seed extract may have deleterious toxic effects on reproductive performance and potential teratogenic effects in fetuses. [95]

Cassia Gum

In a two-generation reproductive toxicity study (OECD Test Guideline 416), semi-refined cassia gum was administered to groups of 25 female Ico:OFA.SD Sprague-Dawley rats (ages not stated) at dietary concentrations of 0; 5,000; 20,000, or 50,000 mg/kg (= doses of, 0; 510; 2,060; and 5,280 mg/kg body weight per day [males] and 0; 510; 2,090; and 6,120 mg/kg body weight [females]).[7,96] An additional group was fed a diet containing purified semi-refined cassia gum (which resulted from an additional isopropanol extraction step) at a dose of 50,000 mg/kg (= dose of 5,430 mg/kg body weight per day [males] and 6,230 mg/kg body weight per day [females]. Parental animals were dosed for approximately 10 weeks prior to mating and during mating, gestation, and lactation. The only effects observed included a slightly reduced pregnancy rate (not observed in a subsequent second mating resulting in an F_{1b} generation), and a slight, non-significant decrease in pup weights in the F_{1a} and F_2 generations . These effects were observed at the highest dose level, and, therefore, 50,000 mg/kg feed (equal to 5280 mg/kg body weight per day) was considered the NOEL.

Groups of 12 pregnant female SD rats (ages not stated) received oral doses of cassia gum (by gavage) at doses of 0; 250; 500; or 1,000 mg/kg body weight per day on days 7 through 16 of gestation.[7,53] There were no treatment-related effects on maternal body weight, the number of resorptions or dead embryos, or the weight and length of fetuses. Also, abnormalities were not observed at skeletal or visceral examination of the fetuses. Therefore, no adverse effects were observed in dams or offspring at doses up to and including 1,000 mg/kg body weight per day.

The developmental toxicity of semi-refined cassia gum (in distilled water) was evaluated using groups of 28 pregnant Crl:CD (SD)BR Sprague-Dawley rats (ages not stated).[7,97] The test substance was administered orally (by gavage) at doses of 0; 250; 500; or 1,000 mg/kg per day on gestation days 6 to 19 post-coitum, in accordance with OECD Test Guideline 414. Purified semi-refined cassia gum (1,000 mg/kg body weight) was administered to a fourth group of 29 pregnant rats according to the same procedure. The animals were killed and examined on day 20 post-coitum. There were no test substance-related effects on pregnancy incidence, implantations, post-implantation loss, or fetal defects at necropsy. In pregnant animals that received semi-refined cassia gum or purified semi-refined dassia gum at the 1,000 mg/kg dose level, a statistically significant reduction in mean daily food consumption and mean body weight gain was noted. These effects were thought to have been related to the viscous nature of the gum and were not considered toxicologically relevant. A statistically significant increase in fetal weight after dosing with purified semi-refined cassia gum was the only finding in offspring. There was no evidence of embryotoxicity or teratogenicity in any of the treatment groups, and the NOAEL was 1,000 mg/kg body weight per day.

In another developmental toxicity study, groups of 20 pregnant New Zealand White rabbits (ages not stated) were dosed with semi-refined cassia gum or purified semi-refined cassia gum, following the procedure (same doses for each) in the preceding study.[7,98] The only deviation from this protocol was that the rabbits were dosed on days 6 to 27 post-coitum and were killed on day 28 post-coitum. The following animals died or were killed moribund during the study: 4 controls, 1 and 4 rabbits dosed with semi-refined cassia gum (350 and 1,000 mg/kg body weight, respectively), and 2 rabbits dosed with purified semi-refined cassia gum (1,000 mg/kg body weight). All deaths were attributed to improper gavaging and/or were incidental. A non-significant reduction in mean daily food consumption was observed in the group dosed with 1,000 mg/kg semi-refined cassia gum. A slight, non-significant reduction in mean fetal weight was also observed in this group, and, possibly, was a secondary effect to the reduced feed consumptin. These effects were probably related to the viscous nature of cassia gum and were not considered to be of toxicological relevance. There were no treatment-related adverse effects relative to the following: pregnancy incidence, implantations, post-implantation loss, or fetal defects upon necropsy. Cassia gum was neither teratogenic nor embryotoxic in rabbits, and an NOAEL of 1,000 mg/kg body weight per day was reported.

In a one-generation reproductive toxicity study (OECD Test Guideline 415), semi-refined cassia gum was administered to groups of cats (10 males, 20 females per group; species not specified) at dietary concentrations of 0; 7,500; or 25,000 mg/kg (= 0; 690; and 2470 mg/kg body weight per day [males] and 0; 860; and 2,950 mg/kg body weight per day [females]) for at least 83 weeks.[7,99] High mortality in the control group resulted in a high litter loss, which impaired appropriate comparison between treatment and control groups. Generally, no obvious effects on short-term toxicity parameters were observed in the parental generation and offspring. Reproductive performance was not affected, and the same was true for growth and development of the offspring. The following effects, possibly related to dosing, were observed only at the highest administered dose: slight decrease in food consumption during late gestation, and a slight increase in absolute and relative ovarian weights in parental females + a significantly higher combined incidence of stillborns and neonatal deaths. Because of the unusually high mortality rate in the control group, the Joint FAO/WHO Expert Committee on

Food Additives (JECFA) considered this study unsuitable for use in evaluating the reproductive toxicity of semi-refined cassia gum.

Dermal Study

Cyamopsis Tetragonoloba (Guar) Gum

The teratogenicity of cyamopsis tetragonoloba (guar) gum (in deionized water) was evaluated using groups of 20 New Zealand White SPF rabbits (4 months old when received).[100] After a 2-month acclimation period, the test substance was administered, via non-occlusive dermal application (6 h), to 3 groups at doses of 2, 10, and 50 mg/kg/day (dose volume = 2 ml/kg), respectively, on days 6 through 18 of gestation. Applications were made to dorsal skin (10 x 20 cm^2). Deionized water was administered to the control group according to the same procedure. Three mortalities were reported, and 3 rabbits produced litters prematurely. A dose-related increase in dermal irritation was noted in 10 and 50 mg/kg dose groups, with frequent observations of erythema, edema, and desquamation. Analyses of behavior observations, body weights, and food consumption were not indicative of test substance-related responses in any of the 3 dose groups. A moderate increase in mean post-implantation loss, accompanied by a decrease in viable fetuses, was noted in the highest dose group (50 mg/kg/day). The increase in early resorptions observed in this group was found to be statistically significant ($p < 0.05$). A slight increase in mean post-implantation loss was noted in the 10 mg/kg/day dose group; however, uncertainty over the significance of this observation was expressed. Increased post-implantation loss was not observed in the 2 mg/kg/day dose group. No other abnormalities were observed in any of the Cesarean section parameters. Analyses of fetal morphological observations were not indicative of test substance-related responses in any of the 3 dose groups.

GENOTOXICITY

Cyamopsis Tetragonoloba (Guar) Gum

The mutagenicity of cyamopsis tetragonoloba (guar) gum (in DMSO) was evaluated in the Ames preincubation assay using the following *Salmonella typhimurium* strains: TA 97, TA 98, TA 100, TA 102, TA 104, TA 1535, TA 1537, and TA 1538. [101] The gum was evaluated at concentrations up to 10,000 µg/plate with metabolic activation. Cyamopsis tetragonoloba (guar) gum was not mutagenic to any of the strains tested.

In the host mediated assay, the mutagenicity of cyamopsis tetragonoloba (guar) gum was evaluated at concentrations of 1% and 5% w/v using *Salmonella typhimurium* strains TA-1530 and G-46 and *Saccharomyces cerevisiae* strain D-3.[55] Untreated cultures served as negative controls and and ethyl methanesulfonate served as the positive control. Cyamopsis tetragonoloba (guar) gum did not produce any measurable mutagenic response or alteration in the recombination frequency. The positive control was mutagenic.

The mutagenicity of cyamopsis tetragonoloba (guar) gum was evaluated in the cytogenetic assay using metaphase chromosomes from rat bone marrow or anaphase chromosomes from *in vitro* cultures of human embryonic lung cells (WI-38).[55] Bone marrow cells were obtained from groups of rats that received oral doses of ceratonia siliqua gum up to 5,000 mg/kg. Cells obtained from the bone marrow of untreated animals served as negative controls, and bone marrow cells from rats dosed with trriethylenemelamine (TEM) served as positive control cultures. WI-38 cells *in vitro* were tested with ceratonia siliqua gum at concentrations up to 1,000 µg/ml. Untreated cultures of these cells served as negative controls and TEM served as the positive control. Cyamopsis tetragonoloba (guar) gum was not mutagenic to metaphase chromosomes from rat bone marrow at any of the doses tested. However, it caused a high increase in the number of aberrant WI-38 anaphase cells. The positive control was mutagenic to bone marrow cells and WI-38 cells.

In the dominant lethal gene test, groups of male rats and pregnant female rats received single and repeated doses of cyamopsis tetragonoloba (guar) gum.[55] Acute, single oral doses (up to 5 g/kg) were administered weekly over an 8-week period and multiple oral doses (up to 5 g/kg) were administered over a 7-week period. Vehicle (not stated) and positive control (TEM) groups were also used. There were no consistent mutagenic responses that were related to test substance administration. Occasional statistical differences between control and test groups (e.g., in numbers of implants, dead implants, copora lutea, and pre-implantation loss in different dose groups) were reported; however, these were considered random occurrences without any suggestion of a time- or dose-response effect. Cyamopsis tetragonoloba (guar) gum was not mutagenic in the dominant lethal test. The positive control was mutagenic.

The test for sex-linked recessive lethals in *Drosophila melanogaster* was used to evaluate the mutagenicity of cyamopsis tetragonoloba (guar) gum.[102] The criterion for a positive response was demonstration of a difference between the mutation frequencies in a treated and a concurrent control group that was statistically at the 5% level. Cyamopsis tetragonoloba (guar) gum (doses not stated) did not produce a mutagenic response in any germ stage or exposure level tested.

Cyamopsis tetragonoloba (guar) gum (in mixed solution of water and glycerin [1:1]) was not mutagenic in the spore rec-Assay when tested at a dose of 2.5 mg/disk with or without metabolic activation.[103]

Hydroxypropyl Guar

The genotoxicity of hydroxypropyl guar (in deionized water or DMSO) with and without metabolic activation was evaluated in the Ames test at concentrations up to 1000 µg/plate, using the following *Salmonella typhimurium* strains: TA-98, TA-100, TA-1535, TA-1537, and TA-1538, and Saccharomyces cerevisiae (yeast) strain D4.[104] The positive controls for the nonactivation assay were methylnitrosoguanidine, 2-nitrofluorene, and quinacrine mustard. In the activation assay, the positive controls were: 2-anthramine, 2-acetylaminofluorene, and 8-aminoquinoline. Without metabolic activation, results were negative in all strains, except for strain TA-100 (positive results at 500 and 1000 µg/plate). With metabolic activation, hydroxypropyl guar was mutagenic to the following strains: TA-1537, TA-1538, TA-98, and TA-100. A dose-related increase in the revertant frequency was observed, with metabolic activation, in strains TA-1538, TA-98, and TA-100. The mutagenic activity of hydroxypropyl guar against strain TA-100 was confirmed with repeated tests at concentrations of 100, 500, and 1000 µg/plate.

Guar Hydroxypropyltrimonium Chloride

A guar hydroxypropyltrimonium chloride trade name material (composition data in Table 3) was evaluated in the Ames test (reverse mutation assay) using the following *Salmonella typhimurium* strains: TA 98, TA 100, TA 1535, TA 1537, and TA 1538.[105] A suspension of the test substance (in Tween 80/water) was evaluated at concentrations up to 1,000 µg/plate with and without metabolic activation. The trade name material was not mutagenic with or without metabolic activation in any of the strains tested.

Hydrolyzed Guar

The mutagenicity of partially hydrolyzed cyamopsis tetragonoloba (guar) gum (in distilled water) was evaluated using *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537, and *Escherichia coli* strain WP2 uvrA.[50] The gum was tested at concentrations up to 5000 µg/plate both with and without metabolic activation. N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), 2-nitrofluorene (2-NF), 9-aminoacridine (9-AA), and 2-aminoanthracene. Partially hydrolyzed cyamopsis tetragonoloba (guar) gum did not induce a 2-fold or greater dose-related increase in revertant colonies, compared to the concurrent negative control. The positive controls were mutagenic.

Ceratonia Siliqua Gum

Ames tests (plate and suspension tests) were used to study the mutagenicity of ceratonia siliqua gum (in DMSO). *Salmonella typhimurium strains* TA-1535, TA-1537, and TA-1538 were used in the plate test and *Saccharomyces cerevisiae* (yeast) strain D4 was used in suspension tests.[106] In the plate test, ceratonia siliqua gum was evaluated at concentrations of 0.45%, 0.90%, and 1.80% with and without metabolic activation. The positive controls for nonactivation plate tests were: ethyl methanesulfonate, 2-nitrofluorene, and quinacrine mustard. Dimethylnitrosamine and 2-Acetylaminofluorene served as activation assay positive controls. DMSO served as the solvent control. Over the range of test concentrations, ceratonia siliqua gum was not mutagenic to any of the *Salmonella typhimurium* strains with or without metabolic activation. The positive controls were mutagenic. In suspension tests, ceratonia siliqua gum was evaluated at concentrations of 1%, 2% , and 3% with and without metabolic activation. The same positive controls were used, and the solvent control was saline or DMSO. Over the range of test concentrations, ceratonia siliqua gum was not mutagenic to *Saccharomyces cerevisiae* strain D4 with or without metabolic activation.

Ceratonia siliqua gum (in DMSO) was evaluated in the Ames test using the following *Salmonella typhimurium* strains: TA 97, TA 98, TA 100, TA 102, TA 104, TA 1535, TA 1537, and TA 1538.[101] The gum was evaluated at concentrations up to 10,000 µg/plate with and without metabolic activation, and results were negative.

In the host mediated assay, ceratonia siliqua gum was evaluated at a concentration of 5% w/v using *Salmonella typhimurium* strain TA-1530 and G-46 and *Saccharomyces cerevisiae* strain D-3.[51] Untreated cultures served as negative

controls and ethyl methanesulfonate served as the positive control. Ceratonia siliqua gum did not produce any measurable mutagenic response or alteration in the recombination frequency. The positive control was mutagenic.

The mutagenicity of ceratonia siliqua gum was tested in the cytogenetic assay using metaphase chromosomes from rat bone marrow or anaphase chromosomes from *in vitro* cultures of human embryonic lung cells (WI-38).[51] Bone marrow cells were obtained from groups of rats that received oral doses of ceratonia siliqua gum up to 5,000 mg/kg. Cells obtained from the bone marrow of untreated animals served as negative controls, and bone marrow cells from rats dosed with triethylenemelamine (TEM) served as positive control cultures. WI-38 cells *in vitro* were tested with ceratonia siliqua gum at concentrations up to 1,000 µg/ml. Untreated cultures of these cells served as negative controls and TEM served as the positive control. Ceratonia siliqua gum was not mutagenic to rat metaphase chromosomes (rat bone marrow) or human anaphase chromosomes (WI-38 lung cells) at any of the doses /concentrations tested. The positive control was mutagenic.

In the dominant lethal gene test, groups of male rats and pregnant female rats received single and repeated doses of ceratonia siliqua gum.[51] Single oral doses (up to 5 g/kg) were administered weekly over an 8-week period and multiple oral doses (up to 5 g/kg) were administered over a 7-week period. Vehicle (not stated) and positive control (TEM) groups were also used. There were no consistent mutagenic responses that were related to test substance administration. Occasional statistical differences between control and test groups (e.g., in numbers of implants, dead implants, copora lutea, and pre-implantation loss in different dose groups) were reported; however, these were considered random occurrences without any suggestion of a time- or dose-response effect. Ceratonia siliqua gum was not mutagenic in the dominant lethal test. The positive control was mutagenic.

Ceratonia Siliqua Gum (in mixed solution of water and glycerin [1:1]) was not mutagenic in the spore rec-Assay when tested at a dose of 2.5 mg/disk with or without metabolic activation.[103]

Caesalpinia Spinosa Gum

The genotoxicity of caesalpinia spinosa gum was evaluated in the Ames test using the following *Salmonella typhimurium* strains: TA 98, TA 100, TA 1535, TA 1537, and TA 1538.[107] The gum was evaluated at test concentrations up to 1,000 µg/plate with and without metabolic activation. During the first and third of 3 assays, there was no significant increase in revertant number, with or without metabolic activation, over the range of concentrations tested. Increases in revertant number were observed in the second assay; however, this finding was not concentration-dependent.

The clastogenic potential of caesalpinia spinosa gum for inducing micronuclei in polychromatic bone marrow cells was evaluated using Swiss Crl mice (5 per sex).[108] The gum (in distilled water, 17.5 mg/ml) was administered by gavage at a dose of 350 mg/kg, and animals were killed at intervals up to 72 h post-dosing. Negative control mice were dosed with distilled water and positive control mice were dosed with cyclophosphamide. In all groups, bone marrow (from femur) was examined for the presence of micronuclei in polychromatic erythrocytes. The number of micronuclei in mice dosed with caesalpinia spinosa gum was not significantly different ($p < 0.05$) from the corresponding negative control values.

Trigonella Foenum-Graecum Seed Extract

In the Ames test, the genotoxicity of trigonella foenum-graecum seed extract was evaluated using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537, and *Escherichia coli* strain WP2*uvr*A.[109] Trigonella foenum-graecum seed extract was tested at concentrations up to 5,000 µg/plate with and without metabolic activation. Water served as the vehicle control and the positive controls were as follows: benzo[α] pyrene, 2-aminoanthracene, 2-nitrofluorene, sodium azide, ICR-91, and 4-nitroquinolone-N-oxide (4-NQO). Trigonella foenum-graecum seed extract was not genotoxic with or without metabolic activation, and the positive controls were genotoxic.

In another Ames test (pre-incubation plate method), the mutagenicity of trigonella foenum-graecum seed extract (alcoholic extract) was evaluated using *Salmonella typhimurium* strain TA 98.[110] Test concentrations up to 50 mg/plate were evaluated without metabolic activation, and trigonella foenum graecum seed extract was found to be non-mutagenic.

The genotoxicity of trigonella foenum-graecum extract was also evaluated in the mouse lymphoma assay using L5178Y mouse lymphoma cells.[109] The ability of this extract to induce forward mutations at the thymidine kinase (TK) locus in these cells was evaluated. In the initial assay, trigonella foenum-graecum extract was tested at concentrations up to 5,000 µg/ml (with metabolic activation) and up to 4,000 µg/ml (without metabolic activation). Trigonella foenum-graecum was then re-evaluated in the confirmatory assay at concentrations up to 5,000 µg/ml (with metabolic activation) and up to 1,500 µg/ml (without metabolic activation). Dose-related increases in cytotoxicity (i.e., reduction in total growth) were observed.

Mutant frequencies induced by cultures treated with trigonella foenum-graecum extract did not meet the minimum criteria for a positive response.

An *in vivo* micronucleus assay was performed using 3 groups of 5 healthy male Crl:CD-1(ICR)BR mice (8 to 10 weeks old).[109] The 3 groups received trigonella foenum-graecum seed extract at oral (gavage) doses of 500, 1,000, and 2,000 mg/kg/day, respectively, for 3 consecutive days. Groups dosed with the vehicle (water) and positive control (cyclophosphamide) were also used. At the end of the dosing period, bone marrow was harvested and at least 2000 polychromatic erythrocytes (PCEs) were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 erythrocytes per animal. At doses up to 2,000 mg/kg/day, trigonella foenum-graecum seed extract did not induce signs of clinical toxicity or evidence of bone marrow cytotoxicity (i.e., no statistically significant decrease in the PCE:NCE ratios). Trigonella foenum-graecum seed extract did not induce any statistically significant increases in micronucleated PCEs at any of the administered doses.

Cassia Gum

The genotoxicity of semi-refined cassia gum (in DMSO) was evaluated in the Ames test (plate incorporation method, OECD Test Guideline 471) using the following bacterial strains, with and without metabolic activation: *Salmonella typhimurium* strains TA98, TA1535, and TA1537 (experiment 1) and *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA (experiment 2).[7,111] In both experiments, cassia gum was tested at concentrations up to 1,000 µg/plate. Slight precipitation occurred at concentrations ≥ 62.5 and ≥ 200 µg/plate in experiments 1 and 2, respectively. Toxicity was not observed in either experiment. In experiment 1, results were negative in *Salmonella typhimurium strains* TA98, TA1535, and TA1537. However, in experiment 2, dose-related increases in the number of revertants at precipitating concentrations were observed in these 3 strains both with (TA 1537 only) and without metabolic activation. However, these increases were within the historical control range. In strain TA100, a dose-related increase in the number of revertants was observed at precipitating concentrations, both with and without metabolic activation, in 2 independent experiments; results were negative for *E.coli* strain WP2uvrA under the same conditions.

In another study, the Ames test (plate incorporation method, OECD Guideline 471) was used to evaluate the genotoxicity of purified semi-refined cassia gum (8.6 mg total anthraquinones/kg) using the following bacterial strains, with and without metabolic activation: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA (experiments 1 and 2) and *Salmonella typhimurium* strain TA100 and *Escherichia coli* strain WP2uvrA (experiment 3).[7,16] In experiments 1 and 2, cassia gum (in ultrapure water) was tested at concentrations up to 100 µg/plate, and cassia gum (in DMSO) was tested in experiment 3 at concentrations up to 5,000 µg/plate. In experiments 1 and 2, slight precipitation occurred at concentrations of 33 and 100 µg/plate; no toxicity was observed. In experiment 3, slight precipitation was observed at all test concentrations; no toxicity was observed. Cassia gum was not genotoxic with or without metabolic activation in this study.

The genotoxicity of cassia gum (in sterilized distilled water) was evaluated in the Ames test (plate incorporation method) using the following bacterial strains, with and without metabolic activation: *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102.[7,53] Results were negative with and without metabolic activation. Information on precipitation or toxicity was not provided.

In the mammalian gene mutation assay (2 experiments, OECD Test Guideline 476), semi-refined cassia gum (in DMSO) was evaluated at concentrations up to 10 µg/ml, with and without metabolic activation, using mouse lymphoma L5178Y TK+/- cells.[7,112] In both experiments, precipitation was observed at a concentration of 10 µg/ml. No toxicity was observed in experiment 1. However, in experiment 2 without metabolic activation, the cell count and cloning efficiency were reduced by 42% and 81% at the highest test concentration (10 µg/ml). Toxicity was not observed with metabolic activation in experiment 2. Study results were considered negative with and without metabolic activation.

In the chromosomal aberrations assay (2 experiments, OECD Test Guideline 473), semi-refined cassia gum (in DMSO) was evaluated at concentrations up to 10 µg/ml, with and without metabolic activation, using human lymphocytes.[7,113] In both experiments, precipitation occurred at a concentration of 10 µg/ml. In experiment 1, the highest test concentration (10 µg/ml) induced mitotic inhibition (22%) with, but not without, metabolic activation. In experiment 2, the highest test concentration induced mitotic inhibition (33%) with, but not without, metabolic activation. Study results were considered negative with and without metabolic activation.

The genotoxicity of cassia gum was evaluated in the micronucleus test using bone marrow from male and female KM mice.[7,53] The animals received oral doses of 625 to 2,500 mg/kg (divided over 2 doses in 30 h) by gavage. Bone marrow was collected 6 h after the second gavage. Micronuclei (1,000 polychromatic erythrocytes [PCE] per mouse) were

counted and the ratio of PCE to normal chromatic erythrocytes (NCE) was determined. Cassia gum was not genotoxic in this assay.

In the sperm abnormality test (KM mouse sperm), mice received oral doses of 625 to 2,500 mg/kg body weight per day by gavage for 5 days.[7,53] Sperm was collected 30 days after the last dose, and aberrations were counted in 1,000 sperm cells per mouse. Cassia gum was not genotoxic in this test.

CARCINOGENICITY

Cyamopsis Tetragonoloba (Guar) Gum, Ceratonia Siliqua Gum, and Caesalpinia Spinosa Gum

The carcinogenicity of cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, and caesalpinia spinosa gum was evaluated using groups of F344 rats (50 males, 50 females/group; 4 weeks old) and groups of B6C3F1 mice (50 males, 50 females/group; 4 to 5 weeks old).[114] The respective groups received either gum in feed at concentrations of 25,000 and 50,000 ppm, respectively, daily for 103 consecutive weeks. Untreated control groups were also used. Animals that were moribund as well as those that survived to the end of the study were killed and necropsied. There were no significant differences in survival between any dose group (rats or mice) and respective control groups. A 10% reduction in body weight gain, compared to controls, was noted in the following dose groups: female mice (50,000 ppm cyamopsis tetragonoloba (guar) gum), male mice (50,000 ppm ceratonia siliqua gum), and male and female mice (50,000 ppm caesalpinia spinosa gum). In all groups, mean daily feed consumption for dosed rats and mice was ≥ 79% of respective control values. A variety of non-neoplastic degenerative and inflammatory lesions was observed in rats and mice of all dose groups. However, these lesions were not related to feeding with any of the gums. A number of significant changes in the incidences of primary tumors ($P < 0.05$, in both positive and negative directions) was reported for rats and mice. In all cases, differences in the tumor incidence observed in one sex were not observed in statistically significant proportions in the other sex of the same species.

A statistically significant increase in the incidence of pheochromocytomas of the adrenal gland was noted in female rats fed both dietary concentrations of cyamopsis tetragonoloba (guar) gum (25,000 and 50,000 ppm). However, the combined incidence of female rats with phaeochromocytoma or malignant phaeochromocytoma of the adrenal gland was not significantly different from control values. Similarly, the incidence of pituitary adenomas in male rats fed cyamopsis tetragonoloba (guar) gum and the incidence of alveolar/bronchiolar adenomas (in lung) in male mice fed ceratonia siliqua gum were not significantly different from control values, when combined with incidences of the corresponding carcinomas at those sites. Alveolar /bronchiolar adenomas of the lung in male mice fed 25,000 ppm ceratonia siliqua gum and pituitary adenomas in female mice fed the same dietary concentration were considered unrelated to feeding, in that the incidences of these tumors were not significantly increased in the respective 50,000 ppm ceratonia siliqua gum groups.

Additionally, the incidence of pituitary adenomas in female mice fed 25,000 ppm ceratonia siliqua gum was not significantly different from the historical control rate of this tumor in control male and female mice. The increased incidence of interstitial tumors of the testis of male rats was not related to dietary administration of caesalpinia spinosa gum because of the high spontaneous rate of this tumor. Therefore, in each case in which a significant incidence ($P < 0.05$) of primary tumors was observed in F344 rats or $B6C3F_1$ mice, a definite association between increased tumor incidence and gum administration could not be made. Reduced incidences of certain other tumor types were also reported for rats and mice fed these gums in the diet. However, when compared to historical control rates, the differences reported were not statistically significant. No histopathologic effects were associated with administration of either of the gums. It was concluded that, under the conditions of these bioassays, cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, and caesalpinia spinosa gum were not carcinogenic in F344 rats or $B6C3F_1$ mice.[114]

Cassia Gum

Carcinogenicity data on cassia gum were not available for review by the Joint FAO/WHO Expert Committee on Food Additives during its evaluation of the safety of cassia gum as a food additive in 2009.[7] However, given the negative results for cassia gum in genotoxicity assays and negative results reported in carcinogenicity assays on ceratonia siliqua gum and caesalpinia spinosa gum, the Committee determined that a long-term toxicity and/or carcinogenicity study was not necessary for the safety evaluation of cassia gum.

Co-Carcinogenicity

The effect of cyamopsis tetragonoloba (guar) gum in the diet on 1,2-dimethylhydrazine (DMH) initiation of colon tumors was studied using groups of 30 male Sprague-Dawley rats (3 weeks old).[115] One group of rats was fed 5% cyamopsis tetragonoloba (guar) gum in the diet during the entire initiation period: 4 weeks of acclimatization, 12-week period of DMH injections, and a 2-week post-injection period. During the last 10 weeks (promotion period), the rats were given standard rat pellets. The control group was fed the basic diet. All animals were killed randomly during the 13th week after the last DMH injection and necropsies performed. None of the animals died spontaneously during the study. Rats fed the cyamopsis tetragonoloba (guar) gum + DMH had more tumors than those fed the basic diet; however, the difference was not statistically significant. It was concluded that cyamopsis tetragonoloba (guar) gum did not significantly influence carcinogenesis in rats initiated with DMH.

Antitumorigenicity

The effect of dietary supplementation with cyamopsis tetragonoloba (guar) gum on 1,2-dimethylhydrazine (DMH)-induced carcinogenesis was studied using groups of 30 male Sprague-Dawley rats (5 weeks old).[116] Each of 60 rats received a basal, fiber free diet (supplemented with 5% cellulose) and a weekly s.c. injection of DMH for 8 weeks (initiation phase), and the animals were then subdivided into 2 groups. One group of 30 rats was then maintained on a basal fiber-free diet supplemented with 10% cyamopsis tetragonoloba (guar) gum for 24 weeks (promotional stage). The other group of 30 (control) was maintained on a basal diet (fiber-free) for 24 weeks. The animals were killed 32 weeks after initiation of the study, and the tumor incidence, location, and frequency in the colon were determined. Compared to the control group, dietary fiber supplementation with 10% cyamopsis tetragonoloba (guar) gum resulted in a 27% ($P = 0.01$) decreased incidence of the total colon adenocarcinomas and a 43% ($P < 0.0005$) decreased incidence of adenocarcinomas in the descending colon.

Anticarcinogenicity

When groups of 6 mice were dosed i.p. with trigonella foenum-graecum seed extract (doses of 100 and 200 mg/kg/day) for 7 days prior to i.p. injection with Ehrlich ascites carcinoma cells (EAC), 86% and 94% inhibiton of tumor cell growth, respectively, was reported when compared to the saline-treated control group.[54] When additional groups of 6 mice received the same doses of trigonella foenum-graecum seed extract, after EAC cell injection, for 3 days, there was approximately 70% inhibition of tumor cell growth, compared to the saline-treated control group.

The effect of trigonella foenum-graecum seed extract on cancer cell growth was evaluated in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT) assay.[117] A 72-h incubation period was included in this assay. During a 72-h incubation period, test concentrations of 10 to 15 µg/ml had a growth inhibitory effect on breast, pancreatic, and prostate cancer cell lines (PCa). At higher test concentrations of 15 to 20 µg/ml, the growth inhibitory effect of trigonella foenum-graecum seed extract continued in PCa cell lines, but not to either primary prostate or hTert-immortalized prostate cells. Part of the growth inhibition observed in assays was due to induction of cell death, as observed in the incorporation of ethidium bromide III into cancer cells exposed to trigonella foenum-graecum seed extract.

In another study, the effect of trigonella foenum-graecum seed extract (aqueous extract) on 7,12-dimethylbenz(α)anthracene (DMBA)-induced breast cancer was evaluated using 4 groups of 8-week-old female Wistar rats (number per group not stated).[118] Oral dosing (gastric intubation) was initiated at 9 weeks of age. The 4 groups were defined as follows: Group A (fed trigonella foenum-graecum seed extract [200 mg/kg body weight] only), Group B (fed trigonella foenum-graecum seed extract + 20 mg DMBA), Group C (fed sesame oil), and Group D (fed DMBA [20 mg]). The extract + DMBA group was dosed orally with DMBA 7 days after daily oral dosing with trigonella foenum-graecum seed extract (in olive oil). Dosing was followed by a 120-day observation period. There was no gross evidence of acute toxicity after administration of the extract or DMBA. The first palpable tumor was observed 110 days after DMBA administration. Mammary tumors were not observed in the sesame oil control group or in the group treated with trigonella foenum-graecum seed extract only. However, mammary tumors were observed in the 2 DMBA-treated groups, and cumulative tumor incidences were 45 % (extract + DMBA) and 80% (DMBA only). The first tumor was initially observed at 5 weeks in these 2 groups, and tumor incidences increased in a time-dependent manner throughout the study. Mammary tumors in the group treated with DMBA only ranged from florid epithelial hyperplasia to fibroadenomas. Only mild-to-moderate hyperplasia was observed in the extract + DMBA group. Metastases at distant sites were not observed in any of the groups. It was concluded that, at a dose of 200 mg/kg body weight, trigonella foenum-graecum seed extract significantly inhibited the DMBA-induced mammary hyperplasia and decreased its incidence.

SUMMARY

The safety of the following ingredients in cosmetics is reviewed in this safety assessment: cyamopsis tetragonoloba (guar) gum, hydroxypropyl guar, C18-22 hydroxyalkyl hydroxypropyl guar, guar hydroxypropyltrimonium chloride, hydroxypropyl guar hydroxypropyltrimonium chloride, carboxymethyl hydroxypropyl guar, hydrolyzed guar, ceratonia siliqua gum, locust bean hydroxypropyltrimonium chloride, hydrolyzed ceratonia siliqua gum extract, caesalpinia spinosa gum, caesalpinia spinosa hydroxypropyltrimonium chloride, hydrolyzed caesalpinia spinosa gum, trigonella foenum-graecum seed extract, trigonella foenum-graecum hydroxypropyltrimonium chloride, hydrolyzed trigonella foenum-graecum seed extract, cassia gum, and cassia hydroxypropyltrimonium chloride. Because of the mannose/galactose chemical make-up of these legume polysaccharides, they are commonly called galactomannans.

Data reported to the Food and Drug Administration's Voluntary Cosmetic Registration Program in 2011 indicated use of the following 10 ingredients in cosmetics: cyamopsis tetragonoloba (guar) gum, hydroxypropyl guar, guar hydroxypropyltrimonium chloride, hydroxypropyl guar hydroxypropyltrimonium chloride, hydrolyzed guar, ceratonia siliqua gum, caesalpinia spinosa gum, hydrolyzed caesalpinia spinosa gum, trigonella foenum-graecum seed extract, and cassia hydroxypropyltrimonium chloride. Results from a survey of ingredient use concentrations conducted by the Personal Care Products Council in 2011 indicate that locust bean hydroxypropyltrimonium chloride and hydrolyzed trigonella foenum-graecum seed extract were also being used in cosmetics. Additionally, results from this survey indicate that galactomannans were being used at concentrations up to 93% (hydroxypropyl guar in a rinse-off product) in cosmetic products.

Production of natural gums consists of various techniques for the milling of seeds, followed by simple purification steps such as dissolving in hot water, filtering and precipitation with isopropanol. Typical production of the derivatized gums in this report involves the reaction of the natural gum with the appropriate epoxide. According to one supplier, trigonella foenum-graecum seed extract produced at that company is a supercritical CO_2 extract, and galactomannans are not extractable with supercritical CO_2.

Impurities that have been detected in cyamopsis tetragonoloba (guar) gum include heavy metal impurities and fluoroacetate. In 2007, the impurities dioxin and pentachlorophenol (PCP) were detected in certain batches of cyamopsis tetragonoloba (guar) gum originating from India. Anthraquinone impurities have been detected in cassia gum and tannins and trypsin inhibitors have been detected in ceratonia siliqua gum. A guar hydroxypropyltrimonium chloride trade name material contains ash as an impurity at concentrations up to 8.7% .

In rats fed cyamopsis tetragonoloba (guar) gum in a basal diet, the galactomannan was fermented quantitatively and approximately 1% of the mannose and 4% of the galactose was excreted in the feces. The feeding of rats with ceratonia siliqua gum in the diet resulted in 98% digestibility of galactomannan; all of the mannose added to the feed in the form of galactomannan was excreted in the feces. Similarly, 85 to 100% of the mannose fed to rats as ceratonia siliqua gum in the diet was excreted in the feces. Based on these study results, it is expected that cassia gum would be largely excreted unchanged and that fermentation by gut microflora may occur to some extent.

In acute oral toxicity studies, the feeding of rats with cyamopsis tetragonoloba (guar) gum did not cause death or test-substance related adverse effects. The following acute oral LD50 values (rats) have been reported for related ingredients: guar hydroxypropyltrimonium chloride (LD50 = 12.5 g/kg), hydroxypropyl guar hydroxypropyltrimonium chloride (LD50 = 12.0 g/kg), carboxymethyl guar (LD50 = 17.8 g/kg), hydrolyzed guar (LD50 > 6 g/kg), cassia gum (LD50 > 5 g/kg), and ceratonia siliqua gum (LD50 = 5 g/kg). LD50s for ceratonia siliqua gum in other species include: 13.1 g/kg (mice), 9.1 g/kg (rabbits), and 10.3 g/kg (hamsters). Acute oral LD50s of > 1 g/kg (mice) and > 10 g/kg (mice) for trigonella foenum-graecum seed extract and cassia gum, respectively, have also been reported.

Most of the repeated dose oral toxicity studies (rats, mostly dietary feeding) involving the following ingredients indicated no test-substance related adverse effects: cyamopsis tetragonoloba (guar) gum, hydrolyzed guar, ceratonia siliqua gum, caesalpinia spinosa gum, and cassia gum. However, reductions in liver and kidney weights in rats after feeding with cyamopsis tetragonoloba (guar) gum were of borderline significance, and 7 of 10 rats died (possibly due to intestinal blockage) after feeding with ceratonia siliqua gum. Increased male kidney-body weight ratios and increased relative weights of the thyroid and testes in rats were observed after feeding with caesalpinia spinosa gum; however, the results of gross and microscopic examination of these organs were not indicative of test substance-related changes. Furthermore, feeding with caesalpinia gum resulted in fewer mature spermatozoa in 4 of 10 male rats. Statistically significant reductions in kidney weight in rats fed cassia gum were not considered treatment-related, in that results of gross and microscopic examinations of the kidneys were negative. No pathological changes in vital organs were observed after repeated i.p. dosing with trigonella foenum-graecum seed extract.

Cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, and cassia gum were minimally irritating when instilled into the eyes of rabbits. In semi-occlusive patch tests, ceratonia siliqua gum was minimally irritating and cyamopsis tetragonoloba (guar) gum, caesalpinia spinosa gum, and cassia gum were non-irritating to the skin of rabbits. The allergenicity of cyamopsis tetragonoloba (guar) gum and ceratonia siliqua gum following inhalation exposure and ingestion has been demonstrated in various case reports. Also, no effects were apparent after ingestion of cyamopsis tetragonoloba (guar) gum in another case report. The allergenicity of trigonella foenum-graecum seed powder following inhalation exposure and ingestion has also been demonstrated in case studies. It was concluded that the prevalence of IgE sensitization to cyamopsis tetragonoloba (guar) gum was between 5% and 8.3% in an occupational study.

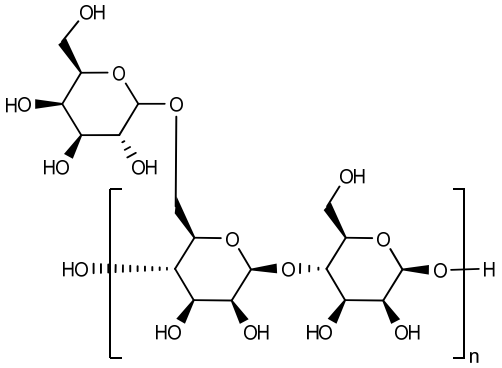
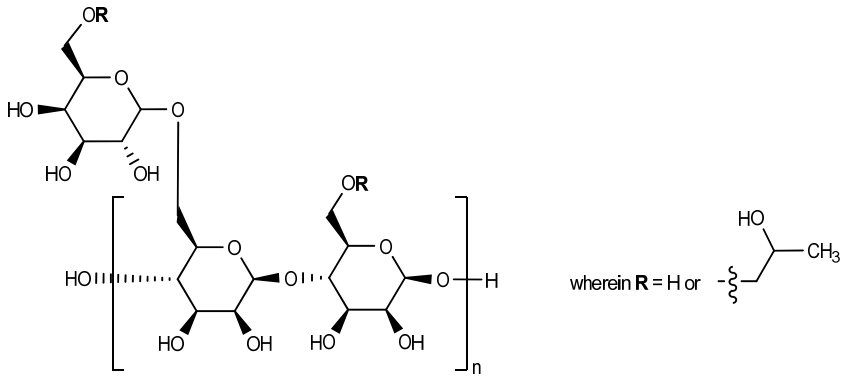
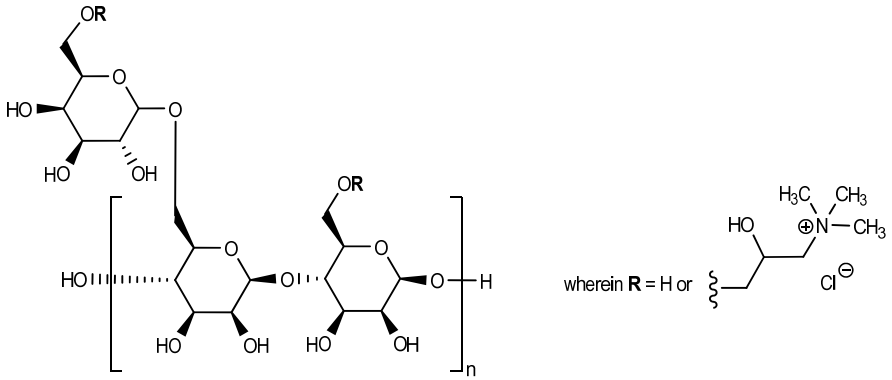
Cyamopsis tetragonoloba (guar) gum was not teratogenic in mice, rats, or hamsters when administered orally, and there was no evidence of maternal toxicity. Ceratonia siliqua gum also was not teratogenic when administered orally to rats, mice, hamsters, and rabbits. However, in one of the studies, significant maternal toxicity was induced by ceratonia siliqua gum in rabbits of the highest dose group. Caesalpinia spinosa gum was not a reproductive toxicant or teratogen when administered orally to rats, and also did not induce maternal toxicity. The oral dosing of mice with trigonella foenum-graecum seed extract caused a significant reduction in the live-birth index in the higher dose group and a significant reduction in litter size and pup body weights in both dose groups. External malformations also occurred in both dose groups.

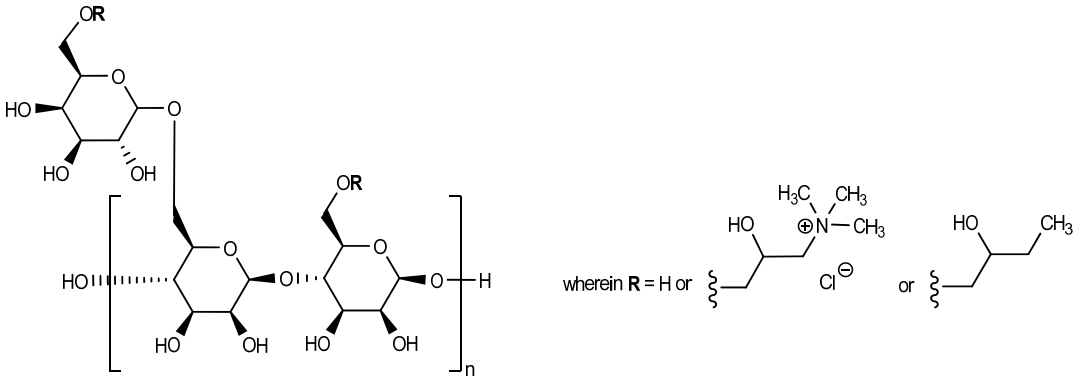
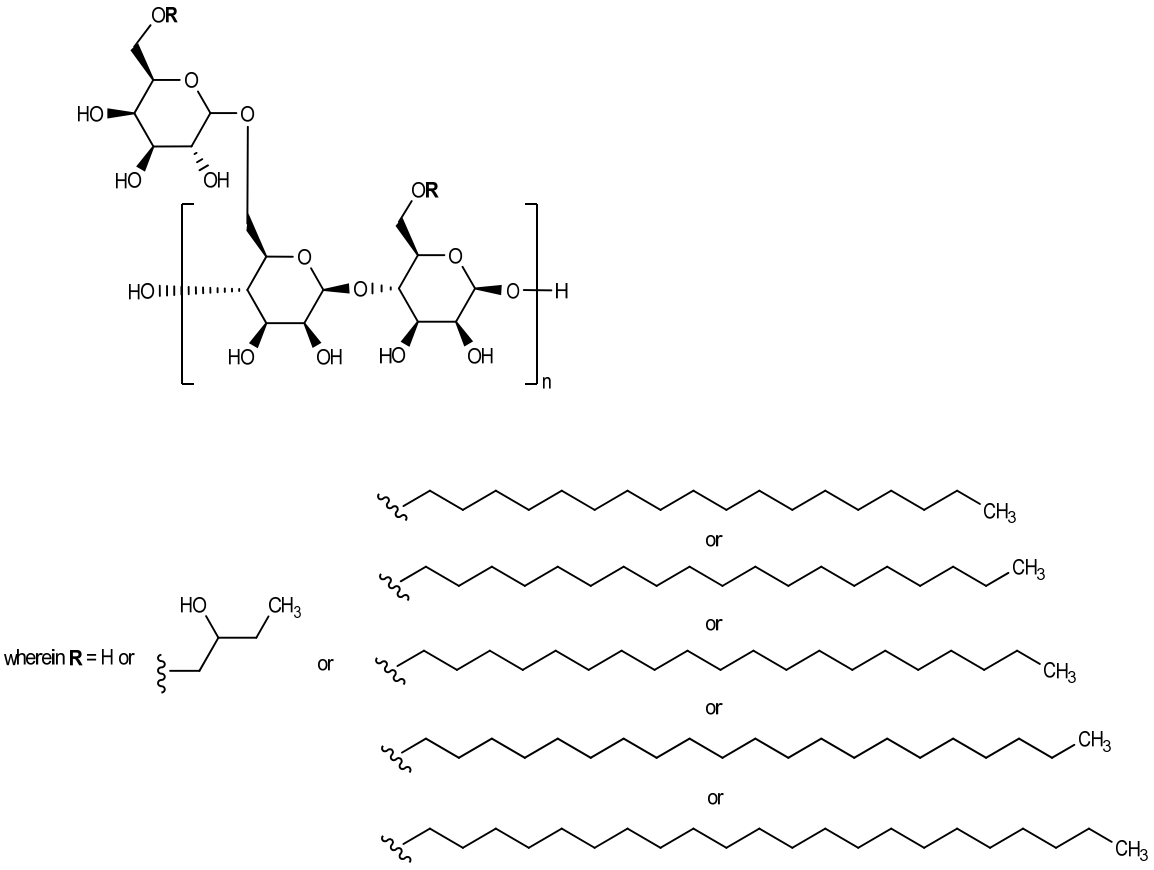
Cassia gum was not teratogenic in rats, rabbits, or cats when dose orally. However, a statistically significant reduction in maternal body weight gain in rats was observed in the highest dose group in one study. This finding was thought to have been related to the viscous nature of the gum and was not considered toxicologically relevant. In another study on cassia gum, non-significant reductions in feed consumption and fetal weight in rabbits were also thought to have been due to the viscous nature of the gum, and were not considered toxicologically relevant. The usefulness of the study involving cats is questionable, given the high mortality rate in the control group. In a dermal teratogenicity study involving rabbits, a statistically significant increase in early resorptions was observed in the highest dose group. However, analyses of fetal morphological observations were not indicative of test substance-related responses at any dose level. A dose-related increase in dermal irritation was noted in the 2 higher dose groups.

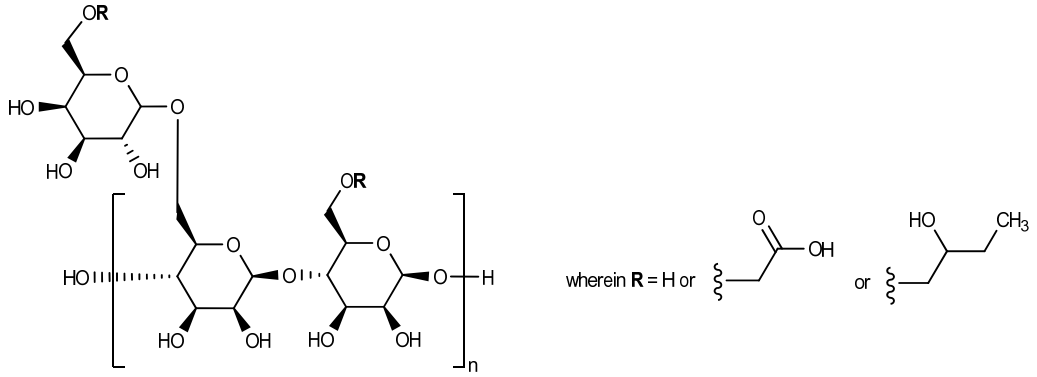
In bacterial and mammalian assays, cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, caesalpinia spinosa gum, trigonella foenum-graecum seed extract, and cassia gum were not found to be genotoxic. In the Ames test, hydroxypropyl guar was mutagenic in 1 of 5 bacterial strains without metabolic activation and in 4 of 5 strains with metabolic activation. Ames test results were negative for hydrolyzed guar and guar hydroxypropyltrimonium chloride (trade name material) with and without metabolic activation. Cyamopsis tetragonoloba (guar) gum, ceratonia siliqua gum, and caesalpinia spinosa gum were not carcinogenic in an oral carcinogenicity study involving F344 rats and B6C3F1 mice.

Rats fed cyamopsis tetragonoloba (guar) gum and 1,2-dimethylhydrazine had more tumors than those fed the basic diet, however, the difference was not statistically significant. The antitumorigenic/anticarcinogenic activity of cyamopsis tetragonoloba (guar) gum and trigonella foenum-graecum extract has been demonstrated *in vivo*.

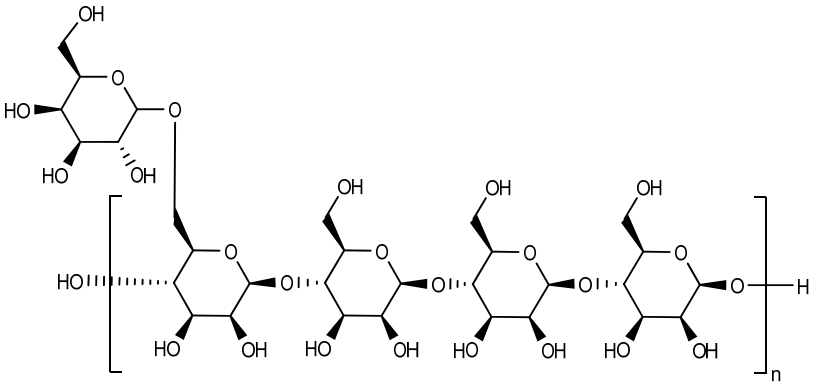
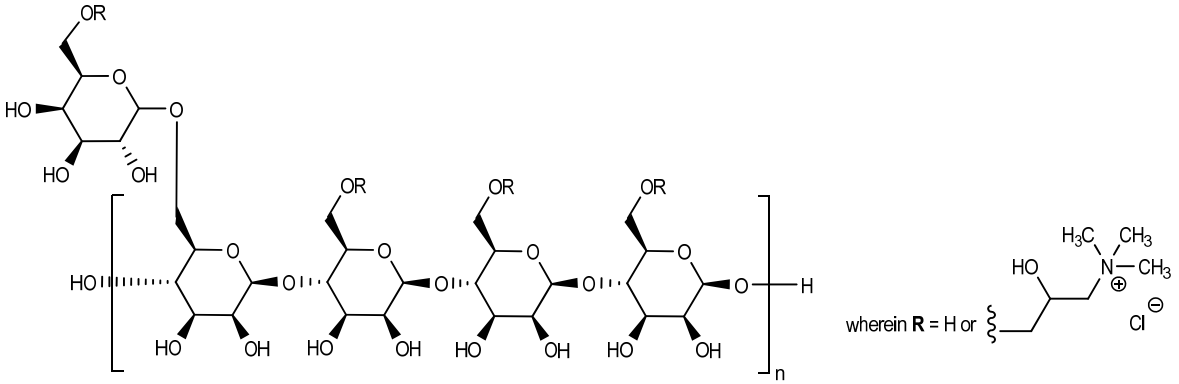
Table 1. Names, CAS Registry Numbers, Definitions and Idealized Structures of the Galactomannan Ingredients

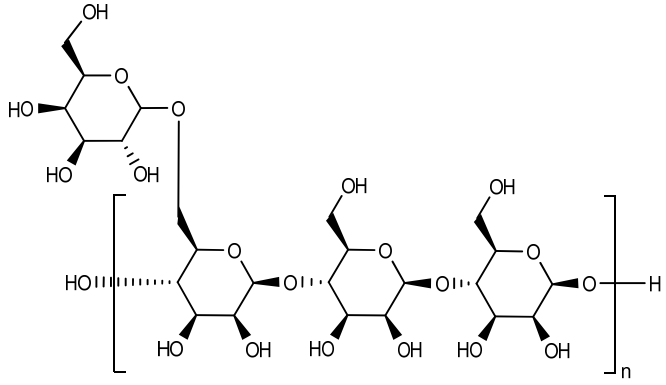
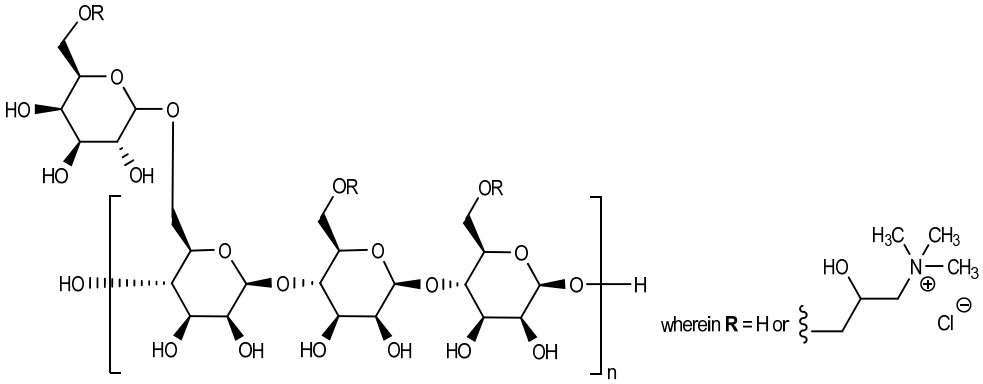
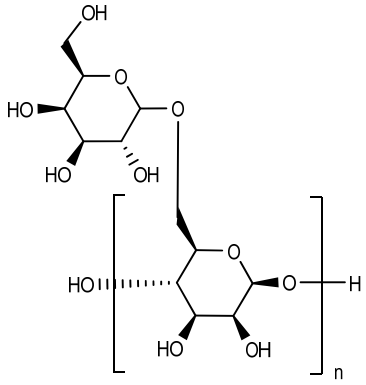
Ingredient CAS No.	Definition and Functions[1]	Formula/structure
Guar gums - mannose:galactose 2:1		
Cyamopsis Tetragonoloba (Guar) Gum 9000-30-0	Cyamopsis Tetragonoloba (Guar) Gum is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 2:1). Functions: binders; emulsion stabilizers; fragrance ingredients; and viscosity increasing agents - aqueous	
Hydroxypropyl Guar 39421-75-5	Hydroxypropyl Guar is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 2:1), derivatized via ether linkages with propylene glycol, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: binders; emulsion stabilizers; film formers; and viscosity increasing agents - aqueous	
Guar Hydroxypropyltrimonium Chloride 65497-29-2	Guar Hydroxypropyltrimonium Chloride is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 2:1), derivatized via ether linkages with trimethylammonium propylene glycol ether, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: antistatic agents; hair conditioning agents; skin-conditioning agents - miscellaneous; and viscosity increasing agents - aqueous	

Ingredient CAS No.	Definition and Functions[1]	Formula/structure
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride 71329-50-5	Hydroxypropyl Guar Hydroxypropyltrimonium Chloride is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 2:1), derivatized via ether linkages with a mixture of propylene glycol and trimethylammonium propylene glycol ether, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: antistatic agents and hair conditioning agents	
C18-22 Hydroxyalkyl Hydroxypropyl Guar	C18-22 Hydroxyalkyl Hydroxypropyl Guar is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 2:1), derivatized via ether linkages with a mixture of propylene glycol and an alkylene glycol that is 18-22 carbons in length, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: hair conditioning agents and skin-conditioning agents - miscellaneous	

Ingredient CAS No.	Definition and Functions[1]	Formula/structure
Carboxymethyl Hydroxypropyl Guar 68130-15-4	Carboxymethyl Hydroxypropyl Guar is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 2:1), derivatized via ether linkages with propylene glycol and carboxymethyl groups, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: binders; emulsion stabilizers; and viscosity increasing agents - aqueous	
Hydrolyzed Guar [70892-12-5]	Hydrolyzed Guar is the hydrolysate of Tetragonoloba (Guar) Gum derived by acid, enzyme or other method of hydrolysis. Function not reported.	Varied segments of Guar Gum

Locust bean gums (Carob gums) - mannose:galactose 4:1

Ceratonia Siliqua Gum 9000-40-2	Ceratonia Siliqua Gum is a polysaccharide, extracted from locust beans, comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 4:1). Functions: adhesives; binders; emulsion stabilizers; fragrance ingredients; and viscosity increasing agents - aqueous	
Locust Bean Hydroxypropyltrimonium Chloride	Locust Bean Hydroxypropyltrimonium Chloride is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 4:1), derivatized, via ether linkages with trimethylammonium propylene glycol ether, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: antistatic agents; hair conditioning agents; and skin-conditioning agents - miscellaneous	

Ingredient CAS No.	Definition and Functions[1]	Formula/structure
Hydrolyzed Ceratonia Siliqua Gum Extract	Hydrolyzed Ceratonia Siliqua Gum Extract is the hydrolysate of an extract of Ceratonia Siliqua Gum (q.v.) derived by acid, enzyme or other method of hydrolysis. Functions: Hair conditioning agents	Varied segments of Locust Bean Gum
Tara gums - mannose:galactose 3:1		
Caesalpinia Spinosa Gum 39300-88-4	Caesalpinia Spinosa Gum is a polysaccharide, obtained from *Caesalpinia spinosa*, comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 3:1). Functions: adhesives; skin-conditioning agents - miscellaneous; and viscosity increasing agents - aqueous	
Caesalpinia Spinosa Hydroxypropyltrimonium Chloride [742071-24-5]	Caesalpinia Spinosa Hydroxypropyltrimonium Chloride is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 3:1), derivatized, via ether linkages with trimethylammonium propylene glycol ether, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: antistatic agents; hair conditioning agents; and skin-conditioning agents - miscellaneous	
Hydrolyzed Caesalpinia Spinosa Gum	Hydrolyzed Caesalpinia Spinosa Gum is the hydrolysate of Caesalpinia Spinosa Gum derived by acid, enzyme or other method of hydrolysis. Functions: absorbents; hair conditioning agents; skin-conditioning agents - emollient	Varied segments of Tara gum.
Fenugreek gums - mannose:galactose 1:1		
Trigonella Foenum-Graecum Seed Extract [73613-05-5]	Trigonella Foenum-Graecum Seed Extract is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 1:1). Functions: fragrance ingredients and skin-conditioning agents - miscellaneous	

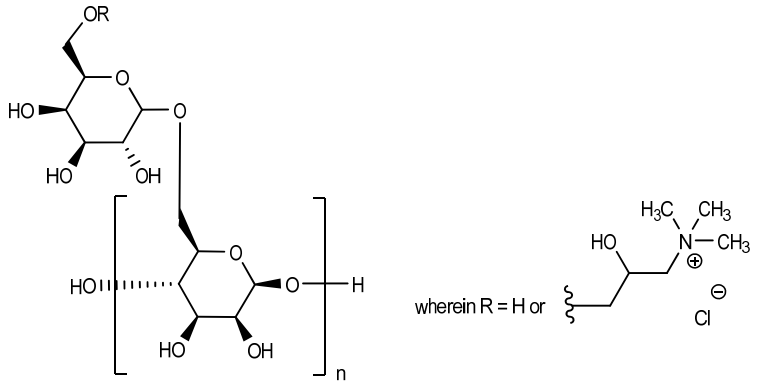
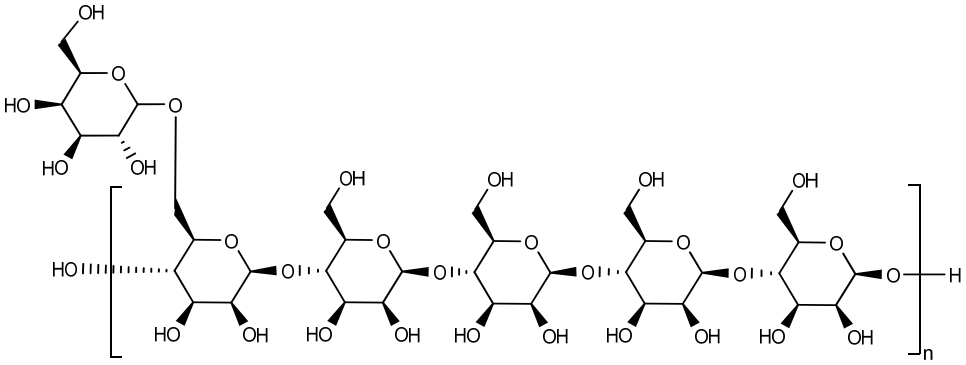
Ingredient CAS No.	Definition and Functions[1]	Formula/structure
Trigonella Foenum-Graecum Hydroxypropyltrimonium Chloride [742071-25-6]	Trigonella Foenum-Graecum Hydroxypropyltrimonium Chloride is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 1:1), derivatized, via ether linkages with trimethylammonium propylene glycol ether, at some of the free hydroxyl groups of the polysaccharide backbone. Functions: antistatic agents; hair conditioning agents; and skin-conditioning agents - miscellaneous	
Hydrolyzed Trigonella Foenum-Graecum Seed Extract	Hydrolyzed Trigonella Foenum-Graecum Seed Extract is the hydrolysate of Trigonella Foenum-Graecum Seed Extract derived by acid, enzyme or other method of hydrolysis. Functions: film formers; humectants; and skin-conditioning agents - miscellaneous	Varied segments of Fenugreek gum.
Cassia Gums - mannose:galactose 5:1		
Cassia Gum	Cassia Gum is a polysaccharide comprised of a polymannose backbone with mono-galactose pendant groups (whereby the mannose:galactose ratio is 5:1). Functions: binders; skin-conditioning agents - miscellaneous; and viscosity increasing agents - aqueous	 OH HO O HO OH HO O HO O O O

Table 2. Specifications and Purity of Gums for Use in Foods/Drugs[2,3,6]

Constituents	Cassia Gum	Ceratonia Siliqua Gum*	Cyamopsis Tetragonoloba (Guar) Gum	Caesalpinia Spinosa Gum
Galactomannan (%)	≥ 75	≥ 75	≥ 70	≥ 82%
Acid-insoluble residue (%)	≤ 2	≤ 4	≤ 7	≤ 2.2
Moisture (%)	≤ 12	NR	NR	NR
Ash (%)	≤ 2	≤ 1.2	≤ 1.5	≤ 1.5
Protein (%)	≤ 7	≤ 7	≤ 10	≤ 3
Fat (%)	≤ 2	NR	NR	≤ 1
Chrysophanic acid (ppm)§	10 (maximum)	NR	NR	NR
Heavy Metals				
Heavy metals	NR	NR	0.002%**	≤ 20 mg/kg
Lead (mg/kg)	< 10	≤ 5	≤ 2	≤ 10
Copper (mg/kg)	< 10	NR	NR	NR
Arsenic (mg/kg)	< 2	≤ 3	3**	≤ 3
Zinc (mg/kg)	< 10	NR	NR	NR
Mercury (mg/kg)	< 1	NR	NR	NR
Impurities				
Mycotoxins (mg/kg)	< 0.001	NR	NR	NR
Pesticides	< 0.001	NR	NR	NR
Total germ count (g)	≤ 5,000	NR	NR	NR
Coliforms (*E. coli*)	Negative	NR	NR	NR
Yeasts (per g)	≤ 100	NR	NR	NR
Moulds (per g)	≤ 100	NR	NR	NR

§A measure for the naturally occurring anthraquinone derivatives in the product
*Specifications for foods and drugs; **Specifications for drugs; NR = Not Reported

Table 3. Properties of Guar Hydroxypropyltrimonium Chloride Trade Name Materials

Properties/Components	Values
Form	White to yellow fine powder[4]
Odor	Characteristic[4]
pH	6.0 to 7.5 (1% solution);[4] 9.0 to 10.5 (1% aqueous, 25°C)[119]
Solubility	Very slightly soluble in water; practically insoluble in ethyl ether, chloroform, petrol ether, ethyl aocohol, and paraffin oil[4]
Active matter	Min. 93%;[4] ≥ 90%[119]
Water	Max. 7%;[4] ≤ 10%[119]
Ash	5.6 to 8.7% (600°C)[4]
Chloride	Max. 6%[4]

Table 4. Properties of Trigonella Foenum-Graecum Seed Extract Trade Name Material[17]

Properties/Components	Values
Form	Clear yellowish liquid
Odor	Faint, herbal
pH	4.5 to 5.5
Refractive Index (20°C)	1.420 to 1.440
Density (20°C)	1.025 to 1.045
Viscosity	~ 60 mPa . s
Solubility	Soluble in water
Trigonella Foenum-Graecum Seed Extract	5 to 10%
Propylene Glycol	75 to 100%
Bactiphen 2506 G preservative	0.60%

Table 5. Current Frequency of Use According to Duration and Type of Exposure Provided in 2011[20]

	Cyamopsis Tetragonoloba (Guar) Gum		Hydroxypropyl Guar		Guar Hydroxypropyl-trimonium Chloride		Hydroxypropyl Guar Hydroxypropyltrimonium - Chloride	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type								
Eye Area	1	NR	NR	NR	1	0.005 to 1	1	NR
Incidental Ingestion	1	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	NR	1	1	NR	4	0.05	NR	NR
Incidental Inhalation-powders	NR	NR	1	NR	NR	NR	NR	NR
Dermal Contact	32	0.02 to 1	73	0.05 to 0.8	203	0.02 to 1	80	0.2 to 0.6
Deodorant (underarm)	NR	1	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	21	0.04 to 0.5	92	0.1 to 93	573	0.07 to 2	46	0.04 to 0.9
Hair-Coloring	14	2 to 5	2	0.1 to 2	38	0.1 to 0.3	1	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	12	0.1 to 1	21	NR	179	0.2 to 0.9	75	0.3 to 0.6
Baby Products	1	0.09	1	NR	2	0.3	NR	NR
Duration of Use								
Leave-On	31	0.02 to 1	111	0.05 to 2	107	0.005 to 2	8	0.04 to 0.9
Rinse off	36	0.09 to 5	56	0.1 to 93	709	0.02 to 2	113	0.2 to 0.8
Diluted for (bath) use	1	NR	NR	NR	2	0.2 to 0.9	6	0.3
Totals/Conc. Range	68	0.02 to 5	167	0.05 to 93	818	0.005 to 2	127	0.04 to 0.9

	Hydrolyzed Guar		Ceratonia Siliqua Gum		Locust Bean Hydroxypropyltrimonium Chloride		Caesalpinia Spinosa Gum	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type								
Eye Area	NR	0.5	4	0.002 to 0.05	NR	NR	2	0.002
Incidental Ingestion	NR	0.2	5	0.07	NR	NR	NR	0.02
Incidental Inhalation-sprays	NR	NR	NR	0.002 to 0.003	NR	NR	NR	NR
Incidental Inhalation-powders	NR	NR	1	NR	NR	NR	NR	NR
Dermal Contact	NR	0.1 to 0.5	41	0.0003 to 0.03	NR	NR	17	0.002 to 0.5
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	1	0.03 to 3	1	NR	NR	0.4	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	0.2	5	0.0003 to 0.07	NR	NR	1	0.02
Baby Products	NR	NR	0	NR	NR	NR	NR	NR
Duration of Use								
Leave-On	1	0.03 to 3	41	0.0003 to 0.05	NR	NR	14	0.002 to 0.5
Rinse-Off	NR	0.2 to 2	7	0.0003 to 0.07	NR	0.4	3	0.03
Diluted for (bath) use	NR	NR	0	NR	NR	NR	NR	NR
Totals/Conc. Range	1	0.03 to 3	48	0.0003 to 0.07	NR	0.4	17	0.002 to 0.5

Table 5. Current Frequency of Use According to Duration and Type of Exposure Provided in 2011[20]

	Hydrolyzed Caesalpinia Spinosa Gum		Trigonella Foenum-Graecum Seed Extract		Hydrolyzed Trigonella Foenum-Graecum Seed Extract		Cassia Hydroxypropyl-trimonium Chloride	
	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)	# of Uses	Conc. (%)
Exposure Type								
Eye Area	1	NR	1	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	0	NR	NR	NR	NR	NR
Incidental Inhalation-sprays	NR	NR	4	0.003 to 0.004	NR	NR	NR	NR
Incidental Inhalation-powders	NR	NR	0	NR	NR	NR	NR	NR
Dermal Contact	11	0.002 to 0.4	22	0.0001 to 0.004	NR	0.003	NR	NR
Deodorant (underarm)	NR	NR	0	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	15	0.03 to 0.3	NR	NR	5	0.06 to 0.4
Hair-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	1	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
Duration of Use								
Leave-On	8	0.002 to 0.4	31	0.00001 to 0.3	NR	0.003	NR	NR
Rinse off	3	0.008	7	0.03 to 0.08	NR	NR	5	0.06 to 0.4
Diluted for (bath) use	NR	NR	NR	NR	NR	NR	NR	NR
Totals/Conc. Range	11	0.0012 to 0.4	38	0.00001 to 0.3	NR	0.003	5	0.06 to 0.4

NR = Not Reported; NS = Not Surveyed; Totals = Rinse-off + Leave-on Product Uses.
Note: Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure type uses may not equal the sum total uses.

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Data

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 28, 2011

SUBJECT: Information on Guar Hydroxypropyltrimonium Chloride

Cognis Corporation. 2011. Data profile: Cosmedia® Guar C261 (Guar Hydroxypropyltrimonium Chloride).

Cognis GmbH. 2011. Data profile: Dehyquart® Guar N (Guar Hydroxypropyltrimonium Chloride).

Henkel KgaA. 1992. Acute oral toxicity in rats Cosmedia Guar C 261 (Guar Hydroxypropyltrimonium Chloride).

Henkel KgaA. 1988. Prüfung auf mutagenität im Ames-test Cosmedia Gura C261 (Guar Hydroxporpyltrimonium Chloride) (includes English summary).

COGNIS ECOHYBRIDS

carechemicals

Data Profile

COSMEDIA® GUAR C 261

Producer / Supplier

Cognis Corporation
Care Chemicals
300 Brookside Avenue
Ambler / PA 19002
Tel. No.: 800-531-0815
FAX No.: 215-628-1353

General characterisation

Chemical description
Quaternised guar flour

Mol weight
approx. 220,000 g / mol

Raw material basis
vegetable (Guar flour)
synthetic (3-chloro-2 hydroxypropyl trimethyl ammonium chloride)

Labeling information

INCI name(s)
Guar Hydroxypropyltrimonium Chloride (EU)
Guar Hydroxypropyltrimonium Chloride (CTFA)

Composition hints for finished product label
Guar Hydroxypropyltrimonium Chloride

Registrations

Ingredient	CASR-No.	EINECS/ELINCS-No.
	65497-29-2	polymer

Officially listed in / Quality conforms to

JCIC: O-{2-Hydroxy-3-(trimethylammonio)propyl} Guar Gum Chloride
(Ingredient Code 523039)

Manufacturing procedure

conversion of Guar with 3 - chloro-2 hydroxypropyl trimethyl ammonium chloride

Product properties

Appearance

COSMEDIA® GUAR C 261 is a white to yellow, fine powder with a characteristic intrinsic odour.

Example of use

Because of its cationic character COSMEDIA® GUAR C 261 is substantive to hair and has a favourable effect on wet and dry combability. Its compatibility with anionic surfactants makes this cationic guar derivative a universally usable conditioning agent in shampoos and hair aftertreatment preparations. COSMEDIA® GUAR C 261 forms slightly cloudy swellings in water. It is therefore preferently used in emulsion-type or pearlescent preparations.

Solubility according to Ph. EUR.

.Ethylether	practically insoluble
Chloroform	practically insoluble
Petrolether	practically insoluble
Ethylalcohol	practically insoluble
Paraffin oil	practically insoluble
Water	very slightly soluble

Solubility

Characteristic values

The specifications stated in the paragraphs 'Quality control data' and 'Additional product descriptive data' finally and conclusively describe the properties of the Product.

Quality control data

(Data which is used for quality release and is certified for each batch.)

Appearance	conforms to standard	
Odour	conforms to standard	
Active matter	min. 93 %	Difference (100%-H2O)
Water	max. 7 %	ISO 760
pH-value 1 %	6.0 - 7.5	DGF H-III 1
Ash (600℃)	5.6 - 8.7 %	DGF C-III 10
Chloride	max. 6.0 %	DGF H-III 9 (92)

Additional product descriptive data

(Data which is proven statistically but not determined regularly.)

Methods of identification

by means of the characteristic values described under section 'Quality Control Data'

Stabilising additives / Auxiliaries
(type and concentration)

Preservatives
not present

Antioxidants
not present

Solvents
not present

Others
not present

Storage and transportation
In original sealed containers and at room temperatures below 30℃ COSMEDIA® GUAR C 261 remains stable for at least one year. Under proper storage conditions germ formations are excluded. COSMEDIA® GUAR C 261 meets the following microbiological criteria:
Aerobic organisms < 1000 colony forming units/g
Fungi/Yeasts < 100 colony forming untits/g
COSMEDIA® GUAR C 261does not contain preservatives.

Miscellaneous

All products in the text marked with an ® are trademarks of the Cognis group.
The information on product specifications provided herein is only binding to the extent confirmed by Cognis in a written Sales Agreement. COGNIS EXPRESSLY DISCLAIMS ANY RESPONSIBILITY FOR THE SUITABILITY OF THE PRODUCTS FOR ANY SPECIFIC OR PARTICULAR PURPOSES INTENDED BY THE USER. Suggestions for the use and application of the products and guide formulations are given for information purposes only and without commitment. Such suggestions do not release Cognis' customers from testing the products as to their suitability for the customer's intended processes and purposes. Cognis does not assume any liability or risk involved in the use of its products as the conditions of use are beyond its control. The user of the products is solely responsible for compliance with all laws and regulations applying to the use of the products, including intellectual property rights of third parties.

Sept. 2011

COGNIS ECOHYBRIDS

carechemicals

Data Profile

DEHYQUART® GUAR N

Address

Supplier
Cognis GmbH
Care Chemicals
D - 40551 Düsseldorf
Tel.: ++49-211-7940-0
Fax.: ++49-211-798-5457

General characterisation

Chemical description
Quaternized Guar Flour

Raw material basis

Vegetable:	(Guar flour)
Synthetic:	(3-chloro-2 hydroxypropyl trimethyl ammonium chloride)

Labeling information

INCI name(s)
Guar Hydroxypropyltrimonium Chloride (EU 2006/257/EC)
Guar Hydroxypropyltrimonium Chloride (CTFA)

Ingredient information

CASR-No.	EINECS/ELINCS/EC/EU NLP-No.
65497-29-2	613-809-4

Product properties

Appearance
DEHYQUART® GUAR N is a white to yellow fine powder with a characteristic intrinsic odour.

Example of use
Because of its cationic character DEHYQUART® GUAR N is substantive to hair and has a favorable effect on wet and dry combability. Its compatibility with anionic surfactants makes this cationic guar derivative a universally usable conditioning agent in shampoos and hair aftertreatment agents. DEHYQUART® GUAR N is cold processable and forms slightly cloudy swellings in water. It is preferentially used with a small amount of citric acid to start the swelling process in water. It is therefore preferently used in emulsion-type or pearlescent preparations.

Characteristic values

The specifications stated in the paragraphs 'Quality control data' and 'Additional product descriptive data' finally and conclusively describe the properties of the product.

Quality control data
(Data which is used for quality release and is certified for each batch.)

Odor evaluation versus standard	corresponds to the standard	
Visual appearance versus standard	corresponds to the standard	
Water	<= 10 %	ISO 4317
Active matter	>= 90 %	Difference 100 % - % Water
pH value 25 °C (1 % in Water)	9,0 - 10,5	ISO 4316

Typical values

(For information purposes only, not part of the guaranteed specifications.)

Nitrogen (Kjeldahl-nitrogen)	1,5 %	DIN EN 25633 (DIN 38409 H11)

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Storage and transportation

Shelf life
12 months

Storage temperature
Keep container tightly sealed.
Store in a dry place.
< + 30 °C

Storage conditions
In original sealed containers and protected from moisture.

Additional information
Under proper storage conditions germ formations are excluded. DEHYQUART® GUAR N meets the following microbiological criteria:
Aerobic organisms <= 1000 colony forming units/g
Moulds/Yeasts <= 100 colony forming units/g

DEHYQUART® GUAR N does not contain preservatives.

Disclaimer

All products in the text marked with an ® are trademarks of the Cognis group. The information on product specifications provided herein is only binding to the extent confirmed by Cognis in a written Sales Agreement. COGNIS EXPRESSLY DISCLAIMS ANY RESPONSIBILITY FOR THE SUITABILITY OF THE PRODUCTS FOR ANY SPECIFIC OR PARTICULAR PURPOSES INTENDED BY THE USER. Suggestions for the use and application of the products and guide formulations are given for information purposes only and without commitment. Such suggestions do not release Cognis´ customers from testing the products as to their suitability for the customer´s intended processes and purposes. Cognis does not assume any liability or risk involved in the use of its products as the conditions of use are beyond its control. The user of the products is solely responsible for compliance with all laws and regulations applying to the use of the products, including intellectual property rights of third parties.

Henkel KGaA

TFB - Toxicology

Cosmedia Guar C 261
Guar Hydroxypropyltrimonium Chloride
Acute Oral Toxicity in Rats

Final Report

March 26, 1992

Henkel KGaA
Postbox 1100
D-4000 Düsseldorf 1
Germany

Dr. W. Pittermann
Expert for Vet. Pathology

RT 920030

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C O N T E N T S

		Page
1.	**General Information**	3
2.	**Statement of Compliance**	4
3.	**Summary**	5
4.	**Objective**	6
5.	**Materials and Methods**	7
	5.1 Test substance	7
	5.2 Test system	7
	5.3 Husbandry	8
	5.4 Treatment	8
	5.5 Observations	8
	5.6 Pathology	8
6.	**Results**	9 - 10
	6.1 Clinical observations	9
	6.2 Mortality	9
	6.3 Mean body weight	9
	6.4 Necropsy findings	10
	6.5 Oral LD_{50}-value	10
7.	**Classification**	10
	Statement of the quality assurance unit	

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1. General Information

Title	**Cosmedia Guar C 261** Acute Oral Toxicity in Rats
Study No.	PT 910118
Sponsor	Henkel KGaA, Postbox 1100 D-4000 Düsseldorf 1 Germany
Test Facility	TFB - Toxicology Henkel KGaA
Archives	TFB - Toxicology Henkel KGaA
File-No.	RT 920030
Dates	Day of application: January 14, 1992 Day of necropsy : January 28, 1992

Personnel involved in the study

Study Director	Dr. W. Pittermann Expert for Vet. Pathology
Laboratory Technicians	Mrs. Kusch, Mrs. Sturm, Mrs. Südkamp, MTA

Dr. W. Pittermann

Date 26.03.1992

Dr. W. Sterzel
TFB - Toxicology

Date 27.03.1992

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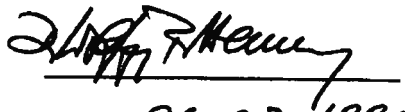
Statement of Compliance

Title	**Cosmedia Guar C 261** Acute Oral Toxicity in Rats
Study No.	PT 910118
Study Director	Dr. med. vet. W. Pittermann

To the best of my knowledge and belief, the study described in this report was conducted in compliance with the regulations of "Good Laboratory Practice".

Study Director

Henkel KGaA
Dr. W. Pittermann
Expert of Vet. Path.



Date 26.03.1992

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3. Summary

The acute oral toxicity of **Cosmedia Guar C 261** was tested in young Wistar rats. The test article was dissolved in arachidis oil, DAB 9, and given once orally by mean of gavage in one dose of 2000 mg/kg body weight to 5 male and 5 female animals.

At frequent intervals at the day of application and twice a day in the following 14 days (working days),the rats were observed for any signs of reaction.

The surviving rats were sacrificed at the end of the observation period and a macroscopic postmortem examination was performed on all rats.

The LD_{50} of **Cosmedia Guar C 261** was

>2000 mg/kg

for male and female rats.

Classification

Applying the EC criteria for classification and labelling requirements for dangerous substances (91/325/EC) **Cosmedia Guar C 261 has neither to be classified as acute toxic nor as acute harmful.**

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4. Objective

The purpose of this acute oral toxicity study was to assess the toxicological profile of **Cosmedia Guar C 261** when administered to rats by single oral gavage, followed by an observation period of either 14 days or for the duration of toxic symptoms, whichever occurs later.

This study should provide a rational basis for risk assessment in man.

The study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

- **Standard Operating Procedures (SOP) T2E001-01**
- **OECD Guideline for Testing of Chemicals, section 4, No. 401 "Acute oral Toxicity", adopted February 24, 1987**
- **Off. J. Europ. Commun. L 251, September 19, 1984; 84/449/EC**
- **Off. J. Europ. Commun. L 180, July 08, 1991; 91/325/EC**
- **OECD Principles of Good Laboratory Practice (GLP), according to the Chemical Act (F.R.G) March 14, 1990**

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5. Materials and Methods

5.1 Test substance

Name	**Cosmedia Guar C 261**
Chemical denomination	Guar gum, 2-hydroxy-3-(trimethylammonio)-propylether, chloride
Batch No.	Charge J 1084 A, produced July 1991
Delivery	December 10, 1991
Aggregate state at 20°C	powder
Colour	slight yellow
Active substance content	90-93%
Analysis data	not given
Stability	
Pure	stable for at least one year
In Solvent	stable in arachidis oil-preferred
Storage	at room temperature
Expiration date	July 1992

5.2 Test system

Test animal	rat, Bor: WISW (SPF) Cpb
Rationale	the recommended test system
Breeder	Winkelmann GmbH, Borchen, Germany
Number of animals	5 male / 5 female
Age at start of treatment	approximately 10 weeks
Body weight at start of treatment	mean male 177 g/female 155 g
Identification	by unique cage number and corresponding color-coded spots on the individual animal.
Acclimatization	6 days under laboratory conditions

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5.3 Husbandry

Standard Laboratory Conditions

Air-conditioned with 10 - 15 air changes per hour, and continously monitored environment with temperature approx. 19 - 22 degrees centigrade, relative humidity approx. 48 - 65%, 12 hours artificial fluorescent light/12 hours dark.

Accommodation

Groups of 5 animals in Makrolon Type-3 cages with standard softwood bedding, ARWI-Center, Essen, Germany.

Diet

Pelleted Altromin Maintenance Diet 1314, Lot No. 151091/1504 (Fa. Altromin GmbH, 4937 Lage, Germany) ad libitum.

Water

Community Tap Water from Düsseldorf ad libitum.

5.4 Treatment

The rats were deprived of food approximately 16 hours before they were dosed once by gavage. 3 hours after the test article application the rats were fed again.
All test animals received the dose level of 2000 mg/kg body weight and the dose volume 10 ml/kg body weight. Arachidis oil, DAB 9, served as vehicle.

5.5 Observations

Mortality/Toxicity symptoms	At frequent intervals during test day one and twice daily during days 2-14 (working days)
Body weight	Animals were weighed the day before the application (-1 day), on the day of application , on day 2, 7 and 14 after application

5.6 Pathology

Necropsy	Macroscopic examination of all external orificies and the organs in thoracic and visceral cavity.

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6. Results

6.1 Clinical observations

Dose: 2000 mg/kg	
male animals	
no symptoms observed	(5)
female animals	
no symptoms observed	(5)

( ) number of animals

6.2 Mortality

Dose: 2000 mg/kg		
number of male animals		(5)
number of female animals		(5)
dead animals after	1 day	(0)
	2 days	(0)
	7 days	(0)
	14 days	(0)

( ) number of animals

6.3 Mean body weight (g)

	male	female
-1 d	187	165
day of appl.	177	155
2 d	194	166
7 d	223	171
14 d	255	184
body weight gain	68	19

-1 d = day before application (appl.)
d = days after application

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6.4 Necropsy findings

Male animals : No macroscopic findings considered to be treatment related

Female animals : No macroscopic findings considered to be treatment related.

6.5 Oral LD_{50}-value

The orientating lethal dose established in the described limit test procedure is

$$LD_{50} > 2000 \text{ mg/kg}$$

7. Classification

Applying the EC criteria for classification and labelling requirements for dangerous substances (91/325/EC) **Cosmedia Guar C 261 has neither to be classified as acute toxic nor as acute harmful.**

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Henkel KGaA
Quality Assurance Unit
D-4000 Düsseldorf

STATEMENT

File No.: RT920030

Author(s): Dr. Pittermann

Report: Cosmedia Guar C 261

Acute oral Toxicity in Rats

Test facilities and conduct of studies were subject to regular inspections by Quality Assurance Unit.

This final report was reviewed.

Dates of Inspections / Audits	Submitted to Testing Facility Management and Study Director
Jan. 08, 1992 Jan. 14, 1992 May 07, 1992	Jan. 08, 1992 Jan. 14, 1992 May 07, 1992

Quality Assurance Unit

Einig

May 07, 1992

Date

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HENKEL

Institut für Toxikologie

Cosmedia Guar C 261
Guar Hydroxypropyltrimonium Chloride

Prüfung auf Mutagenität im Ames-Test

Abschlußbericht

März 1988

Dr. Banduhn

Henkel KGaA, Postfach 1100
D-4000 Düsseldorf

Prüfbericht: 880203

Inhaltsverzeichnis

1.0	Zusammenfassung	1
2.0	Summary	2
3.0	Prüfsubstanz, Datum und Archivierung	3
4.0	Zweck der Untersuchung	4
5.0	Einleitung	5
6.0	Material und Methoden	6
6.1	Bakterielle Testerstämme	6
6.2	Vorbereitung der Testerstämme	6
6.3	Medien	6
6.4	Säugerenzymsystem	7
6.4.1	Darstellung der Rattenleber S-9 Fraktion	7
6.4.2	Darstellung des S-9 Mixes	7
6.5	Positive Kontrollchemikalien und Negativkontrollen	8
6.6	Lösungsmittel	8
6.7	Experimenteller Aufbau	8
6.8	Interpretationskriterien	8
6.8.1	Reproduzierbarkeit	9
		10
7.0	Ergebnisse und Tabellen	11
8.0	Literatur	32

Anhang A : Erklärung der Qualitätssicherungseinheit A.1

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1.0 Zusammenfassung

Das untersuchte Muster von Cosmedia Guar C 261 induzierte k e i n e Rückmutationen in den bakteriellen Testerstämmen TA 1535, TA 100, TA 1537, TA 1538 und TA 98 nach Ames in Abwesenheit und in Gegenwart von Aroclor 1254 aktivierten Rattenleberenzymen (S-9 Mix).

Die untersuchte Prüfsubstanz Cosmedia Guar C 261 zeigte k e i n e Mutagenität in vitro.

Düsseldorf, den 29.03.1988

.....

Dr. rer. nat. N. Banduhn
Prüfleiter

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2.0 Summary

The sample of Cosmedia Guar C 261 was tested for mutagenic activity in the bacterial tester strains Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 1538 and TA 98. The test was conducted on agar plates in the absence or presence of postmitrochondrial supernatant fluids from the liver of male rats treated with Aroclor 1254 (S-9 mix).

Suspensions of the test compound were freshly made up in Tween 80 / water just before use. The following concentrations were tested:

1st and 2nd test : 1.6, 8, 40, 200 and 1000 ug per plate.

Cosmedia Guar C 261 did n o t induce reverse mutations in the presence and absence of S-9 mix in the tester strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98.

Cosmedia Guar C 261 did n o t show mutagenic activity in vitro.

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3.0 Prüfsubstanz, Datum und Archivierung

Die untersuchte Substanz

Cosmedia Guar C 261

wurde am 19.11.1987 dem Institut für Toxikologie der Henkel KGaA, Düsseldorf, übersandt. Es handelt sich bei dieser Substanz um ein hellgelbes, feines Pulver.

Die erste und zweite Versuchsreihe zu diesem Ames-Test wurde unter der Prüfnummer 880106 ausgeführt. Die erste Versuchsreihe wurde mit den Testerstämmen TA 1535, TA 100, TA 1537, TA 1538 und TA 98 in der Zeit vom 15.03. - 17.03.1988 (Tabellen 1 - 10) ausgeführt. Die zweite Versuchsreihe wurde mit den fünf Testerstämmen in der Zeit vom 22.03. - 24.03.1988 ausgeführt (Tabellen 11 - 20). Die erste und zweite Versuchsreihe wurde im Dosisbereich von 1,6 bis 1000 ug Testsubstanz pro Platte ausgeführt.

Das Original dieses Abschlußberichtes mit der Archivnummer 880203 und das Original des Prüfplans 880106 mit den Rohdaten werden im Archiv des Instituts für Toxikologie der Henkel KGaA, Düsseldorf, aufbewahrt. Das Rückstellmuster der untersuchten Substanzprobe von Cosmedia Guar C 261 ist im Rückhaltemusterraum des Instituts untergebracht.

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4.0 Zweck der Untersuchung

Der Zweck dieser Untersuchung war die Prüfung des mutagenen Potentials des Produktes

Cosmedia Guar C 261

im bakteriellen Testsystem nach Ames et al. (1) ohne und mit Enzymen der Säugerleber. Die experimentellen Arbeiten wurden unter Berücksichtigung der OECD Richtlinie Nummer 471 vom 26.05.1983 (2) und unter Beachtung der OECD-Grundsätze für Gute Laborpraxis (GLP), Bundesanzeiger Nr. 42 vom 02.03.1983, ausgeführt.

In diesem Bericht werden die benutzten Methoden und die erzielten Ergebnisse beschrieben.

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5.0 Einleitung

Der sogenannte Ames-Test basiert auf den von B.N. Ames und seinen Mitarbeitern ausgearbeiteten Techniken (1). Speziell entwickelte Bakterienstämme werden mit der Testsubstanz in Kontakt gebracht. Diese Salmonella typhimurium Testerstämme können die zu ihrem Wachstum notwendige Aminosäure Histidin nicht mehr synthetisieren. Mutagene Verbindungen können in diesen Testerstämmen Rückmutationen induzieren, die zu den ursprünglichen, Histidin unabhängigen Formen führen. Diese rückmutierten Bakterien sind durch ihr Wachstum auf einem Histidin-Mangelmedium zu erkennen.

Viele Verbindungen zeigen ihre mutagenen Aktivitäten erst nach der Metabolisierung durch Enzymsysteme, die in der bakteriellen Zelle nicht vorhanden sind. Deshalb wurden die Testsubstanz und die Bakterien auch in Gegenwart von 9000 x g Überstand (S-9 Mix) aus homogenisierten Lebern männlicher Ratten inkubiert. Die Versuchstiere wurden vorher mit einer Verbindung, Aroclor 1254, behandelt, von der eine Stimulierung der Enzymaktivität in der Leber ausgelöst wurde.

Zur Ausführung dieses Tests mit Cosmedia Guar C 261 wurde die OECD Richtlinie Nummer 471 (2) berücksichtigt.

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6.0 Material und Methoden

Die Vorbereitung der Materialien und die gesamten Experimente wurden unter sterilen Versuchsbedingungen ausgeführt.

6.1 Bakterielle Testerstämme

Salmonella typhimurium

Genotyp der verwandten TA-Stämme

Histidin-Mutation				Zusätzliche Mutation	
his G46	his C 3076	his D3052	LPS	Repair	R-factor
TA 1535	TA 1537	TA 1538	rfa	uvrB	-
TA 100		TA 98	rfa	uvrB	+

Alle TA-Stämme wurden von S. typhimurium LT-2 aus entwickelt. Alle Testerstämme sind Histidin-abhängig aufgrund verschiedener Mutationen im Histidin-Operon. Zusätzlich enthalten die Stämme zwei Mutationen, die ihre Sensitivität gegenüber einigen Mutagenen stark steigert:

1. Eine fehlerhafte Lipopolysaccharid-(LPS)-Hülle resultierte aus der deep rough (rfa) Mutation. Die Polysaccharidkette des LPS ist verkürzt, so daß die Bakterienzellwand für Substanzen durchlässiger und die Bakterien selbst für den Menschen vollständig nichtpathogen wurden.

2. Ein Verlust in der UV-Licht empfindlichen Region des Gens (uvrB) führt zum Verschwinden des excision repair-Systems. Diese Mutation schließt außerdem die Nitrat-Reduktase und Biotin Gene ein.

Mit den Stämmen TA 1535 und TA 100 können Mutationen vom Typ einer Basenpaar-Substitution, mit den Stämmen TA 1537, TA 1538 und TA 98 Leseraster-Mutationen erkannt werden. Die genetischen Merkmale der Stämme (his, rfa, uvrB und pkM 101) wurden regelmäßig nach der Empfehlung von Ames et al. (1) überprüft und nur einwandfreie Stammkulturen wurden zur Prüfung von Cosmedia Guar C 261 verwandt.

Die Testerstämme wurden als tiefgefrorene Kulturen in Bouillon im Tiefkühlschrank (-80° C) gelagert.

6.2 Vorbereitung der Testerstämme

Eine kleine Portion (0.5 ml) der aufgetauten Kulturen der Testerstämme wurde in 20 ml einer Standard-I-Nährbouillonlösung überführt, sorgfältig gemischt und anschließend 10 Stunden bei 37° C unter Schütteln bebrütet. Die Bakteriensuspensionen hatten einen Zelltiter von ca. 10^9 lebensfähigen Zellen. Frische Übernacht-Suspensionen wurden für den Versuch hergestellt. Die Zellsuspensionen wurden am Versuchstag bei 4° C (Kühlschrank) aufbewahrt.

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6.3 Medien

Nutrient broth: Standard-I-Nährbouillon für die Mikrobiologie (Merck Art. 7882). 25 g wurden in 1 Liter frisch destilliertem Wasser aufgelöst und im Autoklaven sterilisiert (20 min bei 121° C).

Top agar: 9 g Bacto Agar (Difco) und 5 g NaCl (Merck Art. 6404) wurden in 1000 ml Wasser aufgelöst und 20 Minuten lang bei 121° C autoklaviert. Nach der Sterilisation wurde das Medium am Versuchstag bei 40° C in einem Wasserbad gelagert.

Petrischalen: Die Petrischalen (Durchmesser 9 cm) enthielten 30 ml eines minimalen Glucose-Mediums (1,5 % Bacto Agar im Vogel- Bonner-Medium mit 2 % Glucose). Die Platten wurden von der Firma Merck als Fertigprodukte bezogen und bei 4° C bis zum Gebrauch gelagert. 1 - 2 Tage vor dem Gebrauch wurden die Platten ausgepackt, auf Kontaminationen geprüft und beschriftet.

6.4 Säugerenzymsystem

6.4.1 Darstellung der Rattenleber S-9 Fraktion

Für die Versuche wurde der 9000 x g Überstand (S-9) als Fertigprodukt von Organon (Oberursel) bezogen. Das Material wurde aus Lebern männlicher, 8 - 10 Wochen alter Sprague Dawley Ratten (Charles River Breeders) hergestellt. Die S-9 Fraktion wurde nach dem von Ames et al. (1) beschriebenen Verfahren zubereitet.

Die Tiere, die mit Aroclor 1254 induziert wurden, bekamen 5 Tage vor dem Abtöten eine intraperitoneale (i.p.)-Injektion von in Maisöl suspendiertem Aroclor 1254 (500mg/kg KG). 12 Stunden vor der Leberentnahme wurden die Tiere nicht mehr gefüttert.

Die S-9 Fraktionen wurden als KCl-Homogenisat hergestellt und mit Chargennummern beschriftet. In der ersten Testserie wurde die Charge 09434 mit dem Herstellungsdatum 12.11.1986 eingesetzt.

Folgende biochemischen Informationen wurden vom Hersteller über diese Charge mitgeliefert:

Protein: 24,6 mg/ml nach der Lowry Methode (3).

Mikrosomale Enzymaktivität bei 37° C mit dem Substrat Ethoxyresorufin:

5048 pmol/min/mg Protein.

In der zweiten Testserie wurde die Charge 09434 mit dem Herstellungsdatum 12.11.1986 eingesezt. Folgende biochemischen Informationen wurden über diese Charge vom Hersteller mitgeliefert:

Protein: 24,6 mg/ml nach der Lowry-Methode (3)

Mikrosomale Enzymaktivität bei 37° C mit dem Substrat Ethoxyresorufin:

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5048 pmol/min/mg Protein.

Die S-9 Fraktionen wurden im gefrorenen Zustand auf Trockeneis transportiert und bei -80° C gelagert.

6.4.2 Darstellung des S-9 Mixes

Der S-9 Mix enthielt pro ml: S-9 (0,1 ml), MgCl 2 (8 uMol), KCl (33 uMol), Glucose-6-phosphat (5 uMol, Sigma), NADP (4 uMol, Sigma), und Natriumphosphatpuffer, pH 7,4 (100 uMol) (1). Der S-9 Mix wurde am Versuchstag frisch zubereitet und im Kühlschrank (4° C) bis zum endgültigen Verbrauch gelagert.

6.5 Positive Kontrollchemikalien und Negativkontrollen

Als Positiv-Kontrolle wurde Natriumazid für die Testerstämme TA 1535 und TA 100, 9-Aminoacridin für den Testerstamm TA 1537, sowie 4-Nitro-o-phenylendiamin für die Testerstämme TA 1538 und TA 98 ohne S-9 Mix benutzt.

Die Enzymaktivität der mit Aroclor 1254 induzierten S-9-Fraktionen wurde durch die Prüfung von 2-Aminoanthracen mit allen Stämmen kontrolliert.

Als Negativ-Kontrolle diente das Lösungsmittel Tween 80 / bidest. Wasser und die unbehandelten, frischen Zellsuspensionen.

Die Testansätze mit S-9 Mix (Aroclor 1254) wurden durch Zusätze von jeweils 0,5 ml S-9 Mix Fraktion kontrolliert.

6.6 Lösungsmittel

Cosmedia Guar C 261 wurde unmittelbar vor Versuchsbeginn in beiden Testserien in Tween 80/ bidest. Wasser gelöst und mit bidest. Wasser auf die gewünschten Konzentrationen verdünnt.

6.7 Experimenteller Aufbau

In sterile Reagenzgläser, die 2 ml weichen Top Agar enthielten, wurden nacheinander 0,1 ml der jeweiligen Testerbakteriensuspension und 0,1 ml der entsprechenden Testsubstanzlösung gegeben. Zum weichen Top Agar wurden vor dem Verteilen auf die Reagenzgläser 10 ml einer sterilen Histidin (0,5 mM)- Biotin (0,5 mM)-Lösung pro 100 ml Top Agar hinzugefügt und sorgfältig vermischt.

In den beiden Testreihen ohne Säugerenzymzusatz (ohne S-9 Mix) wurden jeweils 5 Konzentrationen der Testsubstanz Cosmedia Guar C 261 den entsprechenden Reagenzgläsern hinzugefügt. Anstelle des S-9 Mixes wurden 0,5 ml Puffer zu den Testansätzen gegeben.

In den beiden Testreihen mit Säugerenzymzusatz (mit S-9 Mix) wurden ebenfalls jeweils 5 Konzentrationen der Testsubstanz Cosmedia Guar C 261 den entsprechenden Reagenzgläsern mit den Testerbakterien hinzugefügt. Unmittelbar vor dem Ausgießen der Ansätze wurden je 0,5 ml S-9 Mix pro Testansatz hinzugegeben, sorgfältig gemischt und der noch weiche Inhalt innerhalb von 30 Sekunden auf die Minimal-Agar-Platten verteilt.

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Nach Erhärtung dieser Schicht wurden die Platten umgekehrt bei 37° C 48 Stunden lang inkubiert. Die Kolonien wurden mit einem Biotran-III-Counter (Biotronik) auf jeder Platte gezählt. Die Ergebnisse werden in diesem Bericht als Revertanten pro Platte für jeden Testerstamm wiedergegeben. Die Positivkontrollen und die Lösungsmittelkontrollen werden ebenfalls als Vergleichswerte mitgeteilt. Die angegebenen Zahlen für den Prüfstoff Cosmedia Guar C 261 sind die Mittelwerte aus drei Parallelansätzen.

6.8 Interpretationskriterien

Eine Kombination der folgenden Kriterien wurde als positives Ergebnis gewertet:

- Der Plattenhintergrund aus nichtrevertierten Bakterien zeigte im Vergleich zu den entsprechenden Negativ-Kontrollen keine Wachstumsreduktion.
- Die Spontanmutationsraten der Testerstämme lagen pro Platte innerhalb des charakteristischen Spontanmutationsbereiches.

Charakteristischer Spontanmutationsbereich für die Testansätze ohne S-9 Mix

Testerstämme	TA 1535	TA 100	TA 1537	TA 1538	TA 98
Mittelwert	6	87	7	15	20
Extremwerte	1-25	35-201	1-24	6-27	5-39
Ames et al. (1)	20	160	7	25	40
Extremwerte	5-50	60-220	3-25	5-40	15-75

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Charakteristischer Spontanmutationsbereich für die Testansätze mit S-9 Mix

Testerstämme	TA 1535	TA 100	TA 1537	TA 1538	TA 98
Mittelwert	8	105	6	18	27
Extremwerte	1-25	54-252	1-23	3-48	7-76

- Die Positivkontrollen zeigten im Regelfall Mutationsraten, die die Kontrollwerte des TA 100 wenigstens um den Faktor 2,0 und die der anderen Testerstämme wenigstens um den Faktor 3,0 überschritten (5).

- Die Testsubstanz verursachte wenigstens einen 2,0-fachen Anstieg gegenüber den Negativkontrollen im Testerstamm TA 100 bei mehr als einer getesteten Dosierung. Für die anderen Testerstämme wurde ein Anstieg in der Mutationsrate von mehr als 3,0 über den entsprechenden Negativkontrollen als positiv gewertet (5).

6.8.1 Reproduzierbarkeit

Falls die Testsubstanz nur in einem Test Rückmutationen induziert und dieser Effekt in einer oder mehreren Wiederholungen nicht reproduziert werden kann, dann verlieren die anfänglich positiven Testdaten ihre Signifikanz.

Die hier wiedergegebenen Interpretationskriterien gelten nicht absolut. Auch andere Gesichtspunkte, die z. Z. noch zu diesem in vitro Testsystem entwickelt werden, können Eingang in die zusammenfassende Bewertung finden (5).

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7.0 Ergebnisse und Tabellen

Die Prüfsubstanz Cosmedia Guar C 261 wurde im Konzentrationsbereich

1,6 - 1000 ug/Platte (1. und 2. Test)

in zwei unabhängigen Versuchsreihen geprüft.

Cosmedia Guar C 261 wurde in Abwesenheit und Gegenwart von Aroclor 1254 induziertem S-9 Mix geprüft. In beiden Versuchsreihen (Tabellen 1 - 20) wurden die Platten 48 Stunden lang bei 37° C inkubiert.

Die in den Tabellen 1 - 5 und 11 - 15 wiedergegebenen Befunde zeigen, daß die Prüfsubstanz Cosmedia Guar C 261 in den Testerstämmen TA 1535, TA 100, TA 1537, TA 1538 und TA 98 in Abwesenheit von Rattenleberenzymen (ohne S-9 Mix) k e i n e Rückmutationen induzierte.

Ebenso traten k e i n e induzierten Rückmutationen in den Testerstämmen TA 1535, TA 100, TA 1537, TA 1538 und TA 98 in Gegenwart von Aroclor 1254 induziertem S-9 Mix auf (Tabellen 6 - 10, 16 - 20).

Die Prüfsubstanz Cosmedia Guar C 261 war i n a k t i v im in vitro Mutationstest nach Ames.

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Tabelle 1

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 1535	O h n e S - 9 M i x	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	8.0 ±2.7	9 5 7 13 7 7
Positiv-Kontrollen		
Natriumazid 2 ug/Platte	308.6 ±17.2 F = 38.5	328 303 295
2-Aminoanthracen 2.5 ug/Platte	8.3 ±1.1	9 9 7
Testsubstanz		
ug/Platte		
1000	6.3 ±1.1	7 5 7
200	7.0 ±1.7	8 8 5
40	5.6 ±2.0	4 8 5
8	8.0 ±3.6	4 11 9
1.6	8.6 ±3.0	6 8 12
suspendiert in Tween 80/ H2O		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 2

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 100	O h n e S - 9 M i x		
Revertantenzahl/Platte	Mittelwerte	Einzelwerte	
Negativ-Kontrollen	67.5 ±5.6	59 70 76 65 66 69	
Positiv-Kontrollen			
Natriumazid 2.0 ug/Platte	209.0 ±29.1 F = 3.0	184 241 202	
2-Aminoanthracen 5.0 ug/Platte	63.0 ±14.5	62 49 78	
Testsubstanz			
ug/Platte			
1000	85.0 ±10.5	73 89 93	
200	66.3 ±10.1	61 60 78	
40	69.5 ±20.5	84 K 55	
8	75.6 ±8.7	66 83 78	
1.6	69.6 ±9.2	75 59 75	
suspendiert in Tween 80/ H2O			

F : Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle
K : Fremdkeim

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Tabelle 3

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 1537	Ohne S-9 Mix				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	5.3	±1.8	8 3	4 7	5 5
Positiv-Kontrollen					
9-Aminoacridin 80 ug/Platte	161.0 F = 30.1	±32.9	199	143	141
2-Aminoanthracen 2.5 ug/Platte	6.0	±1.0	6	7	5
Testsubstanz					
ug/Platte					
1000	3.3	±1.5	5	3	2
200	2.5	±0.70	3	2	K
40	3.0	±1.0	3	4	2
8	2.6	±0.57	3	3	2
1.6	3.0	±1.0	3	2	4
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle
K: Fremdkeim

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Tabelle 4

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 1538	O h n e S - 9 M i x	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	8.0 ±2.9	7 5 5 8 11 12
Positiv-Kontrollen		
4-Nitro-o-phenylendiamin 40 ug/Platte	810.3 ±51.7 F =101.2	834 846 751
2-Aminoanthracen 5 ug/Platte	8.0 ±3.0	8 5 11
Testsubstanz		
ug/Platte		
1000	8.0 ±1.0	7 9 8
200	7.6 ±1.1	7 7 9
40	7.0 ±1.0	7 8 6
8	7.0 ±1.7	6 9 6
1.6	7.0 ±5.1	13 4 4
suspendiert in Tween 80/ H2O		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 5

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 98	Ohne S-9 Mix	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	12.5 ±3.5	9 14 19 11 11 11
Positiv-Kontrollen		
4-Nitro-o-phenylendiamin 40 ug/Platte	330.3 ±37.7 F = 26.4	348 287 356
2-Aminoanthracen 5 ug/Platte	11.6 ±2.5	14 12 9
Testsubstanz		
ug/Platte		
1000	14.6 ±2.8	13 13 18
200	13.3 ±4.0	17 14 9
40	14.6 ±5.5	20 15 9
8	10.0 ±4.3	8 7 15
1.6	15.0 ±3.0	12 18 15
suspendiert in Tween 80/ H2O		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 6

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 1535	Mit S-9 Mix				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	10.0	±2.4	12 11	6 11	8 12
Positiv-Kontrollen					
Natriumazid 2 ug/Platte	51.3 F =	±5.0 5.1	52	56	46
2-Aminoanthracen 2.5 ug/Platte	89.3 F =	±13.4 8.9	95	99	74
Testsubstanz					
ug/Platte					
1000	14.0	±4.0	14	10	18
200	12.0	±2.6	14	9	13
40	10.0	±1.0	11	10	9
8	8.0	±1.0	7	9	8
1.6	8.6	±0.57	9	9	8
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 7

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 100	M i t S - 9 M i x	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	77.0 ±6.5	79 81 87 70 74 71
Positiv-Kontrollen		
Natriumazid 2 ug/Platte	129.6 ±4.7 F = 1.6	128 126 135
2-Aminoanthracen 5 ug/Platte	761.3 ±50.0 F = 9.8	747 720 817
Testsubstanz		
ug/Platte		
1000	92.6 ±7.2	101 88 89
200	78.3 ±10.6	66 84 85
40	81.3 ±9.7	92 79 73
8	75.3 ±7.0	68 82 76
1.6	70.3 ±3.5	67 70 74
suspendiert in Tween 80/ H2O		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 8

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 1537	Mit S-9 Mix				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	5.5	±2.1	8 6	4 8	3 4
Positiv-Kontrollen					
9-Aminoacridin 80 ug/Platte	74.0 F = 13.4	±7.0	77	79	66
2-Aminoanthracen 2.5 ug/Platte	43.6 F = 7.9	±5.8	46	48	37
Testsubstanz					
ug/Platte					
1000	5.0	±2.0	7	5	3
200	5.0	±1.7	3	6	6
40	5.6	±3.5	2	6	9
8	6.6	±3.5	10	7	3
1.6	6.3	±1.5	8	5	6
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 9

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 1538	Mit S-9 Mix	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	11.8 ±3.0	11 10 14 9 10 17
Positiv-Kontrollen		
4-Nitro-o-phenylendiamin 40 ug/Platte	900.6 ±67.1 F = 76.1	953 924 825
2-Aminoanthracen 5 ug/Platte	446.0 ±82.1 F = 37.6	420 538 380
Testsubstanz		
ug/Platte		
1000	12.6 ±4.0	15 15 8
200	15.6 ±3.5	19 16 12
40	11.6 ±2.5	9 12 14
8	11.0 ±4.3	6 13 14
1.6	12.3 ±9.5	3 12 22
suspendiert in Tween 80/ H2O		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrol e

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Tabelle 10

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

1. Versuchsreihe: 15.03. - 17.03.1988

Testerstamm TA 98	M i t S - 9 M i x	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	18.3 ±4.7	15 22 17 12 19 25
Positiv-Kontrollen		
4-Nitro-o-phenylendiamin 40 ug/Platte	560.6 ±34.5 F = 30.5	561 595 526
2-Aminoanthracen 5 ug/Platte	351.3 ±64.0 F = 19.1	286 354 414
Testsubstanz		
ug/Platte		
1000	19.3 ±5.8	15 17 26
200	23.3 ±8.6	25 31 14
40	18.3 ±8.5	15 12 28
8	21.6 ±6.3	18 18 29
1.6	14.6 ±4.0	19 11 14
suspendiert in Tween 80/ H2O		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 11

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 1535	O h n e S - 9 M i x		
Revertantenzahl/Platte	Mittelwerte	Einzelwerte	
Negativ-Kontrollen	8.6 ±3.3	5 10 10 12 4 11	
Positiv-Kontrollen			
Natriumazid 2 ug/Platte	197.0 ±16.0 F = 22.7	210 202 179	
2-Aminoanthracen 2.5 ug/Platte	4.3 ±1.5	4 3 6	
Testsubstanz			
ug/Platte			
1000	6.3 ±4.1	5 3 11	
200	6.3 ±1.5	8 5 6	
40	6.6 ±1.1	6 8 6	
8	7.0 ±1.7	8 5 8	
1.6	7.3 ±1.1	8 8 6	
suspendiert in Tween 80/ H2O			

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 12

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 100	O h n e S - 9 M i x				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	76.5	±8.4	65 85 78 83 81 67		
Positiv-Kontrollen					
Natriumazid 2.0 ug/Platte	183.0 F = 2.3	±15.6	173	201	175
2-Aminoanthracen 5.0 ug/Platte	54.6	±14.9	59	38	67
Testsubstanz					
ug/Platte					
1000	70.5	±14.8	60	K	81
200	56.3	±9.0	48	66	55
40	57.3	±2.5	55	60	57
8	73.6	±11.9	64	70	87
1.6	58.6	±2.3	56	60	60
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle
K: Fremdkeim

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Tabelle 13

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 1537	O h n e S - 9 M i x				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	4.5	±2.5	2 5	9 5	2 4
Positiv-Kontrollen					
9-Aminoacridin 80 ug/Platte	84.6 F = 18.8	±9.2	77	95	82
2-Aminoanthracen 2.5 ug/Platte	4.6	±1.5	6	5	3
Testsubstanz					
ug/Platte					
1000	5.5	±0.70	6	5	K
200	5.6	±2.3	3	7	7
40	3.6	±1.5	4	2	5
8	3.3	±2.3	2	2	6
1.6	3.0	±1.0	3	2	4
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle
K: Fremdkeim

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Tabelle 14

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 1538	O h n e S - 9 M i x				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	7.8	±3.0	7 11	7 4	12 6
Positiv-Kontrollen					
4-Nitro-o-phenylendiamin 40 ug/Platte	503.6 F = 64.2	±73.3	546	419	546
2-Aminoanthracen 5 ug/Platte	8.3	±0.57	8	9	8
Testsubstanz					
ug/Platte					
1000	9.6	±2.3	11	7	11
200	5.6	±0.57	6	5	6
40	6.0	±4.0	2	6	10
8	6.6	±1.5	5	8	7
1.6	7.6	±4.6	13	5	5
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 15

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 98	O h n e S - 9 M i x				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	14.1	±4.2	12	18	15
			11	20	9
Positiv-Kontrollen					
4-Nitro-o-phenylendiamin 40 ug/Platte	348.0	±28.0	360	368	316
	F = 24.5				
2-Aminoanthracen 5 ug/Platte	14.0	±2.6	11	16	15
Testsubstanz					
ug/Platte					
1000	10.6	±2.0	10	13	9
200	13.0	±1.0	12	14	13
40	13.0	±2.6	11	12	16
8	13.0	±2.6	16	11	12
1.6	10.3	±4.1	7	15	9
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 16

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 1535	M i t S - 9 M i x		
Revertantenzahl/Platte	Mittelwerte	Einzelwerte	
Negativ-Kontrollen	12.3 ±3.2	18 13 9 10 11 13	
Positiv-Kontrollen			
Natriumazid 2 ug/Platte	45.6 ±3.0 F = 3.7	43 45 49	
2-Aminoanthracen 2.5 ug/Platte	64.0 ±17.0 F = 5.1	83 50 59	
Testsubstanz			
ug/Platte			
1000	9.0 ±1.7	8 11 8	
200	11.3 ±3.0	8 14 12	
40	10.6 ±4.0	10 15 7	
8	10.0 ±4.5	14 5 11	
1.6	8.3 ±4.0	6 6 13	
suspendiert in Tween 80/ H2O			

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 17

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 100	Mit S-9 Mix				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	57.3	±10.5	41	70	61
			59	49	64
Positiv-Kontrollen					
Natriumazid 2 ug/Platte	78.0	±10.3	72	72	90
	F =	1.3			
2-Aminoanthracen 5 ug/Platte	532.3	±62.5	514	481	602
	F =	9.2			
Testsubstanz					
ug/Platte					
1000	66.0	±9.8	69	74	55
200	80.6	±14.5	79	96	67
40	82.6	±3.0	80	86	82
8	75.3	±11.8	89	68	69
1.6	84.6	±5.1	89	79	86
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 18

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 1537	Mit S-9 Mix				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	7.3	±2.8	7 8	5 4	12 8
Positiv-Kontrollen					
9-Aminoacridin 80 ug/Platte	65.0 F =	±17.3 8.8	61	84	50
2-Aminoanthracen 2.5 ug/Platte	40.6 F =	±4.0 5.5	43	36	43
Testsubstanz					
ug/Platte					
1000	4.6	±1.5	3	5	6
200	7.6	±1.5	8	9	6
40	6.0	±2.6	4	5	9
8	5.6	±2.5	3	6	8
1.6	5.3	±0.57	5	5	6
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 19

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 1538	Mit S-9 Mix	
Revertantenzahl/Platte	Mittelwerte	Einzelwerte
Negativ-Kontrollen	12.1 ±3.0	13 14 12 7 11 16
Positiv-Kontrollen		
4-Nitro-o-phenylendiamin 40 ug/Platte	891.0 ±62.1 F = 73.2	959 837 877
2-Aminoanthracen 5 ug/Platte	352.6 ±71.6 F = 28.9	347 284 427
Testsubstanz		
ug/Platte		
1000	7.3 ±2.5	7 5 10
200	6.6 ±2.3	8 4 8
40	15.6 ±2.0	15 14 18
8	9.6 ±2.0	12 9 8
1.6	13.3 ±5.7	10 10 20
suspendiert in Tween 80/ H20		

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

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Tabelle 20

Ames-Test mit der Testsubstanz Cosmedia Guar C 261

2. Versuchsreihe: 22.03. - 24.03.1988

Testerstamm TA 98	M i t S - 9 M i x				
Revertantenzahl/Platte	Mittelwerte		Einzelwerte		
Negativ-Kontrollen	15.0	±3.3	17 15 11 11 19 17		
Positiv-Kontrollen					
4-Nitro-o-phenylendiamin 40 ug/Platte	677.6 F = 45.1	±65.6	746	615	672
2-Aminoanthracen 5 ug/Platte	373.3 F = 24.8	±38.0	336	372	412
Testsubstanz					
ug/Platte					
1000	14.0	±6.0	18	17	7
200	22.0	±4.5	27	18	21
40	14.0	±3.6	11	13	18
8	15.6	±0.57	16	16	15
1.6	17.3	±5.1	16	23	13
suspendiert in Tween 80/ H2O					

F: Mutationssteigerungsfaktor gegenüber der Negativ-Kontrolle

8.0 Literatur

(1) Ames, B.N., J. McCann, and E. Yamasaki
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test.
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"Genetic Toxicology: Salmonella typhymurium,
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(3) Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall
J. Biol. chem. 193 (1951) 265 - 275
Protein measurement with the Folin phenol reagent.

(4) Nebert, D.W., and H.V. Gelboin
J. Biol. chem. 243 (1968) 6242 - 6249
Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme.

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Mutation Res. 65 (1979) 133 - 226
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Henkel KGaA
Qualitätssicherung

ERKLÄRUNG DER QUALITÄTSSICHERUNG

Archiv-Nr.: 880203 **Prüf-Nr.:** 880106

Autor: Dr. Banduhn

Titel: Cosmedia Guar C 261

Prüfung auf Mutagenität im Ames-Test

Die Qualitätssicherung der Henkel KGaA, Düsseldorf, bestätigt, daß in diesem Abschlußbericht alle Methoden, Verfahren und Beobachtungen genau beschrieben worden sind und daß die berichteten Ergebnisse die Rohdaten der Prüfung genau wiedergeben.

Von der Qualitätssicherung wurden Inspektionen der Prüfeinrichtung und Überprüfungen der laufenden Prüfung durchgeführt am

24.02.1988
22.04.1988

Die Ergebnisse der Inspektionen und Überprüfungen wurden der Leitung der Prüfeinrichtung und dem Prüfleiter umgehend mitgeteilt.

Leiter der QS

i.V. Dr. Waldhoff

Datum 25.4.88

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: August 16, 2011

SUBJECT: Data Sheet Trigonella Foenum-Graecum Seed Extract

Grau Aromatics GmbH & Co. KG. 2009. Data sheet Fenugreek Extract HS 2447 G.

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

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GRAU AROMATICS GmbH & Co. KG

PRODUCT: FENUGREEK EXTRACT HS 2447 G **Date: 03 / 2009**

Product-No: 520 163

Botanical name: Trigonella foenum-graecum

INCI name (CTFA/Linné):

Propylene Glycol and	75 - 100%
Trigonella Foenum-Graecum Seed Extract	5 - 10%

Parts used: Seeds

Plant composition:

- Trigonellin
- Flavonoids
- Proteins
- Tannins
- Choline
- Enzymes
- Bitter substances
- Essential oil
- Fatty oil
- Mucilages
- Steroid saponins
- Nicotinic acid

Plant properties:

- wound healing (gathering, furuncle, glandular swelling)
- demulcent
- tonic
- fungicidal

Literature:

"Drogenkunde", H.A. Hope, Bd. 1, 8. Aufl., Verlag WdeG
"Lexikon der offizinellen Arzneipflanzen", Verlag WdeG
"Les plantes dans la thérapeutique moderne", maloine s.a. éditeur
"Seifen-Öle-Fette-Wachse", 115. Jg., Nr. 2/89

Solvent of extraction: 1,2-Propylene glycol

Preservatives: 0,6 % Bactiphen 2506 G (Phenoxyethanol 75-100% and Methylparaben 10-25% and Ethylparaben 1-5% and Propylparaben 1-5% and Butylparaben 1-5%)

Analytical data:

Aspect:	clear, yellowish coloured liquid	
Odour:	faint herbal odour	
pH-value:	4,5 - 5,5	
Proof of identity:	HPLC	
Bacteriological control:	max. 100 germs/ml	
Refraction index:	1,420 - 1,440	(20° C)
	This is a natural product which can change in colour with age.	
Density:	1,025 - 1,045	(20° C)
Colour number:	1 - 2	(Lovibond)
	This is a natural product which can change in colour with age.	
Viscosity:	ca. 60 mPa . s	(20° C/Viscotester VT-02)
Solubility:	in water clear soluble	

Dosage/Use: 1 - 10% in cosmetic products. Not for use in food.
Storage: In closed containers, a temperature of 10°-25°C should be maintained, protected from light.
Shelf life: At least 30 months.

C.A.S.: 84625-40-1
Einecs: 283-415-1
Brussels nomenclature: 1302 1980

All our extracts are filtered clear after fabrication. It is generally accepted, that a longer period of storage may cause sediments. These, however, influence neither the quality nor the usability of the extract, and are no reason for complaints.
These information's are given to the best of our knowledge, as an advice for our customers, and without any legal liability.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: October 26, 2011

SUBJECT: Concentration of Use by FDA Product Category: Galactomannans

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use by FDA Product Category*

Cyamopsis Tetragonoloba (Guar) Gum
Hydroxypropyl Guar
C18-22 Hydroxyalkyl Hydroxypropyl Guar
Guar Hydroxypropyltrimonium Chloride
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride
Carboxymethyl Hydroxypropyl Guar
Hydrolyzed Guar
Ceratonia Siliqua Gum
Locust Bean Hydroxypropyltrimonium Chloride
Hydrolyzed Ceratonia Siliqua Gum Extract
Caesalpinia Spinosa Gum
Caesalpinia Spinosa Hydroxypropyltrimonium Chloride
Hydrolyzed Caesalpinia Spinosa Gum
Trigonella Foenum-Graecum Seed Extract
Trigonella Foenum-Graecum Hydroxypropyltrimonium Chloride
Hydrolyzed Trigonella Foenum-Graecum Seed Extract
Cassia Gum
Cassia Hydroxypropyltrimonium Chloride

Ingredient	Product Category	Maximum Concentrations of Use
Cyamopsis Tetragonoloba (Guar) Gum	Baby shampoos	0.09%
Cyamopsis Tetragonoloba (Guar) Gum	Hair conditioners	0.3-0.5%
Cyamopsis Tetragonoloba (Guar) Gum	Shampoos (noncoloring)	0.5%
Cyamopsis Tetragonoloba (Guar) Gum	Tonics, dressings and other hair grooming aids	0.04-0.5%
Cyamopsis Tetragonoloba (Guar) Gum	Hair dyes and colors (all types requiring caution statement and patch testing)	3%
Cyamopsis Tetragonoloba (Guar) Gum	Hair lighteners with color	2%
Cyamopsis Tetragonoloba (Guar) Gum	Hair bleaches	5%
Cyamopsis Tetragonoloba (Guar) Gum	Bath soaps and detergents	0.1-1%
Cyamopsis Tetragonoloba	Deodorants (underarm)	1%

(Guar) Gum		
Cyamopsis Tetragonoloba (Guar) Gum	Face and neck creams, lotions and powders	0.02%
Cyamopsis Tetragonoloba (Guar) Gum	Moisturizing creams, lotions and powders	0.02%
Hydroxypropyl Guar	Hair conditioners	0.1-0.8%
Hydroxypropyl Guar	Hair straighteners	93%
Hydroxypropyl Guar	Tonics, dressings and other hair grooming aids	0.5-2%
Hydroxypropyl Guar	Hair bleaches	0.1%
Hydroxypropyl Guar	Other hair coloring preparations	2%
Hydroxypropyl Guar	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.3%
Hydroxypropyl Guar	Body and hand creams, lotions and powders	0.05%
Hydroxypropyl Guar	Other skin care preparations	0.8%
Guar Hydroxypropyltrimonium Chloride	Baby shampoos	0.3%
Guar Hydroxypropyltrimonium Chloride	Other baby products	0.3%
Guar Hydroxypropyltrimonium Chloride	Bubble baths	0.4%
Guar Hydroxypropyltrimonium Chloride	Other bath preparations	0.2-0.9%
Guar Hydroxypropyltrimonium Chloride	Mascara	0.005-1%
Guar Hydroxypropyltrimonium Chloride	Hair conditioners	0.07-1%
Guar Hydroxypropyltrimonium Chloride	Rinses (noncoloring)	0.1-0.3%
Guar Hydroxypropyltrimonium Chloride	Shampoos (noncoloring)	0.09-2%

Guar Hydroxypropyltrimonium Chloride	Tonics, dressings and other hair grooming aids	0.5-2%
Guar Hydroxypropyltrimonium Chloride	Other hair preparations (noncoloring)[1]	0.3-0.4%
Guar Hydroxypropyltrimonium Chloride	Hair dyes and colors (all types requiring caution statement and patch test)	0.3%
Guar Hydroxypropyltrimonium Chloride	Hair shampoos coloring	0.1%
Guar Hydroxypropyltrimonium Chloride	Bath soaps and detergents	0.2-0.8%
Guar Hydroxypropyltrimonium Chloride	Other personal cleanliness products	0.2-0.3%
Guar Hydroxypropyltrimonium Chloride	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.02-1%
Guar Hydroxypropyltrimonium Chloride	Body and hand creams, lotions and powders	0.1-0.5%
Guar Hydroxypropyltrimonium Chloride	Foot powders and sprays	0.05%
Guar Hydroxypropyltrimonium Chloride	Paste masks and mud packs	0.1%
Guar Hydroxypropyltrimonium Chloride	Other skin care preparations	0.5%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Bubble baths	0.3%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Other bath preparations	0.3%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Shampoos (noncoloring)	0.3-0.8%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Tonics, dressings and other hair grooming aids	0.04-0.9%

Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Bath soaps and detergents	0.6%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.2-0.5%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Face and neck creams, lotions and powders	0.3%
Hydroxypropyl Guar Hydroxypropyltrimonium Chloride	Body and hand creams, lotions and powders	0.2%
Hydrolyzed Guar	Eye makeup remover	0.5%
Hydrolyzed Guar	Hair conditioners	0.4-2%
Hydrolyzed Guar	Tonics, dressings and other hair grooming aids	0.03-3%
Hydrolyzed Guar	Foundations	0.1%
Hydrolyzed Guar	Lipstick	0.2%
Hydrolyzed Guar	Shaving cream (aerosol, brushless and lather)	0.2%
Hydrolyzed Guar	Skin cleansing (cold creams, cleansing lotions liquids and pads)	0.4%
Ceratonia Siliqua Gum	Eye lotion	0.002-0.01%
Ceratonia Siliqua Gum	Mascara	0.05%
Ceratonia Siliqua Gum	Other eye makeup preparations	0.02%
Ceratonia Siliqua Gum	Colognes and toilet waters	0.002%
Ceratonia Siliqua Gum	Other fragrance preparations	0.003%
Ceratonia Siliqua Gum	Other oral hygiene products	0.07%
Ceratonia Siliqua Gum	Bath soaps and detergents	0.0003%
Ceratonia Siliqua Gum	Face and neck creams, lotions and powders	0.0008-0.03%

Ceratonia Siliqua Gum	Body and hand creams, lotions and powders	0.0003-0.01%
Ceratonia Siliqua Gum	Night creams, lotions and powders	0.003%
Ceratonia Siliqua Gum	Other skin care preparations	0.002%
Locust Bean Hydroxypropyltrimonium Chloride	Shampoos (noncoloring)	0.4%
Caesalpinia Spinosa Gum	Eye lotion	0.002%
Caesalpinia Spinosa Gum	Foundations	0.002%
Caesalpinia Spinosa Gum	Lipstick	0.02%
Caesalpinia Spinosa Gum	Other makeup preparations	0.003%
Caesalpinia Spinosa Gum	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.03%
Caesalpinia Spinosa Gum	Face and neck creams, lotions and powders	0.005-0.5%
Caesalpinia Spinosa Gum	Moisturizing creams, lotions and powders	0.006%
Caesalpinia Spinosa Gum	Night creams, lotions and powders	0.1%
Caesalpinia Spinosa Gum	Other skin care preparations	0.3%
Hydrolyzed Caesalpinia Spinosa Gum	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.008%
Hydrolyzed Caesalpinia Spinosa Gum	Face and neck creams, lotions and powders	0.4%
Hydrolyzed Caesalpinia Spinosa Gum	Moisturizing creams, lotions and powders	0.002%
Hydrolyzed Caesalpinia Spinosa Gum	Night creams, lotions and powders	0.4%
Trigonella Foenum-Graecum Seed Extract	Perfumes	0.004%
Trigonella Foenum-Graecum Seed Extract	Other fragrance preparations	0.003%

Trigonella Foenum-Graecum Seed Extract	Shampoo (noncoloring)	0.03-0.08%
Trigonella Foenum-Graecum Seed Extract	Tonics, dressings and other hair grooming aids	0.3%
Trigonella Foenum-Graecum Seed Extract	Body and hand creams, lotions and powders	0.00001%
Hydrolyzed Foenum-Graecum Seed Extract	Moisturizing creams, lotions and powders	0.003%
Cassia Hydroxypropyltrimonium Chloride	Hair conditioners	0.06%
Cassia Hydroxypropyltrimonium Chloride	Shampoos (noncoloring)	0.4%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.
[1]0.3% in a rinse-off product

Information collected in 2011
Table prepared October 26, 2011

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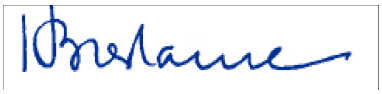
Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel



DATE: October 3, 2011

SUBJECT: Comments on the Scientific Literature Review on the Safety Assessment of Galactomannans

The following are the most important issues identified for this report:

Use of Definitions Other than Those Included in the Dictionary

p.28-32, Table 1 - As this is a review of cosmetic ingredients, the definitions included in the *Cosmetic Ingredient Dictionary and Handbook* (Dictionary) need to be included (and referenced) in the report. Although it acceptable to include other definitions in the report, the additional definitions should indicate the references from which they were obtained.

The Dictionary defines Carboxymethyl Hydroxypropyl Guar as a sodium salt which is not indicated in the definition or structure in Table 1

Defining Trigonella Foenum-Graecum Seed Extract as a gum is not correct. If the ingredients was predominantly a polysaccharide it would have been named as a gum. Extracts of other species included in this report, such as Caesalpinia Spinosa Fruit Pod Extract, are not included in the report. Why is it appropriate to include the seed extract of *Trigonella foenum-graecum*? Evidence that Trigonella Foenum-Graecum Seed Extract is not primarily a polysaccharide includes:

1. The GRAS listing is for an "Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)"
2. Fragrance is listed as a function
3. One supplier has indicated that their CO_2 extract contains no polysaccharides (the Council is trying to get additional composition information from suppliers and companies reporting use of this ingredient)
4. Reference 103 (discussed on p.22) indicated that the Trigonella Foenum-

Graecum Seed Extract they studied contained a minimum of 40% 4-hydroxy isoleucine

The gum of *Trigonella foenum-graecum* can be included in Table 1, but it should be indicated that it is not (at least not yet) a cosmetic ingredient. This company provides some information about fenugreek gum on their website at http://www.emeraldseedproducts.com/canafen_gum_info.

The Dictionary defines Cassia Gum as being derived from two specific species, *Cassia tora* or *Cassia obtusifolia*. Apparently this is important as p.5 of the report suggests another species, *Cassia occidentalis* has higher levels of anthraquinones. The species from which Cassia Gum is derived should not be omitted from the definition of this gum.

Identification of type of Trigonella Foenum-Graecum Seed Extract Studied

If Trigonella Foenum-Graecum Seed Extract is left in the report, the type of extract needs to be identified for every study of this material. The type of extract studied should also be stated in the Summary.

Additional comments

p.1 - Although the Dictionary is given as the reference for the sentence under Definition and Structure, most of the information in Table 1 is not from the Dictionary

p.2, 3, Figures 2 and 3 - Please provide references for these figures.

p.4 - Please provide a reference for the method of manufacture for Hydroxypropyl Guar.

p.4 - The second paragraph under Composition/Impurities for Cyamopsis Tetragonoloba (Guar) Gum states that method 4 results in the total elimination of protein twice.

p.5 - Before the discussion of impurities of Cassia Gum, there needs to be a discussion of the species from which it is derived, as it appears that a related species, *Cassia occidentalis*, contains higher levels of anthraquinones than *Cassia tora* or *Cassia obtusifolia*.

p.9 - If the authors of reference 52 identified a NOAEL for the 91 day dietary study of Cyamopsis Tetragonoloba (Guar) Gum, please include it in the report.

p.12 - In the first paragraph, please delete "body weight" in the following: "at a dietary dose of 7,500 or 25,000 mg/kg body weight" (the mg/kg body weight doses are then provided).

p.13 - References 69 and 72 are not a case reports and should not be presented under case reports. They are experimental studies done in humans.

p.15 - The heading "Hyperplastic Effect" does not seem appropriate for reference 49. The investigators appear to be studying an inflammatory (immunologic) response to the i.p. injection of Trigonella Foenum-Graecum Seed Extract.

p.16-17 - In the description of the developmental studies, there does not appear to be an understanding of why hamsters were treated for "a shorter dosing period". It appears that all the species were treated during the major period of organogenesis - this period is shorter for the hamster.

p.20, 21 - Please delete the word "gene" from "dominant lethal gene test". The difference between the dosing regimen in the 8 week and the 7 week study in reference 50 is not clear. It appears that

in the 8 week study the rats given one dose each week. The frequency of dosing in the 7 week study is not stated (it says they were given multiple oral doses).

p.21 - Please move reference 98 (spore rec-Assay) up before the mammalian cell assays.

p.23-24 - Please indicate that these were NTP carcinogenicity studies.

p.24, 25 - What is the difference between “antitumorigenicity” and “anticarcinogenicity” and is it really necessary to include both subheadings?

p.25 - It should be indicated that anthraquinones have been found in Cassia Gum when a related species is included as a contaminant.

p.26 - Please give some indication of the doses used (the lowest resulting in an effect, or the highest dose that did not a cause an effect) in the Summary.

p.33, Table 2 - Please also include the specification “Loss on Drying” (would include “moisture” plus other volatiles) that is in the Food Chemical Codex, e.g., not more than 15% for guar gum. Are anthraquinone levels relevant for the other ingredients? Perhaps NR should be “not relevant” and use NA for “not available” (or use some other means to distinguish between “not relevant” and “not reported”.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: September 9, 2011

SUBJECT: Trigonella Foenum-Graecum Seed Extract

One supplier indicates that the Trigonella Foenum-Graecum Seed Extract they supply is a supercritical CO_2 extract. They indicate that galactomannans (polysaccharides) are not extractable with supercritical CO_2. Only fat soluble components, such as essential oils and fatty oils are extractable using supercritical CO_2.

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Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: November 7, 2011

SUBJECT: Composition Trigonella Foenum-Graecum Seed Extract

Based on GC analysis, one vendor reports that the total polysaccharides in the Trigonella Foenum-Graecum Seed Extract are <0.1 mg/g.

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