Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to an application

on the use of cassia gum as a food additive

QUESTION N° EFSA-Q-2003-134

Adopted on 26 September 2006

SUMMARY

The European Commission has asked the European Food Safety Authority to provide a scientific opinion on the safety of cassia gum when used as a gelling agent and as a thickener.

Cassia gum is the flour from the purified endosperm of seeds from *Cassia tora* and *Cassia obtusifolia*. Cassia gum is comprised primarily of a linear chain of 1,4-β-D mannopyranose units with 1,6-linked α-D galactopyranose units attached to every fifth mannose.

Galactomannans are recognised as components of dietary fiber and are resistant to digestive enzymes in the gastrointestinal tract. The absorption and distribution of cassia gum have not been studied.

It is expected that cassia gum would be excreted unchanged. Fermentation of cassia gum by gut microflora may occur to a small extent. However, the panel notes that any hydrolysed material would represent oligo- or monosaccharides that can be expected to be absorbed and metabolised in normal biochemical pathways.

The petitioner requests the authorisation of cassia gum for use in ice cream and frozen milk desserts for stabilising and controlling overrun, for the improvement of water retention in certain baked goods (including cheese-crème filling in pastries and other cheese-crème filled desserts), for use as a thickening agent for soup mixes, sauces and selected oil-free salad dressings, and for the improvement of texture and water retention in yogurt and yogurt drinks, sausages, corned beef, and canned poultry meats. Cassia gum is intended to be used in sausages, corned beef, and canned poultry meats at levels up to 1.5 g/kg and in all other applications at levels up to 2.5 g/kg.

Based on the petitioner's proposed use levels of cassia gum as a food additive and on conservative assumptions daily exposure to cassia gum was estimated to be 2.1 mg/kg bw/day at the mean, and 4.9 mg/kg bw/day at the 90th percentile (US data for consumers only).

Most acute, sub-chronic and reproductive and developmental toxicity studies reported for cassia gum have been performed with a cassia gum preparation with specifications different from those defined in the present application. These studies used an older test sample of cassia gum containing approximately 70 mg/kg of total anthraquinones. The petitioner has recently implemented an isopropanol extraction purification step that reduces the total anthraquinones levels from 70 mg/kg to below the 0.5 mg/kg detection limit, the latter being in line with the specifications of the present submission.

Cassia gum containing approximately 70 mg/kg anthraquinones has been tested in acute, sub-chronic, reproductive and developmental toxicity studies and it does not have significant toxicological properties.

The Panel notes that the margin between the no-observed-adverse-effect level (NOEL) in the 28-day rat study of 230 mg/kg bw/day and the exposure estimates of 2.1 mg/kg bw/day at the mean, and 4.9 mg/kg bw/day at the 90th percentile, based on the proposed levels of use, is between 50 and 110. However, the Panel also notes that the slight biochemical and haematological effects occurring at the higher dose levels in this 28-day rat study were generally not dose related and of limited toxicological significance. Furthermore, in a 90-day dog study and a 13-week cat study there were no effects up to doses of 3500 and 1250 mg/kg bw/day respectively.

Cassia gum of the old specifications was not mutagenic or clastogenic in mammalian cells. Based on the results of recent genotoxicity studies, cassia gum, prepared by the newly defined production method, did not increase the number revertants in any of the four Ames tester strains (S. *typhimurium*), nor in the E. *coli* WP_2uvrA test strain both in the presence and absence of S9-metabolic activation. It is concluded that cassia gum complying with the newly defined specifications does not give rise to safety concern with respect to genotoxicity.

Long-term carcinogenicity studies on cassia gum were not available. Other related galactomannan gums, including locust (carob) bean, guar gum and tara gum were not carcinogenic when fed to mice and rats. Given that cassia gum is not genotoxic, and that many other related galactomannan gums are not carcinogenic, the Panel does not consider long-term carcinogenicity studies essential for the safety assessment of cassia gum.

The toxicological data on cassia gum are insufficient to establish an acceptable daily intake (ADI). On the other hand, the existing data do not give reason for concern.

The Panel wishes to stress the importance of inspection of the seeds for cassia gum preparation for the presence of seeds of C. *occidentalis* which has to be less than 0.1% by selection based on color and shape.

Given these results from the toxicological studies, the very low absorption of cassia gum and the fact that, if hydrolysed at all, cassia gum would be degraded to compounds that will enter normal metabolic pathways, the Panel concludes that the use of cassia gum complying with the newly defined specifications as an additive for the proposed food uses is not of safety concern.

KEY WORDS

Cassia gum, food additive, CAS Registry Number 11078-30-1.

BACKGROUND

Cassia gum is the flour from the purified endosperm of the seeds from *Cassia tora* and *Cassia obtusifolia*, which belong to the *leguminosae* family.

A petitioner has requested the authorisation of cassia gum for use in ice cream and frozen milk desserts for stabilising and controlling overrun, for the improvement of water retention in certain baked goods (including cheese-crème filling in pastries and other cheese-crème filled desserts), for use as a thickening agent for soup mixes, sauces and selected oil-free salad dressings, and for the improvement of texture and water retention in yogurt and yogurt drinks, sausages, corned beef, and canned poultry meats. Cassia gum is intended to be used in sausages, corned beef, and canned poultry meats at levels up to 1.5 g/kg and in all other applications at levels up to 2.5 g/kg.

This question was previously considered by the EC Scientific Committee on Food (SCF) at which time further information was requested. EFSA is asked to consider the request in light of the additional data submitted by the petitioner.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion on the safety of cassia gum when used as a gelling agent and as a thickener.

ASSESSMENT

Chemistry

Cassia gum (CAS number: 11078-30-1) is the flour from the purified endosperm of the seeds from *Cassia tora* and *Cassia obtusifolia*, which belong to the *leguminosae* family. Cassia gum is comprised of at least 75% galactomannan, a polysaccharide which consists primarily of a linear chain of 1,4-β-D mannopyranose units with 1,6-linked α-D galactopyranose units attached to every fifth mannose. Accordingly the ratio of mannose to galactose is 5:1. The molecular mass is 200,000-300,000 Daltons.

Manufacturing Process

The manufacturing process has been adequately described by the petitioner. Cassia gum is produced from seeds from C. *obtusifolia* along with C. *tora* as raw material. The seeds consist of an outer husk, an endosperm (split) and the ovary or germ. Only the endosperm or split, which contains mainly polysaccharides, is used for the production of the cassia gum.

C. occidentalis, another species of Cassia that has been associated with muscle toxicity, generally does not grow in conjunction with C. *tora* or C. *obtusifolia*, but is an occasional impurity for which the collected seeds need to be inspected. The petitioner has a specification to limit the presence of C. *occidentalis* in the cassia seeds that are the raw material used to produce cassia gum. The petitioner indicates that the content of C. *occidentalis* in seeds for cassia gum has to be less than 0.1% by selection based on color and shape.

The concentration of anthraquinones originally in the seeds is approximately 10,000 mg/kg. After splitting, polishing and brushing, the concentration of anthraquinones in the split can be reduced to below 250 mg/kg as measured by a UV test method. The reduction of the anthraquinone content from 250 mg/kg to below the level of detection of the HPLC analytical method (< 0.5 mg/kg)(detected by HPLC with diode array detection and quantification on the basis of peak area at 435 nm) is done by a specific extraction with isopropanol. The drying process drives off residual isopropanol and water.

Specifications

The specifications of cassia gum suggested by the petitioner are as follows (see also Hallagan *et al.*, 1997): galactomannan $\geq$ 75%, acid-insoluble residue $\leq$ 2%, moisture $\leq$ 12%, ash $\leq$ 2%, proteins $\leq$ 7%, fat $\leq$ 2%, heavy metals $\leq$ 0.002% of which lead at $\leq$ 1 mg Pb/kg and arsenic at $\leq$ 3 mg As/kg, which is in accordance with the limits for the similar gums, guar gum and locust (carob) bean gum, in the Food Chemicals Codex V (2004), and isopropanol $\leq$ 0.075%, which is in accordance with the FDA regulations for xanthan gum and gellan gum. The proposed levels are more or less in line with the provisions for similar gums in the Directive on the specification for food additives other than colours and sweeteners except that no limits are proposed for mercury or cadmium or for microbiological parameters (EC 1996). The petioner also proposes a limit for anthraquinones below the detection limit of 0.5 mg/kg.

The following composition of the sugar components was revealed upon hydrolysis of the galactomannan (75% or higher of the total): mannose (77.2-78.9%), galactose (15.7-14.7%) and glucose (7.1-6.3%).

Methods of analysis in foods

There are several different AOAC methods for analyzing cassia gum in foods (AOAC, 2003). The specific method used depends on the particular food matrix and the ease of removing potentially interfering substances.

Reaction and fate in foods, stability

Cassia gum is claimed by the petitioner to be stable during food processing and storage but test results to support this statement were only provided for stability during storage.

To confirm the stability of cassia gum, samples from six master batches were tested for stability, measuring protein content, fat content, ash production, pH, percentage of the sample lost on drying and gel strength. These batches were manufactured according to the procedure described for cassia gum by the petitioner, including extraction with isopropanol. The experiments reveal that the product is stable for at least 20 months. All changes in the values were within the limits of the specifications. The only significant change observed was an increase in the loss on drying value. This is due to the fact that the product is hygroscopic until a final moisture content of 8-12% is achieved.

Case of need and proposed uses

The galactomannan from cassia gum as specified by the petitioner differs from related galactomannan gums, derived from locust bean gum, tara gum and guar gum, in having fewer galactose molecules next to the long mannose chain. According to the petitioner this fact has a significant effect on the synergy of cassia gum with anionic food gums like carrageenan or xanthan gum. A high number of galactose side chains inhibits the synergistic gelling effect with anionic polymers. As a result, a smaller amount of hydrocolloid blend containing cassia gum is needed in a food product to achieve the same effect as with carrageenan alone or blends of carrageenan with other related galactomannans. The break strength and gelling properties of pure carrageenan, for example, can be achieved with 2/3 less of a mixture of carrageenan and cassia gum.

The petitioner claims that by replacing carrageenan and/or a galactomannan by cassia gum in a given food recipe, the amount of total hydrocolloid can be reduced significantly.

The applications for which cassia gum is intended to be used include the use of cassia gum in ice cream and frozen milk desserts for stabilising and controlling overrun; to improve water retention in certain baked goods (including cheese-crème filling in pastries and other cheese-crème filled desserts); as a thickening agent for soup mixes, sauces and selected oil-free salad dressings; and to improve texture and water retention in yogurt and yogurt drinks, sausages, corned beef, and canned poultry meats. Cassia gum would be used in sausages, corned beef, and canned poultry meats at levels up to 1.5 g/kg and in all other applications at levels up to 2.5 g/kg.

Exposure

The petitioner provided two estimates of the potential daily exposure based on the proposed applications and usages as a food additive in the different foodstuffs. The two estimates are based on data for respectively EU (availability figures) and US (consumption figures). For the EU, overall consumption data for the various food types from Euromonitor/IMIS were divided by the 2003 population of the EU (455,000,000) to obtain the average per capita exposure. For the US, consumer use and food frequency intake data from USDA Continuing Survey of Food Intakes by Individuals (CSFII) were used to calculate the mean and 90th percentile average daily exposure for each selected food category. Based on the similar food habits in both geographical locations, the petitioner assumes that the intended applications for cassia gum will lead to similar exposures in the EU and in the US.

EU Data

The estimated potential daily exposure to cassia gum was based on the per capita (p.c.) amounts of yoghurt and yoghurt drinks (31 g p.c. /day), ice cream (22.5 g p.c. /day), desserts (9 g p.c. /day), processed cream cheese (4.5 g p.c. /day) and canned/preserved items (19 g p.c. /day), available for consumption and the maximum intended concentrations of cassia gum. The overall exposure was estimated to amount to approximately 195 mg/person/day. Assuming a 60 kg adult body weight, the average daily exposure to cassia gum would be 3.2 mg/kg bw/day. The estimates obtained from these calculations are based on the conservative assumption that all the food products listed are prepared with cassia gum. On the other hand the Panel underlined a critical limitation of “per capita” approaches when used in exposure assessment: such approaches do not allow quantification of exposure in the population of interest, i.e. high

consumers of products containing the substance of interest. These data were therefore not used for further quantification of the exposure to cassia gum.

US Data

The petitioner indicated that cassia gum is intended for use in the US in the same food groups as in the EU and with the same maximum use level. A detailed exposure assessment was performed based on the currently available database from the USDA's Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96, which includes detailed food consumption data collected on two non-consecutive days (USDA, 1997). The Panel noted that average consumption in consumers only, based on a 2 day survey, overestimates consumption for rarely consumed foods. The proposed maximum use levels for cassia gum were combined with the CSFII consumption data for a selection of foods from the 9 broad food categories in which cassia gum may be used.

The 2-day daily exposure for consumers of cassia gum, from all foods groups, was estimated to be 2.1 mg/kg bw/day at the mean, and 4.9 mg/kg bw/day at the 90th percentile. The highest exposure to cassia gum on a per consumer basis was from yoghurt and yoghurt drinks as well as ice cream.

The levels of consumption were considered by the Panel to be sufficiently conservative and their combination with maximum use level ensured the overall conservativeness of the exposure assessment.

Existing authorisations and evaluations

Cassia gum is authorised as a food additive in Japan (Japanese Ministry of Health and Welfare 1995). Within the EU cassia gum has been authorised as a feed additive in animal nutrition (canned pet foods) in 1991 and is included in the Annexes to Directive 70/524/EC. The substance is not included as an authorised feed additive for food producing animals.

TOXICOLOGICAL DATA

The petitioner indicates that most of the toxicological studies described were performed with older test samples of cassia gum, which were samples that still contained approximately 70 mg/kg total anthraquinones. The petitioner has recently implemented an isopropanol extraction purification step that substantially reduces the total anthraquinones levels compared to the levels found in the samples fed in the previously performed animal studies. After isopropanol extraction anthraquinones are reduced from 70 mg/kg to below the 0.5 mg/kg detection limit in the product, the latter being in line with the newly defined specifications.

Absorption, Bioavailability and Metabolism

Galactomannans are recognised components of dietary fiber and are resistant to digestive enzymes in the gastrointestinal tract. Galactomannans were shown to be stable in artificial gastric juice (Nurnberg and Bleimuller, 1981). No information is available on the bioavailability of galactomannans from cassia gum: other galactomannans appear to undergo no or only minimal hydrolysis by the digestive juices. This does not depend upon the specific mannose:galactose ratio (Trowell *et al*., 1976).

Data on other glucomannan-based additives (such as konjac gum assessed by SCF) (SCF, 1997) indicated that these substances are non-digestible by human intestinal enzymes, but are susceptible to fermentation by enzymes of the colonic microflora.

Fermentation of the galactomannans from cassia gum by gut microflora may occur to a small extent (Nyman and Asp, 1982). Any hydrolysed material would be absorbed and metabolised in normal biochemical pathways (Mathews and van Holde, 1990).

Data on the related guar gum indicate that high (5%) concentrations in food may decrease the efficiency of nutrient digestion and absorption. No such data are available for cassia gum.

Acute Oral Toxicity

The oral (gavage) LD_{50} value of cassia gum was reported to be >5 g/kg bw in male rats (Schering, 1986; Hallagan *et al*., 1997).

Short-term and sub-chronic toxicity

The petitioner reports that cassia gum as a dietary admixture is well tolerated by animals and did not produce evidence of toxicity in three repeated dose studies in dogs, cats and rats. A decrease in food consumption was commonly observed and is attributed to the absorption of water by cassia gum within the gastrointestinal tract and subsequent increase in volume and bulk. The three studies summarised below were conducted according to the OECD guidelines (OECD, 1981).

In a 28-day study, groups of five male and five female Sprague-Dawley rats were administered cassia gum as part of a powdered diet at concentrations of 0, 2.5 g/kg, 10 g/kg, 25 g/kg, or 50 g/kg (Zühlke, 1990; Hallagan *et al*., 1997). The calculated overall mean intakes reported by the petitioner amounted to 0, 252, 1032, 2591 and 4958 mg/kg bw/day for male rats and to 0, 231, 1107, 2358 and 4589 mg/kg bw/day for female rats. A sixth group, which was fed a control diet, was treated by gavage with cassia gum as a suspension in distilled water, two times a day at a dose of 1000 mg/kg bw/day.

Clinical, haematological, urinary and biochemical parameters were evaluated. The petitioner indicates that generally, all haematological parameters examined were considered to be within the normal range of the background data for the species tested. The following minor changes were observed which were not considered by the petitioner to be treatment-related because they did not occur in the high oral 50 g/kg diet dose group: a statistically significant decrease in the red blood cell count and packed cell volume of males and females at the 10 g/kg and 25 g/kg dietary levels; a statistically significant decrease in mean haemoglobin values of females in the 10 g/kg and 25 g/kg dietary level groups; and a statistically significant increase in white blood cell count for males in the groups receiving 10 g/kg, 25 g/kg and 50 g/kg dietary levels.

Clinical chemistry findings included statistically significant increases in mean glucose concentrations of males and females in the 10 g/kg and 25 g/kg dietary level groups, and mean triglyceride concentrations of males only in the 10 g/kg and 25 g/kg dietary level groups. The petitioner indicates that these changes are not considered dose-related because they were not observed at the highest dose. A decrease in blood urea nitrogen was statistically significant but not dose-dependent for males in the 10 g/kg (-26.3%), 25 g/kg (-23.4%) and 50 g/kg (-39.1%) dietary level groups, but was not statistically significant in females in the 25 and 50 g/kg diet

groups. Statistically significant, dose-dependent decreases in sodium (from -1.6% to -2.9%) were observed in males in the 10 g/kg, 25 g/kg and 50 g/kg dietary level groups, and the 1000 mg/kg bw/day intragrastic suspension group (-2.9%), and females at the 25 g/kg and 50 g/kg dietary level only (-3.9% and -3.3.% respectively). A statistically significant, dose-dependent increase in group mean triglyceride concentrations was reported in females in the 10 g/kg (46%) and 25 g/kg (67%) dietary level groups. Statistically significant but not dose-dependent reduced mean total protein (6.2%) and albumin levels (7.7%) of males in the 50 g/kg dietary level group, statistically significant elevated potassium levels (13.0%) of males in the 25 g/kg group, and decreased chloride levels (6.8%) of males treated in 1000 mg/kg bw/day group intragastrically were also observed. Body weight gain was statistically significantly reduced (20%) in males in the 50 g/kg dietary level group and in females (17% each) in groups fed 10 g/kg and 25 g/kg in the diet, and the group given 1000 mg/kg bw/day intragastrically (gavage). Such weight decrements have previously been observed when high dose levels of carbohydrate-based gums are administered to rats (Takahashi *et al.*, 1994; Hallagan *et al*., 1997) and the petitioner concludes that they are not considered to be biologically significant.

All animals were necropsied and all tissues and organs were weighed and examined macroscopically. There were no treatment related necropsy or histopathological findings in animals necropsied at study termination. Histological examination was performed on major organs for animals in the control group, high dose group, and group treated intragastrically. Group mean absolute kidney weights were statistically significantly decreased in males fed 10 g/kg in the diet (-8.0%), 50 g/kg in the diet (-15.3%) and 1000 mg/kg bw/day (-6.9%) intragastrically. Group mean relative kidney weights were statistically significantly increased in females fed the 50 g/kg diet (+11.3%). A dose-relationship was not reported. The petitioner indicates that these changes in the kidney weights were not considered treatment-related and/or toxicologically significant since there were no changes in relative weight in males and in absolute weight in females and no histopathological changes were seen (Zühlke, 1990; Hallagan *et al*., 1997).

The Panel concluded that a clear NOEL was the 2.5 g/kg dietary exposure group that resulted in calculated exposures of 230-250 mg/kg bw/day, but noted that the effects observed at higher dose levels were generally not dose-related and of limited toxicological relevance.

In a 90-day study, three groups, each composed of four beagle dogs of each sex, were exposed to cassia gum through the diet at levels of 0, 7.5 g/kg and 25 g/kg , equivalent to 0, 1000 or 3500 mg/kg bw/day. The only effect was a statistically significant and dose-dependent increase of water consumption probably due to water retention in the gastrointestinal tract by colloidally dissolved gum. This was not considered of toxicological relevance. The Panel concluded that the no-observed-adverse-effect level (NOAEL) was at least 3500 mg/kg bw/day (Schuh, 1990; Hallagan *et al*., 1997).

The petitioner also reported a 13-week subchronic toxicity study in cats. Three groups of five male and five female cats were provided with diets containing 0, 5 g/kg, or 25 g/kg (w/w) cassia gum calculated by the FDA (FDA, 1993) to be equivalent to 0, 250 or 1250 mg/kg bw/day as part of a standard canned-food diet for 13 weeks. Clinical, haematological, urinary and biochemical parameters were evaluated and were in the normal range. No consistent treatment related, dose-dependent adverse effects were reported. Histological examination was

performed on all major organs in all animals. There were no adverse or compound-related histopathological findings. Survival was 100% (Virat, 1984; Hallagan *et al*., 1997).
The Panel concluded that the NOAEL was at least 1250 mg/kg bw/day.

Reproductive and developmental toxicity

Four reproductive or developmental toxicology studies have been conducted in which cassia gum was presented as a dietary admixture or administered by gavage. These include a one-generation study in cats, a two-generation study in rats and developmental toxicity studies in rats and rabbits. The four studies, discussed in more detaill below, were conducted according to OECD guidelines (1981).

In a one-generation study in cats three groups of 10 males and 20 females each were exposed to cassia gum through the diet at levels of 0, 7.5 g/kg and 25 g/kg diet , equivalent to 0, 375 or 1250 mg cassia gum/kg bw/day. The quality of the study was somewhat impaired by an unusually high litter loss in the control group. Statistically significant findings at the highest dose included reduced food consumption and increased absolute and relative ovarian weight in dams, and increased combined incidence of stillborns and neonatal deaths. The NOAEL was 375 mg/kg bw/day (Virat, 1989; Hallagan *et al*., 1997).

In a two-generation reproductive study using Sprague-Dawley rats (McIntyre, 1990; Hallagan *et al*., 1997), five groups of 25 males and 25 females were given diets containing 0, 5, 20, or 50 g cassia gum/kg, or a diet containing 50 g of cassia gum prepared according to the new specifications per kg. Findings at the highest dose included slightly reduced pregnancy rate (P0 and P1) and slightly reduced weight gain of pups (F1 and F2). The NOAEL was 20 g cassia gum/kg diet equal to about 1000 mg/kg bw/day.

In a developmental toxicity study, three groups of 28 pregnant Sprague-Dawley rats were administered cassia gum in distilled water by gavage at doses of 0, 350 or 1000 mg/kg bw/day for 14 days from days 6 to 19 post-coitum (inclusive) (Hallagan *et al*., 1997). The control animals received the vehicle (distilled water) alone. A fourth group containing 29 pregnant rats were administered cassia gum of the new specifications by gavage in distilled water at 1000 mg/kg bw/day over the same period. All animals were sacrificed and examined on day 20 post-coitum (Müller, 1989; Hallagan *et al*., 1997). No compound-related adverse effects were reported for pregnancy incidence, implantation, post-implantation loss, and fetal defects upon necropsy. A statistically significant reduction in mean daily food consumption and mean body weight gain was observed in the pregnant animals dosed at 1000 mg/kg bw/day of cassia gum or cassia gum of the new specifications, and was considered to be a treatment related effect. However, these effects were not accompanied by any evidence of embryotoxicity or teratogenicity (Müller, 1989; Hallagan *et al*., 1997). Maternal toxicity was evidenced at the top dose level by significantly reduced weight gain and food consumption (NOAEL 350 mg/kg bw/day). No effects on intrauterine growth or development were observed by gavage up to the top dose level of 1000 mg/kg bw/day.

In a related developmental toxicity study, three groups of 20 pregnant New Zealand White rabbits were administered cassia gum in distilled water by gavage at 0, 350 or 1000 mg/kg bw/day for 22 days from days 6 to 27 post-coitum (inclusive) (Hallagan *et al*., 1997). The control animals received the vehicle (distilled water alone). A fourth group of 20 pregnant rabbits were administered 1000 mg/kg bw/day of cassia gum of the new specifications by gavage in distilled water over the same period. All animals were sacrificed and examined on

day 28 post-coitum (Müller, 1989: Hallagan *et al*., 1997). No compound-related adverse effects were reported in pregnant animals for clinical observations, morality, necropsy findings, pregnancy incidence, implantation, post-implantation loss, or fetal defects. A reduction in mean daily food consumption was reported in animals administered 1000 mg/kg bw/day of cassia gum, and was accompanied by a reduction in mean fetal weights. However, these effects were not statistically significant. No indication of teratogenicity was reported (Müller, 1989; Hallagan *et al.,* 1997).

Mutagenicity

A battery of *in vitro* studies have been conducted to evaluate the genotoxic potential of cassia gum. These include bacterial reverse mutation assays (Ames tests and *Escherichia coli (E. coli)* reverse mutation assays), a chromosome aberration assay, and an *in vitro* mammalian cell gene mutation assay. The first series of *in vitro* studies were conducted in compliance with the OECD guidelines existing at the time (OECD, 1981).

In the first series of studies, cassia gum (old specifications) was tested, with and without metabolic activation, in the *Salmonella typhimurium* (S. *typhimurium*) reverse mutation assay (Ames test) with strains TA1535, TA1537 and TA98 at concentrations of 4, 16, 62.5 and 1000 μg/plate. Cassia gum precipitated on the plates at concentrations of 62.5 μg/plate and higher. Increases in the number of revertant colonies in strains TA1535, TA1537 and TA98 were seen only at precipitating concentrations and were within the historical control data range. Strain TA100 was tested at concentrations of 3, 10, 33, 100, 333 and 5000 μg/plate. Increases in the number of revertant colonies were related to the amount added and outside the historical control data range, but were seen only at precipitating concentrations (Verspeek-Rip *et al*., 1998a).

A second experiment in the study described above involved testing cassia gum (old specifications) at concentrations of 1.6, 8, 40, 200, and 1000 μg/plate in the same S. *typhimurium* strains and in E. *coli* strain WP_2uvrA. Cassia gum precipitated on the plates at concentrations of 200 μg/plate and higher. The results were negative (i.e. no evidence of mutagenic activity) in strains TA1535, TA98 and WP_2uvrA. Dose-related increases in the number of revertant colonies were observed in strains TA1537 and TA100, but again only at precipitating concentrations (Verspeek-Rip *et al*., 1998a). Only the results in TA100 were outside the range of historical controls showing a 5.4-fold and 2.7-fold increase in the number of revertant colonies in the absence and presence of S9 respectively at the highest dose tested. Based on the results from the two experiments, it was concluded that cassia gum tested positive for mutagenic activity in S. *typhimurium* strain TA100, and was negative for mutagenicity in strains TA1535, TA1537, TA98 and E. *coli* strain WP_2uvrA (Verspeek-Rip *et al*., 1998a).

Following the above described mutagenicity tests the petitioner decided to conduct a new series of Ames tests to attempt to avoid the solubility/precipitation problem. In addition, the purification method used to prepare the cassia gum was enhanced to reduce potential impurities with the addition of an extraction step with isopropyl alcohol. The Ames mutagenicity tests were thus redone on more stringently purified samples (new specifications), which are representative of the current production method

The second series of studies, the results of which were provided by the petitioner, was conducted in the same laboratory by the same principal investigator as the previous studies to more fully assess the mutagenic potential of cassia gum using the current, more highly refined

product, and using the most recent international guidelines (OECD, 1997). The same tester strains were used as described above for the first assay. The main part of the assay was conducted using water as the solvent in an effort to minimise precipitation. A bridging study with DMSO, which was previously used as the solvent in the first Ames test, was conducted at higher precipitating concentrations (100, 300, 1000, 3330 and 5000 μg/plate) to enable comparisons with the first Ames test results, which were previously positive in strain TA100 as noted above.

The petitioner reports that when cassia gum was tested up to concentrations of 5000 μg/plate using DMSO as a vehicle in the absence and presence of S9-mix cassia gum precipitated on the plates at all concentrations tested (100-5000 μg/plate). Cassia gum did not induce an increase in the number of revertant colonies in any of the tester strains (TA1535, TA1537, TA98, TA100 and WP_2uvrA) either in the presence or the absence of S9-metabolic activation.

Following the current OECD guidelines for selection of concentrations (OECD, 1997), the concentrations in water were established so that the top doses were limited to two concentrations that caused precipitation (33 and 100 μg/plate). The amounts added to the plates in this part of the study were 0.3, 1.3, 10, 33 and 100 μg/plate both with and without metabolic activation. The Panel notes that following the current OECD guidelines the highest precipitating concentrations of 300 and 1000 μg/plate were no longer included in the studies. Cassia gum did not increase the number revertants in any of the four Ames tester strains nor in the E. *coli* WP_2uvrA test strain either in the presence or the absence of S9-metabolic activation.

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

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

Carcinogenicity

There are no carcinogenicity studies on cassia gum. Other related galactomannan gums, including locust (carob) bean, guar gum and tara gum were not carcinogenic when fed to mice and rats. Guar gum when given to F344/N rats or B6C3F1 mice at dietary levels of 25 or 50 g/kg (amounting to about 1250 or 2500 mg/kg bw/day for rats and 3600 and 7200 mg/kg bw/day for mice) for 103 weeks did not induce cancer (NTP, 1982a). Similarly when locust (carob) bean gum was given to F344/N rats or B6C3F1 mice at dietary levels of 25 or 50 g/kg (equal to about 1250 or 2500 mg/kg bw/day for rats and and 3600 and 7200 mg/kg bw/day for mice) for 103 weeks it did not induce cancer (NTP, 1981). F344/N rats fed diets containing tara gum at 0, 25 or 50 g/kg diet (equal to about 0, 1250 or 2500 mg/kg bw/day for rats and 0, 3600 and 7200 mg/kg bw/day for mice) for 2 years exhibited no tumorigenic effects related to the test material (Borzelleca *et al*., 1993; NTP, 1982b).

Human data

Human data refer especially to studies on hypersensitivity, and do not include oral intake studies. There are studies in industrially exposed and pharmaceutical workers with results indicative of sensitisation by cassia gum upon skin exposure (Satheesh *et al*. 1994; Steger *et al*., 1999). No data are available on food allergies or intolerance following oral intake in humans.

DISCUSSION

Galactomannans are recognized as components of dietary fiber and are resistant to digestive enzymes in the gastrointestinal tract. The absorption and distribution of cassia gum have not been studied, but is expected to be similar to structurally related galactomannans.

It is expected that cassia gum would be excreted unchanged. Fermentation of cassia gum by gut microflora may occur to a small extent. However, the Panel notes that any hydrolysed material would represent oligo- or monosaccharides that can be expected to be absorbed and metabolised in normal biochemical pathways.

Based on the petitioner's proposed use levels of cassia gum as a food additive and on conservative assumptions, daily exposure to cassia gum was estimated to be 2.1 mg/kg bw/day at the mean, and 4.9 mg/kg bw/day at the 90th percentile (US data for consumers only).

Most acute, short-term, sub-chronic and reproductive and developmental toxicity studies reported for cassia gum have been performed with cassia gum with specifications different from those of the present application. These studies used an older test sample of cassia gum containing approximately 70 mg/kg total anthraquinones. The petitioner has recently implemented an isopropanol extraction purification step that reduces the total anthraquinones levels compared to the levels from 70 mg/kg to below the 0.5 mg/kg detection limit, the latter being in line with the specifications of the present submission.

Cassia gum is devoid of significant toxicological properties, including reproductive and developmental toxicity. The Panel noted that a NOEL of 230 mg/kg bw/day could be derived from the 28-day rat study, with slight biochemical and haematological effects occurring at the higher dose levels which were generally not dose-related and of limited toxicological significance.

This study was performed with a cassia gum preparation with high anthraquinone levels. Although there were haematological effects in the 28-day rat study, these were not observed in the one-generation reproductive study with cats, which showed a NOAEL of 375 mg/kg bw/day based on reduced food consumption, an increase in absolute and relative ovarian weight in dams and increased neonatal mortality, all at the higher dose of 1250 mg/kg bw/day. The quality of the latter study was impaired by the unusually high litter loss in the control group. Furthermore, in a 90-day dog study and a 13-week cat study there were no effects up to doses of 3500 and 1250 mg/kg bw/day, respectively.

Cassia gum (old specifications) was not mutagenic or clastogenic in mammalian cells. In the bacterial reversion assay, a positive response was obtained in the *Salmonella* strain TA 100

only: the response was elicited both in the presence and in the absence of metabolic activation, but at concentrations greatly exceeding the solubility limit.

Based on the results of more recent genotoxicity studies, using the current, more highly refined cassia gum, it did not increase the of number revertants in any of the four Ames tester strains (S. *typhimurium*), nor in the E. *coli* WP_2uvrA test strain both in the presence and absence of S9-metabolic activation.

From the available studies it is concluded that cassia gum, as prepared by the newly defined production method, complying with the defined specifications, does not give rise to safety concern with respect to genotoxicity.

Long-term carcinogenicity studies on cassia gum were not available. Other related galactomannan gums, including locust (carob) bean, guar gum and tara gum were not carcinogenic when fed to mice and rats. Given that cassia gum does not give rise to safety concern with respect to genotoxicity, and that other related galactomannan gums are not carcinogenic, the Panel does not consider long-term carcinogenicity studies essential for the safety assessment of cassia gum.

The toxicological data on cassia gum are insufficient to establish an ADI, because i) the toxicity data base for the cassia gum of the old specifications is weak, ii) the toxicitity studies were not performed with the purified cassia gum of the present specifications, iii) the toxicity data set on the cassia gum of the present specifications does not provide a long-term study and iv) the 28-day study and other studies provided are performed with only a limited number of animals. On the other hand, the existing data do not give reason for concern.

The Panel notes that the margin between the NOEL of 230 mg/kg bw/day in the 28-day rat study and the exposure estimates of 2.1 mg/kg bw/day at the mean, and 4.9 mg/kg bw/day at the 90th percentile, based on the proposed levels of use is between 50 and 110. However, the Panel also notes that the slight biochemical and haematological effects occurring at the higher dose levels in this 28-day rat study were generally not dose-related and of limited toxicological significance. Furthermore, in a 90-day dog study and a 13-week cat study there were no effects up to doses of 3500 and 1250 mg/kg bw/day respectively.

Given these results from the toxicological studies, the very low absorption of cassia gum and the fact that, if hydrolysed at all, cassia gum would be degraded to compounds that will enter normal metabolic pathways, the Panel concludes that the use of cassia gum complying with the newly defined specifications as an additive for the proposed food uses is not of safety concern.

CONCLUSIONS AND RECOMMENDATIONS

Dietary exposure estimates based on the petitioner's proposed use levels of cassia gum as a food additive and on conservative assumptions lead to a calculated exposure of cassia gum of 2.1 mg/kg bw/day at the mean, and 4.9 mg/kg bw/day at the 90th percentile (US data for consumers only).

Cassia gum complying with the newly defined specifications does not give rise to safety concern with respect to genotoxicity. Cassia gum preparations of less high quality were tested

in acute, sub-chronic, reproductive and developmental toxicity studies and did not have significant toxicological properties.

The Panel wishes to stress the importance of inspection of the seeds for cassia gum preparation for the presence of seeds of C. *occidentalis* which has to be less than 0.1% by selection based on color and shape.

The Panel concludes that the use of cassia gum complying with the newly defined specifications as an additive for the proposed food uses is not of safety concern.

DOCUMENTATION PROVIDED TO EFSA

Submission to the European Commission for the Safety Evaluation of Cassia Gum by the European Food safety Authority (EFSA). For the inclusion in Annex 1 to the EU Directive 95/2/EC listing the food additives which are permitted for use in foodstuffs. Dossier submitted by Keller and Heckman LLP for the petitioner Noveon Inc.

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