GR

Original Submission

LAW OFFICES
HYMAN, PHELPS & MCNAMARA, P.C.

JAMES R. PHELPS
PAUL M. HYMAN
ROBERT A. DORMER
STEPHEN H. MCNAMARA
ROGER C. THIES
THOMAS SCARLETT
JEFFREY N. GIBBS
BRIAN J. DONATO
FRANK J. SASINOWSKI
DIANE B. MCCOLL
A. WES SIEGNER. JR.
ALAN M. KIRSCHENBAUM
DOUGLAS B. FAROUHAR
JOHN A. GILBERT. JR.
JOHN R. FLEDER
MARC H. SHAPIRO

ROBERT T. ANGAROLA
(1945-1996)

DIRECT DIAL (202) 737-4291

,700 THIRTEENTH STREET, N.W.
SUITE 1200
WASHINGTON, D. C. 20005-5929
(202) 737-5600
FACSIMILE
(202) 737-9329
www.hpm corn

SAMIA N RODRIGUEZ
MARY KATE WHALEN
OF COUNSEL

JENNIFER B DAVIS
FRANCES K WU
DAVID B. CLISSOLD
KATE DUFFY MAZAN
HOLLY M BAYNE
CASSANDRA A SOLTIS
JOSEPHINE M TORRENTE
MICHELLE L. BUTLER
PATRICIA A A VANSTORY
THOMAS R. GIBSON
LEIGH E KENNEDY.
ANNE MARIE MURPHY'
PAUL L. FERRARI
JEFFREY N. WASSERSTEIN
BRIAN J. MALKIN

*NOT ADMITTED IN DC

May 11,2001

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

Subject: Notice of a GRAS exemption for D-tagatose

Dear Sir/Madam:

Pursuant to the proposed rule outlined at 62 Fed. Reg. 18939 (April 17, 1997), Arla Foods Ingredients amba ("Arla") hereby submits notification that the use of D-tagatose as **a** bulk sweetener, humectant, texturizer or stabilizer in foods is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because Arla has determined that such uses are generally recognized as safe ("GRAS") based on scientific procedures.

To facilitate your review, this notification is submitted in the format suggested under proposed 21 C.F.R. § 170.36(c) (see 62 Fed. Reg. at 18961). Also enclosed is an electronic copy (Microsoft Word 97) of the Notification Claim (GRAS Exemption Claim.doc) and Additional Information (GRAS Notice Additional Information.doc) documents.

Sincerely,

B. McColl
Counsel to Arla Foods Ingredients amba

DBM/dmh
Enclosure

2603 MAIN STREET
SUITE 650
IRVINE. CALIFORNIA 92614
19491553-7400
FAX: (949) 553-7433

RECEIVED
MAY 11
OFFICE OF

4819 EMPEROR BOULEVARD
SUITE 400
DURHAM, NORTH CAROLINA 27703
19191313-4750
FAX 19191313-4751

GRAS Exemption Claim

GRAS EXEMPTION CLAIM

We hereby claim that the use of D-tagatose as a bulk sweetener, humectant, texturizer or stabilizer in diet/sugarfree carbonated beverages, low calorie tea beverages, ready-to-eat breakfast cereals, icings and frostings, low/reduced fat diet, energy or nutrient fortified bars, regular and dietetic hard candies, regular and low-fat/non-fat frozen dairy desserts, sugarfree chewing gum and meal replacements is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because we have determined that such use of D-tagatose is generally recognized as safe (GRAS).

(1) Name and address of the notifier:

Kristian Eriknauer
Business Development Manager
Arla Foods Ingredients amba
Skanderborgvej 277
P.O. Box 25 10
DK-8260 Viby
Denmark
01 145-89-38-15-36(phone)
01 145-89-38-12-90(fax)

(2) Common or usual name of the substance that is the subject of the GRAS exemption claim:

D-tagatose

(3) Applicable conditions of use of the notified substance:

(a) Foods in which the substance is to be used:

Diet and/or sugar free carbonated beverages and presweetened low calorie ready to drink teas, ready to eat breakfast cereals, sugarless chewing gum, icings and frostings for baked goods, frozen dairy desserts including light ice creams, frozen milk desserts, low- and non-fat yogurt and frozen novelties, low/reduced fat, diet, energy, or nutrient-fortified nutrient bars, regular and dietetic hard candies, dietetic soft candies, and meal replacements.

(b) Levels of use in such foods:

Regulatory Category	Food Type	Use Level (%)
Beverages and beverage bases	Diet and/or sugar free carbonated beverages	1
Breakfast cereals	Ready to eat cereals	5.45-20 (serving size dependent)
Chewing gum	Sugar less and sugar free chewing gum	60
Coffee and tea	Ready to drink teas presweetened with low-calorie sweeteners	1
Confections and frostings	Icings or glazes used on baked goods (cookies, pastries, brownies, and angel food, chiffon and pound cakes)	30
Frozen dairy desserts	Light ice cream (ice milk), frozen milk desserts, low-fat and non-fat frozen yogurt and related frozen novelties	3
Grain products	Low-fat, reduced-fat, diet, energy or nutrient fortified bars	10
Hard candy and cough drops	Regular and dietetic hard candies	15
Soft candy	Dietetic soft candies	10
Meal replacements	Powdered products prepared with milk	5 g/serving

(c) Purposes for which the substance is used:

Bulk sweetener, humectant, texturizer or stabilizer

(d) Description of the population expected to consume the substance:

Members of the general population who consume at least one of the food categories described above.

(4) Basis for the GRAS determination:

The basis of the GRAS determination is through scientific procedures.

(5) Review and Copying Statement:

The data and information that are the basis for Arla Foods Ingredients' GRAS determination are available for the Food and Drug Administration's (FDA's) review and copying at reasonable times at the offices of the notifier, or will be sent to FDA upon request.

Kristian Eriknauer
Arla Foods Ingredients amba

Please address correspondence to:

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth Street, N.W.
Washington, D.C. 20005

Additional Information

ADDITIONAL, INFORMATION

I. Identity of the notified substance

A. Chemical name

D-tagatose

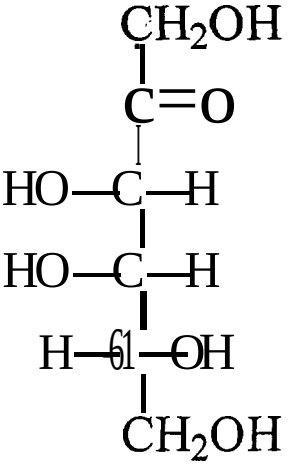
B. Chemical Abstracts Service (CAS) Registry Number

87-81-0

C. Empirical formula

$C_6H_{12}O_6$

D. Structural formula



E. Method of manufacture

Crystalline D-tagatose is manufactured from food grade lactose by a two-step process involving enzymatic hydrolysis (immobilized *A. oryzae* lactase) to D-galactose followed by chemical isomerization **of** D-galactose to D-tagatose, induced by calcium hydroxide, and recrystallization.

All processing aids used in the manufacturing process are considered safe and suitable. The method of manufacture uses processes common to other food ingredients. Potential impurities such as residual D-galactose, talose, aldol condensation products and other impurities arising during the manufacturing process are removed largely by

demineralization, chromatographic purification, and in the final crystallization of D-tagatose. None of the compounds present are of toxicological concern.

F. Characteristic properties

D-Tagatose is a stereo-isomer of D-fructose, which is a common six carbon sugar. It is recognized as a component of the regular diet, found in low amounts in heated milk products (Troyano *et al.*, 1991, 1996). Its relative sweetness in comparison to sucrose is about 75 and 92% based on a 1 and 10% aqueous solution, respectively. Like other sugars, it can be used as a bulk sweetener, humectant, texturizer or stabilizer (Levin *et al.*, 1995). D-tagatose has a reduced physiological energy value (Livesey and Brown, 1996), is non-cariogenic (Imfeld, 1996; Noguchi and Muhlemann, 1976), exerts a prebiotic effect (Bertelsen *et al.*, 2001; Jensen and Buemann, 1998; Jensen and Laue, 1998), and is not associated with a glycemic response (Donner *et al.*, 1996; Zehner, 1994). Additional information concerning chemical and physical properties is provided in the attached "D-Tagatose Specifications."

G. Any content of potential human toxicants

None

H. Specifications for food-grade material

Appropriate food grade chemical specifications supported by analytical data from several manufactured lots have been derived for crystalline D-tagatose and adopted by the Joint Expert Committee on Food Additives (JECFA) at its fifty-fifth meeting in 2000 (WHO, 2000). Potential microbiological contaminants have been addressed by the development of appropriate internal microbiological specifications. See attached "D-Tagatose Specifications."

11. Information on any self-limiting levels of use

None.

111. Detailed summary of the basis for the notifier's determination that a particular use of the notified substance is exempt from the premarket approval requirements of the Federal Food, Drug and Cosmetic Act because such use is GRAS.

Based on a critical review of the scientific evidence, including, e.g., physical and chemical identity information, manufacturing process, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, an independent panel of experts qualified by scientific training and national and international experience concluded that D-tagtose is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of use in foods described

herein. A summary of the basis for the Expert Panel's determination of GRAS status is provided in the enclosed "Expert Panel Consensus Statement" and "Supporting Documentation" by Herbert Blumenthal, Ph.D., John Doull, M.D., Ph.D., Walter Glinsmann, M.D., Michael Levitt, M.D., Ronald Thurman, Ph.D. and Ian Munro, Ph.D. As requested during the pre-submission meeting with FDA, also enclosed is a review of the evidence supporting the safety of D-Tagatose for consumption by persons with hyperuricemia.

IV. Probable Consumption of the Substance

D-Tagatose is intended to be used as a bulk sweetener in the preparation of low- and reduced-calorie foods although it has other technical effects similar to other sugars. Intended food uses and use levels include diet and/or sugar free carbonated beverages and presweetened low calorie ready to drink teas at levels up to 1%, ready to eat breakfast cereals at levels up to 20% (up to 3 g D-tagatose per Reference Amounts Customarily Consumed (RACC), as defined in 21 CFR § 101.12), sugarless chewing gum at levels up to 60%, icings and frostings for baked goods at levels up to 30%, frozen dairy desserts including light ice creams, frozen milk desserts, low- and non-fat yogurt and frozen novelties at levels up to 3%, low/reduced fat, diet, energy, or nutrient-fortified nutrient bars at levels up to 10%, regular and dietetic hard candies at levels up to 15%, dietetic soft candies at levels up to 10%, and meal replacements at levels providing 5 g D-tagatose per serving.

The consumption of D-tagatose from most of the proposed food categories was estimated using the United States Department of Agriculture Continuing Survey of Food Intake By Individuals food consumption database (USDA, 2000). Consumption from sugarless chewing gum was estimated separately using a Market Facts survey of sugarless chewing gum consumption. Consumption from use in meal replacements was also determined separately on the basis of consumption of 5 g D-tagatose per serving and replacement of 1 to 2 meals per day. Since sugarless chewing gum intake estimates were determined in a separate survey, and meal replacements were determined separately, the contribution of sugarless chewing gum and meal replacements on the total D-tagatose consumption was determined by normalization of the sugarless chewing gum and meal replacement consumption estimates.

The estimated mean consumption of users in the total population is 6.6 g/day (0.12 g/kg body weight/day). Heavy consumers (90th percentile) may consume up to 14.9 g/day (0.27 g/kg body weight/day). Heavy users in children aged 2 to 5 years and children aged 6 to 12 years have the largest consumption on a body weight basis, 0.42 g/kg body weight/day and 0.32 g/kg body weight/day, respectively, as a result of their higher requirement for energy. Consumption of D-tagatose was evenly split between meals and snacks consumed over an entire day.

V. Basis for concluding, in light of the data and information described above, that there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food that there is reasonable certainty that the substance is not harmful under the intended conditions of use.

See the attached "Expert Panel Consensus Statement: The Generally Recognized as Safe (GRAS) Status of D-Tagatose" by Herbert Blumenthal, Ph.D., John Doull, M.D., Ph.D., Walter Glinsmann, M.D., Michael Levitt, M.D., Ronald Thurman, Ph.D. and Ian Munro, Ph.D.

VI. References

Bertelsen, H., Andersen, H., and Tvede, M. 2001. Fermentation of D-tagatose by human intestinal bacteria and dairy lactic acid bacteria. Microbial Ecology Health Disease (accepted for publication).

Blumenthal, H., Doull, J., Glinsmann, W., Levitt, M., Thurman, R. and Munro, I. 2001. Expert Panel Consensus Statement: The Generally Recognized as Safe (GRAS) Status of D-Tagatose. (unpublished).

Donner, T., Wilber, J., and Ostrowski, D. 1996. D-Tagatose: A novel therapeutic adjunct for non-insulin-dependent diabetes. Diabetes 45(Suppl. 2):125A.

Imfeld, T. 1996. Telemetric evaluation of D-tagatose provided by MD foods Ingredients Amba, Denmark, with regard to the product's qualification as being "safe for teeth". Dental Institute, University of Zurich, Switzerland.

Jensen, B.B., and Buemann, B. 1998. D-Tagatose. The influence of D-tagatose on bacterial composition and fermentation capacity of faecal samples from human volunteers. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Jensen, B.B., and Laue, A. 1998. D-Tagatose. Absorption of tagatose and fermentation products of tagatose from the gastrointestinal tract of pigs. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba (now Arla Foods amba).

Levin, G.V., Zehner, L.R., Saunders, J.P., and Beadle, J.R. 1995. Sugar substitutes: Their energy values, bulk characteristics, and potential health benefits. Am J Clin Nutr 62(Suppl.):1161S-1168S.

Livesey, G., and Brown, J.C. 1996. D-tagatose is a bulk sweetener with zero energy determined in rats. J Nutr 126:1601-1609.

Noguchi, T., and Muhlemann, H.R. 1976. The effects of some carbohydrates on in vitro growth of Streptococcus mutans and Actinomyces viscosus. Schweiz Mschr Zahnheilk 86:1361/45.

Troyano, E., Olano, A., Fernandez-Diaz, M., Sanz, J., and Martinez-Castro, I. 1991. Gas chromatographic analysis of free monosaccharides in milk. Chromatographia 32(7/8):379-382.

Troyano, E., Villamiel, M., Olano, A., Sanz, J., and Martinez-Castro, I. 1996. Monosaccharides and myo-inositol in commercial milks. J Agric Food Chem 44:815-817.

USDA 2000. 1994-96, 98 Continuing Survey of Food Intakes by Individuals [CD-ROM], data and documentation. Agricultural Research Service, United States Department of Agriculture. National Technical Information Service, Accession No. PB2000-500027.

WHO 2000. D-Tagatose. Safety evaluation of certain food additives and contaminants. Fifty-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series. World Health Organization, Geneva.

Zehner, L.R. 1994. Human exposure to D-tagatose in two lactulose-based drugs. Internal memorandum of Biospherics Inc., Beltsville, MD.

D-TAGATOSE SPECIFICATIONS

Parameter	Specification
Identification	
Solubility	Very soluble in water, very slightly soluble in ethanol
Specific rotation	$[\alpha]^{20}_{D}$ -4 to -5.6° (1% aqueous solution)
Melting range	133-137°C
Reaction with alkaline cupric tartrate	Formation of red cuprous oxide
Chromatography	Retention time of major peak same as standard in chromatographic assay
Purity	
Assay	Not less than 98% on dry basis
Loss on drying	Not more than 0.5% (102oC, 2hr)
Total ash	Not more than 0.1%
Lead	Not more than .5 mg/kg *

* According to FDA's Advanced Notice of Proposed Rulemaking on Reducing Lead Levels in Foods, D-Tagatose is a moderately high-poundage ingredient (estimated **20** million pounds per year). See 59 Fed. Reg. 5363,5368 (Feb. 4,1994).

GRAS Consensus Statement

000031

EXPERT PANEL CONSENSUS STATEMENT: THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE

000032

April 2,2001

EXPERT PANEL CONSENSUS STATEMENT: THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE

As an independent panel of recognized experts (the "Expert Panel"), qualified by our scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, we were asked by Arla Foods Ingredients amba ("Arla") to conduct a comprehensive and critical evaluation of the available pertinent data and information to determine whether the intended use of the D-tagatose in foods would be "generally recognized as safe" ("GRAS") based on scientific procedures. Copies of ***curricula vitae*** evidencing our scientific training and experience, and qualifications for evaluating the safety of food ingredients are provided in Appendix 1.

D-tagatose is intended for use as a bulk sweetener, humectant, texturizer or stabilizer in diet/sugarfree carbonated beverages, low calorie tea beverages, ready-to-eat breakfast cereals, icings and frostings, low/reduced fat diet, energy or nutrient fortified bars, regular and dietetic hard candies, regular and low-fatlnon-fat frozen dairy desserts, sugarfree chewing gum and meal replacements.

We independently and critically evaluated a comprehensive package of publicly available scientific data and information compiled from literature and other published sources, as well as a number of unpublished studies deemed appropriate or necessary. In addition, we evaluated data and information provided by Arla with respect to the chemical and physical properties of D-tagatose, the method of manufacture and stability of D-tagatose, the product specifications and analytical data, and conditions of the intended uses of D-tagatose in foods, including estimated daily intakes and functionality information. A dossier describing in detail the data and information, including confidential manufacturing information, examined by the Expert Panel is provided in Appendix **2.**

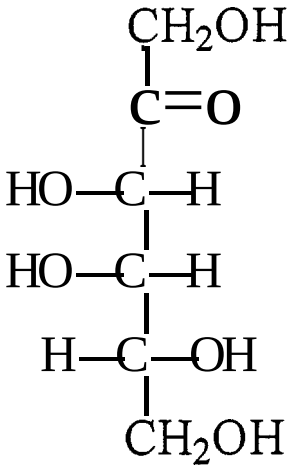
Following independent, critical evaluation of such data and information, we conclude that under the conditions of intended use in foods, D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is "generally recognized as safe" ("GUS") based on scientific procedures. **A** summary of the basis for our conclusion is provided below.

000033

Chemical Identity and Properties

D-Tagatose is a stereo-isomer of D-fructose, which is a common **six** carbon sugar. It is recognized as a component of the regular diet, found in low amounts in heated milk products (Troyano ***et al.***, 1991, 1996). Its relative sweetness in comparison to sucrose is about **75** and **92%** based on a 1 and 10% aqueous solution, respectively. Like other sugars, it can be used as a bulk sweetener, humectant, texturizer or stabilizer (Levin ***et al.***, 1995). D-tagatose has a reduced physiological energy value (Livesey and Brown, 1996), is non-cariogenic (Imfeld, 1996; Noguchi and Muhlemann, 1976), exerts a prebiotic effect (Bertelsen ***et al.***, **2001;** Jensen and Buemann, 1998; Jensen and Laue, 1998), and is not associated with a glycemic response (Donner ***et al.***, 1996; Zehner, 1994).

The empirical formula for D-tagatose is $C_6H_{12}O_6$. The chemical structure of D-tagatose is shown below.



Manufacturing, Composition and Estimated Exposure

Crystalline D-tagatose is manufactured from food grade lactose by a two-step process involving enzymatic hydrolysis (immobilized ***A. oryzae*** lactase) to D-galactose followed by chemical isomerization of D-galactose to D-tagatose, induced by calcium hydroxide, and recrystallization.

All processing aids used in the manufacturing process are considered safe and suitable. The method of manufacture uses processes common to other food ingredients. Potential impurities such as residual D-galactose, talose, aldol condensation products and other impurities arising during the manufacturing process are removed largely by demineralization, chromatographic purification, and in the final crystallization of D-tagatose. None of the compounds present are of toxicological concern.

Appropriate food grade chemical specifications supported by analytical data from several manufactured lots have been derived for crystalline D-tagatose and adopted by the Joint Expert Committee on Food Additives (JECFA) at its fifty-fifth meeting in 2000. Potential microbiological contaminants have been addressed by the development of appropriate internal microbiological specifications.

D-Tagatose is intended to be used as a bulk sweetener in the preparation of low- and reduced-calorie foods although it has other technical effects similar to other sugars. Intended food uses and use levels include diet and/or sugar free carbonated beverages and presweetened low calorie ready to drink teas at levels up to 1%, ready to eat breakfast cereals at levels up to 20% (up to 3 g D-tagatose per Reference Amounts Customarily Consumed (RACC), as defined in 21 CFR § 101.12), sugarless chewing gum at levels up to 60%, icings and frostings for baked goods at levels up to 30%, frozen dairy desserts including light ice creams, frozen milk desserts, low- and non-fat yogurt and frozen novelties at levels up to 3%, low/reduced fat, diet, energy, or nutrient-fortified nutrient bars at levels up to 10%, regular and dietetic hard candies at levels up to 15%, dietetic soft candies at levels up to 10%, and meal replacements at levels providing 5 g D-tagatose per serving.

The consumption of D-tagatose from most of the proposed food categories was estimated using the United States Department of Agriculture Continuing Survey of Food Intake By Individuals food consumption database (USDA, 2000). Consumption from sugarless chewing gum was estimated separately using a Market Facts survey of sugarless chewing gum consumption. Consumption from use in meal replacements was also determined separately on the basis of consumption of 5 g D-tagatose per serving and replacement of 1 to 2 meals per day. Since sugarless chewing gum intake estimates were determined in a separate survey, and meal replacements were determined separately, the contribution of sugarless chewing gum and meal replacements on the total D-tagatose consumption was determined by normalization of the sugarless chewing gum and meal replacement consumption estimates.

The estimated mean consumption of users in the total population is 6.6 g/day (0.12 g/kg body weight/day). Heavy consumers (90th percentile) may consume up to 14.9 g/day (0.27 g/kg body weight/day). Heavy users in children aged 2 to 5 years and children aged 6 to 12 years have the largest consumption on a body weight basis, 0.42 g/kg body weight/day and 0.32 g/kg body weight/day, respectively, as a result of their higher requirement for energy. Consumption of D-tagatose was evenly split between meals and snacks consumed over an entire day.

000035

Metabolism

The available data on D-tagatose demonstrate some absorption of an ingested dose from the small intestine with the remainder passing into the large intestine where it is fermented to short chain fatty acids and gaseous products (CO_2, methane and hydrogen) by the intestinal bacteria (Saunders *et al.*, 1999a; Laerke and Jensen, 1999). Studies of D-tagatose conducted in pigs and in germ-free and conventional rats indicate that about 20 to 30% of ingested D-tagatose is absorbed from the small intestine (Jensen and Laue, 1998; Laerke and Jensen, 1999; Saunders *et al.*, 1999a). Unlike fructose, the absorption of D-tagatose appears to be passive. D-Tagatose does not interfere with the absorption of fructose since it does not bind to the fructose transporters/carriers (Sigrist-Nelson and Hopfer, 1974; Crouzoulon, 1978; Tatibouet *et al.*, 2000).

Absorption data for D-tagatose in humans is limited to a study with ileostomic patients in which subjects ingested single 15 g doses (Normén *et al.*, 2001). About **20%** of the ingested D-tagatose was recovered from the 24-hour ileal effluent. The usefulness of these data with respect to estimating absorption levels in healthy populations is questionable given that similarly high absorption results have been obtained from studies in ileostomates for other polyols including sorbitol, maltitol and isomalt that are generally found to be poorly digested and absorbed (Bär, 1990; Langkilde, 1994).

The D-tagatose absorbed from the small intestine is metabolized by the same biochemical pathway as fructose (Raushel and Cleland, 1977). In the initial step in this pathway, D-tagatose is phosphorylated to D-tagatose-1-phosphate by fructokinase (now termed ketohexokinase). This intermediate is then split by aldolase B to D-glyceraldehyde and dihydroxyacetone phosphate (now termed glycerone phosphate). The rate at which D-tagatose-1-phosphate is split is only about half the rate for D-fructose-1-phosphate due to a lower affinity of aldolase B for this tagatose derivative. In spite of the lower affinity of aldolase B for D-tagatose-1-phosphate, in comparison to D-fructose-1-phosphate, the intracellular concentration **of** this metabolite remains low. This was evidenced by a single dose study, employing magnetic resonance spectroscopy, in healthy humans in which liver concentrations of D-tagatose-1-phosphate did not exceed 1 mM following the ingestion of 30 *g* D-tagatose (Buemann *et al.*, 2000a).

Safety Studies

Animal studies

The safety of D-tagatose has been evaluated in a number of toxicological studies including acute, subchronic (up to six months), genotoxicity and developmental toxicity studies. In addition, special mechanistic studies investigating the potential for liver enlargement associated with high dietary exposure to D-tagatose were conducted. D-Tagatose did not exhibit any genotoxic potential nor were there any indications of

000036

developmental or teratogenic effects associated with dietary exposures. The principle treatment-related effect was increased liver weights in Sprague Dawley rats administered D-tagatose at 10 to 20% in the diet for 90 days (Kruger ***et al.,*** 1999a) and in dams in the reproduction study given D-tagatose at levels upwards of 20% in the diet (Kruger ***et al.,*** 1999c). Hepatocellular hypertrophy was also-observed histopathologically at dietary levels of 15 and 20% in the 90-day study. The no-observed-effect level (NOEL) determined from this study was ***5%.*** Although chronic studies are not available, there is no expectation, given the negative results from genotoxicity assays and its structural similarity to the common sugar fructose, that D-tagatose would possess any carcinogenic potential.

The toxicity of D-tagatose was investigated in a 90-day study in Sprague Dawley rats in which groups of animals (20/sex) were administered diets containing 0, 5, 10, 15 or 20% D-tagatose (Kruger ***et*** *al.,* 1999a,c). An additional "isocaloric" control group was administered a diet containing 10% fructose plus 10% cellulose. Based on the findings, following sacrifice in the fasted condition, of increased liver weights at the 10, 15, and 20% dose levels, and minimal liver hypertrophy at the 15 and 20% dose levels, the NOEL for this study was the ***5%*** dietary level, which corresponded to dose levels of **2.3** to 6.7 g/kg/day in males and 2.8 to 6.3 g/kg/day in females. This range of intakes is because young (6-week old) animals have a higher food intake per unit body weight compared to mature animals. As a result, young animals would receive higher doses than would mature animals.

A six-month sub-chronic oral toxicity study with D-tagatose was conducted in male Wistar rats (60 rats per group) in which treatment groups were fed diets containing 0, ***5,*** or 10% D-tagatose, 10% tagatose and 10% fructose, or 20% fructose (Lina and de Bie, 2000d). Following overnight fasting, ten males of each group were sacrificed after 3 days (subgroup 1), 7 days (subgroup 2), 14 days (subgroup 3), 28 days (subgroup 4), **3** months (subgroup 5), and 6 months (subgroup 6) of treatment. Administration of D-tagatose in the diet at levels up to 10% was not associated with any adverse effects including changes in absolute or relative liver weights or histopathological changes at any stage.

Effects of D-tagatose on the livers of pigs were evaluated as part of an energy balance study (Mann, 1997). In this study, four groups of pigs (n=2) were fed diets containing regular pig diet (0% added sucrose/D-tagatose), 20% sucrose, 20% D-tagatose ***(–5*** g/kg bw/day), or a mixture of 10% sucrose and 10% D-tagatose for 33 days. Animals were killed about ***6*** hours after their last feeding. No histopathological changes were observed in liver tissues in either of the groups receiving D-tagatose.

D-Tagatose **was** found to be inactive in genotoxicity tests (Kruger ***et al.,*** 1999b), including: (i) Ames ***Salmonella typhimurium*** reverse mutation assay, (ii) ***Escherichia*** *coli*/mammalian microsome assay, (iii) mammalian chromosomal aberration assay in

Chinese hamster ovary cells, (iv) forward mutation assay in mouse lymphoma L5**178** Y cells, and (v) ***in vivo*** mouse micronucleus assay.

In a developmental study (Kruger ***et al.,*** 1999c), four groups of 24 pregnant Sprague-Dawley rats were administered D-tagatose ***via*** gastric intubation at doses of 0, 4, 12, or 20 g/kg/day on Days 6 to 15 of gestation. No adverse effects on reproductive performance, fetal weight, sex distribution, liver weight, or on external, skeletal, or visceral malformations, were reported at any dose level. Maternal absolute liver weights were increased at the high dose and mean relative liver weights were increased in the mid- and high-dose groups.

In a series of three Sprague-Dawley rat feeding studies (Lina and de Bie, 1998a; Lina and de Bie, 1998b; Lina ***et al.,*** 1998), published in Bar ***et al.*** (1999), that investigated the characteristics of D-tagatose-induced liver enlargement, it was found that dietary administration of ***5*** to 20% D-tagatose for up to 28 days resulted in significant liver enlargement and glycogen deposition in rats sacrificed in the non-fasted condition. However, at the 5% level, no significant liver enlargement was observed in rats sacrificed in the fasted condition (Lina and de Bie, 1998a,b). Further, it appears that liver enlargement in rats treated with D-tagatose at 20% in the diet for 4-weeks and not subject to fasting prior to sacrifice was partly reversible upon cessation of administration for 14 days. Glycogen deposition alone cannot explain the liver enlargement/hypertrophy in fasted Sprague-Dawley rats administered D-tagatose at concentrations greater than 5% in the diet. In Sprague-Dawley rats administered D-tagatose at 20% in the diet for 4-weeks and not fasted prior to sacrifice, reported increases in DNA and protein per liver (expressed as mg/liver) are consistent with the conclusion that growth of the liver tissue occurred (Lina and de Bie, 1998a). Also, in the livers of rats administered D-tagatose at 15 and 20% in the diet in the 90-day subchronic toxicity study (Kruger ***et al.,*** 1999a), the use **of** periodic acid-Schiff stain demonstrated that despite depletion of liver glycogen, microscopic evidence of hepatocellular hypertrophy remained.

The results **of** three additional unpublished rat feeding studies (Lina and de Bie, 2000a,b,c) similarly indicate that liver enlargement can be explained only in part by glycogen deposition.
Liver enlargement is also seen in rats fed large amounts of fructose, although at about 4 times higher doses than D-tagatose (Bar, 1999). It should be noted; however, that it is not known if the liver enlargement produced by fructose ingestion is solely related to glycogen deposition.

Human data

Effects associated with dietary consumption of D-tagatose were investigated in several short duration single dose and repeated dose clinical trials (up to **28** days exposure) in which healthy and/or diabetic subjects were administered doses between 15 and 75 g/day

(Buemann *et al.*, 1999a,b,c, 2000b; Donner *et al.*, 1999; Lee and Storey, 1999; Saunders *et al.*, 1999b; Boesch *et al.*, 2001). The predominant effects associated with excessive consumption were physiological gastrointestinal effects attributed to an osmotic effect from incomplete absorption of D-tagatose. Such effects also are commonly associated with excessive consumption of other poorly digestible sugars and polyols. At doses of up to 25 g D-tagatose per meal, flatulence was generally the only side effect reported. Other symptoms noted at higher consumption levels included nausea, borborygmi (*i.e.*, rumbling or gurgling noises in the gut), colic, and laxation. Some of these symptoms are characteristically observed following the consumption of poorly absorbed carbohydrates.

Effects of D-tagatose on the volume of the human liver and postprandial liver glycogen concentration were assessed in a clinical trial involving 12 healthy male volunteers and employing a double-blind, two-way crossover study design (Boesch *et al.*, 2001). D-Tagatose (test substance) or sucrose (placebo) was administered in the diet in amounts of 15 g at each meal (total of **45** g/day) for **28** days each with a washout period of **28** days. Liver volume and glycogen levels were measured using magnetic resonance imaging and magnetic resonance spectroscopy, respectively. No significant differences in clinical chemistry including plasma uric acid levels, hematological parameters, or urinary endpoints were noted between sucrose and tagatose fed subjects. Liver volumes increased significantly over time with both treatments, however, there was no difference between individuals given D-tagatose and those given sucrose. A comparison of liver volumes on Day 1, as measured before and after breakfast, indicated no significant changes associated with short term exposures for either D-tagatose or sucrose. Post-prandial liver glycogen concentrations after the prolonged consumption of D-tagatose or sucrose did not differ significantly from those recorded during pretreatment.

Consumption of D-tagatose, as with fructose, has the potential to increase plasma concentrations of uric acid as a result of the activity of fructokinase, in the presence of ATP, on these substrates. Phosphorylation of D-tagatose or fructose transiently decreases the hepatic ATP and P_i pools. Since P_i is an inhibitor of adenosine deaminase, a rate-limiting enzyme in the catabolism of adenosine monophosphate, phosphorylation of D-tagatose or fructose may result in increased degradation of purine nucleotides and increased release **of** uric acid from the liver.

Three clinical studies including single and repeated dose studies with D-tagatose in comparison to fructose, sucrose or glucose have evaluated potential uricemic effects. Ingestion of a single 15-gram dose of D-tagatose had no effect on serum uric acid concentrations in comparison to a similar dose of sucrose (Boesch *et al.*, 2001). Ingestion of single oral doses of 30 **grams** of D-tagatose (in solution) by 8 fasted volunteers increased serum uric acid. However, this increase remained within the stated normal range (Buemann *et al.*, 1999b). By comparison, fructose was reported to produce smaller increases in plasma uric acid. Larger, single oral doses of 75 grams of D-tagatose

in healthy volunteers and type 2 diabetics did elevate serum uric acid levels (Saunders *et al.*, 1999b).

In a repeat dosing phase in which D-tagatose (to both diabetics and normals) or glucose (normals only) was administered as 3 separate 25 gram doses at meal times for an 8-week period, no significant effects on fasting plasma uric acid levels were found compared to baseline values. Consumption of 45 grams of D-tagatose by healthy male volunteers as 3 separate 15 gram doses did not alter fasting serum uric acid concentrations in comparison to consumption of equal amounts of sucrose (Boesch *et al.*, 2001). As consumed in food, the dosing pattern of the Boesch *et al.* (2001) study is most relevant to the assessment of D-tagatose on serum uric acid in humans.

Conclusions

The estimated mean consumption of D-tagatose from the proposed food uses is approximately 6.6 g/day with heavy users (90th percentile) consuming up to 14.9 g/day. On a body weight basis, consumption of D-tagatose is highest in children aged 2 to 5 years (0.42 g/kg bw/day). The corresponding 90th percentile g/day intake estimate for this age group is 7.4 g/day.

Data supporting the safety of D-tagatose include several clinical trials in healthy and diabetic volunteers, and toxicology studies including acute, subchronic, genotoxicity and developmental toxicity studies. In animals, the only notable treatment-related effects were increased liver weights observed in Sprague Dawley rats administered 10 to 20% D-tagatose in the diet for 90 days and hepatocellular hypertrophy at dietary levels of 15 and 20%, in this same study. The NOEL determined from this study was 5% (2.3 to 6.7 g/kg/day). For liver enlargement, specifically, although the mechanism is not entirely clear, it is important to note that (i) this effect was not associated with any adverse clinical symptoms, (ii) there was no histopathological evidence of overt hepatotoxicity at any dose, and (iii) the results of the clinical trial in humans did not indicate a difference in liver weight or glycogen concentration between D-tagatose and sucrose exposures. Moreover, all effects noted in rats for D-tagatose have been reported for fructose as well, and, although D-tagatose was demonstrated to be about 4 times more potent than fructose, the anticipated consumption levels of D-tagatose are almost 6 times lower.

Other issues requiring consideration were the potential for increased plasma uric acid concentration (this effect has been associated with fructose) and the possibility of metabolic interactions between fructose and D-tagatose when co-ingested. With respect to uric acid concentrations, the results of clinical trials demonstrated that fasting plasma uric acid levels were not affected by consumption of D-tagatose in food at doses up to 45 grams per day, and that the changes observed at single doses in the range of 75 grams per day were mild and did not approach serum concentrations of 15 to 20 mg/100 ml associated with uric acid nephropathy. There was no difference in post-prandial serum

uric acid levels after intake of single doses of 15 grams D-tagatose or 15 grams sucrose (Boesch *et al.*, 2001). With regard to metabolic interactions, enzyme kinetic considerations and the results from a liver perfusion study indicate that the levels of D-tagatose from the proposed uses would not be sufficient to interfere with the metabolism of fructose. Finally, based on the available animal studies in which fructose and D-tagatose were co-administered, there is no indication of an additive or synergistic effect of D-tagatose and fructose on clinical measurements of liver function (Saunders, 1992; Lina and de Bie, 2000d).

In summary, the safety assessment of D-tagatose, under the intended conditions of use and consequent consumption levels, was based upon the presence of a 10- to 20-fold safety factor between estimated intake levels and the 5% dietary NOEL for liver enlargement established in Sprague-Dawley rats. In addition, the results of a human clinical trial in which groups of individuals were given 45 grams of D-tagatose per day for 28 days did not indicate any significant increase in liver volume in comparison to controls given sucrose, nor did D-tagatose have any effect on post-prandial liver glycogen concentrations.

Supporting corroborative data, indicative of a possible species- and or strain-specific effect of D-tagatose on the liver, include the finding of no liver enlargement in either Wistar rats fed D-tagatose at 10% in the diet for 6 months or in pigs administered D-tagatose at **20%** in the diet for **33** days. It should be noted, however, that differences in the durations of the studies and in the dose levels used in these studies do not permit a direct quantitative comparison of the liver effects in Wistar and Sprague-Dawley rats. Finally, although the mechanism for potential effects of D-tagatose on uric acid is conjectural, any possible effect of D-tagatose on uric acid, even of a mild nature, would occur only at exposures several fold in excess of the 90th percentile estimated daily intake.

In conclusion, the available data demonstrate that the anticipated consumption levels of D-tagatose from the proposed uses in food, even at the 90th percentile, are not associated with any adverse health effects in humans and should be considered safe.

Expert Panel Consensus Statement

Based on our critical review of the scientific evidence, including, e.g., physical and chemical identity information, the manufacturing process, stability data, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, we, as members of an Expert Panel specifically convened for this purpose, conclude that D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of intended use in foods specified herein.

BY:

Herbert Blumenthal, Ph.D.	Date 4/2/01
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date
Ronald G. Thurman, Ph.D.	Date

000042

Expert Panel Consensus Statement

Based on our critical review of the scientific evidence, including, e.g., physical and chemical identity information, the manufacturing process, stability data, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, we, as members of an Expert Panel specifically convened for this purpose, conclude that D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of intended use in foods specified herein.

BY:

Herbert Blumenthal	Date
[redacted]	
John Doull, M.D., Ph.D.	April 3, 2001 Date
[redacted]	
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date
Ronald G. Thurman, Ph.D.	Date

000043

Expert Panel Consensus Statement

Based on our critical review of the scientific evidence, including, e.g., physical and chemical identity information, the manufacturing process, stability data, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, we, as members of an Expert Panel specifically convened for this purpose, conclude that D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of intended use in foods specified herein.

BY:

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date 05 April 2001
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date
Ronald G. Thurman, Ph.D.	Date

000044

Panel Consensus Statement

Based on our critical review of the scientific evidence, including, e.g., physical and chemical identity information, the manufacturing process, stability data, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, we, as members of an Expert Panel specifically convened for this purpose, conclude that D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of intended use in foods specified herein.

BY:

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt,	4/9/01 Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date
Ronald G. Thurman, Ph.D.	Date

000045

Expert Panel Consensus Statement

Based on our critical review of the scientific evidence, including, e.g., physical and chemical identity information, the manufacturing process, stability data, publicly available safety data, comborating unpublished safety data, intended uses and consumption estimates, we, as members of an Expert Panel specifically convened for this purpose, conclude that D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of intended use in foods specified herein.

BY:

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	April 9, 2001 Date
Ronald G. Thurman, Ph.D.	Date

000046

Pane, Consensus Statement

Based on our critical review of the scientific evidence, including, e.g., physical and chemical identity information, the manufacturing process, stability data, publicly available safety data, corroborating unpublished safety data, intended uses and consumption estimates, we, as members of an Expert Panel specifically convened for this purpose, conclude that D-tagatose, meeting appropriate food grade specifications and manufactured in accordance with current good manufacturing practices, is generally recognized as safe ("GRAS"), based on scientific procedures, under the conditions of intended use in foods specified herein.

BY

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date
Ronald G. Thurman, Ph.D.	4-7-01 Date

000047

References

Bar, A. 1990. Factorial calculation model for the estimation of the physiological caloric value of polyols. Proc. Int. Symposium on Caloric Evaluation of Carbohydrates. Hosoya, N. (Ed.), The Japan Assoc. Dietetic & Enriched Foods, Tokyo, pp. 209-257.

Bar, A. 1999. Characteristics and significance of D-tagatose-induced liver enlargement in rats: An interpretative review. Regul Toxicol Pharmacol 29(2, 2/2):S83-S93.

Bertelsen, H., Andersen, H., and Tvede, M. 2001. Fermentation of D-tagatose by human intestinal bacteria and dairy lactic acid bacteria. Microbial Ecology Health Disease (accepted for publication).

Boesch, C., Diamantis, I., and Bär, A. 2001. Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. Regul Toxicol Pharmacol, accepted for publication.

Buemann, B., Toubro S., and Astrup, A. 1999a. Human gastrointestinal tolerance to D-tagatose. Regul Toxicol Pharmacol 29(2, 2/2):S71-S77.

Buemann, B., Toubro, S., Holst, J.J., Rehfeld, J., and Astrup, A. 1999b. D-Tagatose, a stereoisomer of D-fructose, increases blood uric acid concentration. Metabolism 49(8):969-976.

Buemann, B., Toubro, S., Raben, A., and Astrup, A. 1999c. Human tolerance to single, high dose of D-tagatose. Regul Toxicol Pharmacol 29(2, 2/2):S66-S70.

Buemann, B., Gesmar, H., Astrup, A., and Quistorff, B. 2000a. Effects of oral D-tagatose, a stereoisomer of D-fructose, on liver metabolism in man as examined by ^{31}PMRS. Metabolism 49(10):1335-1339.

Buemann, B., Toubro, S., Raben, A., Blundell, J., and Astrup, A. 2000b. The acute effect of D-tagatose on food intake in human subjects. Br J Nutr 84:227-231.

Crouzoulon, G. 1978. Les proprietes cinétiques du flux d'entrée du fructose a travers la bordure en brosse du jéjunum du rat. Arch Int Physiol. Biochim 86:725-740.

000048

Donner, T., Wilber, J., and Ostrowski, D. 1996. D-Tagatose: A novel therapeutic adjunct for non-insulin-dependent diabetes. Diabetes 45(Suppl. 2):125A.

Donner, T.W., Wilber, J.F., and Ostrowski, D. 1999. D-Tagatose, a novel hexose: Acute effects on carbohydrate tolerance in subjects with and without type 2 diabetes. Diabetes, Obesity and Metabolism 1:285-291.

Imfeld, T. 1996. Telemetric evaluation of D-tagatose provided by MD foods Ingredients Amba, Denmark, with regard to the product's qualification as being "safe for teeth". Dental Institute, University of Zurich, Switzerland.

Jensen, B.B., and Buemann, B. 1998. D-Tagatose. The influence of D-tagatose on bacterial composition and fermentation capacity of faecal samples from human volunteers. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Jensen, B.B., and Laue, A. 1998. D-Tagatose. Absorption of tagatose and fermentation products of tagatose from the gastrointestinal tract of pigs. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Kruger, C.L., Whittaker, M.H., Frankos, V.H., and Trimmer, G.W. 1999a. 90-Day oral toxicity study of D-tagatose in rats. Regul Toxicol Pharmacol 29(2, 2/2):S1-S10.

Kruger, C.L., Whittaker, M.H., and Frankos, W.H. 1999b. Genotoxicity tests on D-tagatose. Regul Toxicol Pharmacol 29(2, 2/2):S36-S42.

Kruger, C.L., Whittaker, M.H., Frankos, V.H., and Schroeder, R.E. 1999c. Developmental toxicity study of D-tagatose in rats. Regul Toxicol Pharmacol 29(2, 2/2):S29-S35.

Laerke, H.N., and Jensen, B.B. 1999. D-Tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. J Nutr 129:1002-1009.

Langkilde, A.M. 1994. Digestion and absorption of sorbitoll, maltitol and isomalt form the small bowel. A study in ileostomy subjects. Eur J Clin Nutr 48:768-775.

Lee, A., and Storey, D.M. 1999. Comparative gastrointestinal tolerance of sucrose, lactitol, or D-tagatose in chocolate. Regul Toxicol Pharmacol 29(2, 2/2):S78-S82.

Levin, G.V., Zehner, L.R., Saunders, J.P., and Beadle, J.R. 1995. Sugar substitutes: Their energy values, bulk characteristics, and potential health benefits. Am J Clin Nutr 62(Suppl.):1161S-1168S.

Lina, B.A.R., Appel, M.J., de Groot, D.M.G., and Salmon, F.G.Ch. 1998. Oral toxicity study with D-tagatose in rats. Unpublished report No. V98.728 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 1998a. Short-term oral feeding study with tagatose in rats. Unpublished report No. V98.500 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 1998b. Oral toxicity study with D-tagatose in rats. Unpublished report No. V98.927 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000a. Investigation into the consequences of administering D-tagatose spaced from, and simultaneously with, the ingestion of precursors of glycogen in Wistar rats (7-day study). Unpublished report No. V99.1094 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000b. Investigation into the consequences of administering D-tagatose spaced from, and simultaneously with, the ingestion of precursors of glycogen in male Sprague Dawley rats. Unpublished report No. V2529 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000c. Investigation into the consequences of administering D-tagatose spaced from, and simultaneously with, the ingestion of precursors of glycogen in male Sprague-Dawley rats (28-day study). Unpublished report No. V2678 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000d. Investigation into the consequences of feeding D-tagatose and fructose on liver parameters in Wistar rats. Unpublished report No. V99.1123 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Livesey, G., and Brown, J.C. 1996. D-tagatose is a bulk sweetener with zero energy determined in rats. J Nutr 126:1601-1609.

Mann, P.C. 1997. Light microscopic and ultrastructural analysis of livers of pigs fed tagatose. Unpublished pathology report of Experimental Pathology Laboratories Inc., Research Triangle Park, NC, USA.

Noguchi, T., and Muhlemann, H.R. 1976. The effects of some carbohydrates on in vitro growth of Streptococcus mutans and Actinomyces viscosus. Schweiz Mschr Zahnheilk 86:1361/45.

Normén, L., Laerke, H.N., Langkilde, A.-M., and Anderson, H. 2001. Small bowel absorption of D-tagatose and related effects on carbohydrate digestibility. An ileostomy study. Am J Clin Nutr 73(1):105-110.

Raushel, F.M., and Cleland, W.W. 1977. Bovine liver fructokinase: Purification and kinetic properties. Biochem 16(10):2169-2175.

Saunders, J.P. 1992. Multiple fat rat study. Unpublished report of Biospherics, Inc., Beltsville, MD.

Saunders, J.P., Zehner, L.R., and Levin, G.V. 1999a. Disposition of D-[U-^{14}C] tagatose in the rat. Regul Toxicol Pharmacol 29(2, 2/2):S46-S56.

Saunders, J.P., Donner, T.W., Sadler, J.H., Levin, G.V., and Makris, N.G. 1999b. Effects of acute and repeated oral doses of D-tagatose on plasma uric acid in normal and diabetic humans. Regul Toxicol Pharmacol 29(2,2/2):S57-S65.

Sigrist-Nelson, K., and Hopfer, U. 1974. A distinct D-fructose transport system in isolated brush border membrane. Biochim Biophys Acta 367:247-254.

Tatibouet, A., Yang, J., Morin, C., and Holman, G.D. 2000. Synthesis and evaluation of fructose analogues as inhibitors of the D-fructose transporter GLUTS. Bioorg Med Chem 8:1825-1833.

Troyano, E., Olano, A., Fernández-Díaz, M., Sanz, J., and Martinez-Castro, I. 1991. Gas chromatographic analysis of free monosaccharides in milk. Chromatographia 32(7/8):379-382.

Troyano, E., Villamiel, M., Olano, A., Sanz, J., and Martinez-Castro, I. 1996. Monosaccharides and myo-inositol in commercial milks. J Agric Food Chem 44:815-817.

USDA 2000. 1994-96, 98 Continuing Survey of Food Intakes by Individuals [CD-ROM], data and documentation. Agricultural Research Service, United States Department of Agriculture. National Technical Information Service, Accession No. PB2000-500027.

Zehner, L.R. 1994. Human exposure to D-tagatose in two lactulose-based drugs. Internal memorandum of Biospherics Inc., Beltsville, MD.

000052

Supporting Documentation

000053

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE AS A FOOD INGREDIENT

000054

March 30, 2001

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE AS A FOOD INGREDIENT

Table of Contents

		Page
EXECUTIVE SUMMARY		i
1.0	INTRODUCTION	1
2.0	MANUFACTURING AND COMPOSITION	1
3.0	SPECIFICATIONS	3
4.0	PROPOSED USES AND ESTIMATED CONSUMPTION	3
5.0	SUMMARY OF SAFETY DATA	5
5.1	Metabolic Data	5
5.2	Toxicological Studies	7
5.2.1	*Animal Data*	7
5.2.2	*Human Data*	14
5.3	Special Studies	19
5.3.1	*Special Studies on Liver Enlargement*	19
6.0	CRITICAL ENDPOINTS FOR THE SAFETY OF D-TAGATOSE	24
6.1	GI Effects	24
6.2	Liver Effects	25
6.3	Plasma Uric Acid Effect	26
6.4	D-Tacatosc and Fructose Interaction	27
6.5	Glycation of Proteins	29
7.0	DISCUSSION AND CONCLUSION	30
8.0	REFERENCES	32

List of Tables

Table 4-1	Summary of Estimated D-tagatose Users Consumption (G/day) from **All** Proposed Food Uses in Different U.S. Population Groups	5
Table 4-2	Summary of Estimated D-Tagatose Users Consumption (g/kg bw/day) from **All** Proposed Food Uses in Different U.S. Population Groups	5
Table 5-1	Dose Levels Associated with Dietary Concentration of D-Tagatose in a 90 Day Study in Sprague Dawley Rats	9
Table 5-2	Relative Liver Weight Data of Sprague Dawley Rats Fed Diets Containing up to 20% D-Tagatose or 20% Fructose/Cellulose for 90 Days	11
Table 5-3	Summary ol'Genotoxicity Studies with D-Tagatose	13
Table 5-4	Treatment Groups (Lina and de Bie, 2000a)	21
Table 5-5	Mean Liver Weights and Liver Glycogen Levels (Lina and de Bie, 2000a)	21
Table 5-6	Treatment Groups (Lina and de Bie, 2000b)	22
Table 5-7	Treatment Groups (Lina and de Bie, 2000c)	23
Table 5-8	Absolute Values lor Liver Parameters (Lina and de Bie, 2000c)	24
Table A-1	Specifications for D-Tagatose	A-1
Table B-3	Proposed Food Uses and Use-Levels for D-Tagatose	B-1

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE AS A FOOD INGREDIENT

EXECUTIVE SUMMARY

D-Tagatose is a stereo-isomer of D-fructose, which is a common six carbon sugar. It is recognized as a component of the regular diet, found in low amounts in heated milk products (Troyano et *al*., 1991, 1996). Its relative sweetness in comparison to sucrose is about 75 and 92% based on a 1 and 10% aqueous solution, respectively. Like other sugars, it can be used as a bulk sweetener, humectant, texturizer or stabilizer (Levin *et* al., 1995). D-Tagatose has a reduced physiological energy value, is non-cariogenic, exerts a prebiotic effect, and is not associated with a glycemic response (Zehner, 1994; Donner *et* ul., 1996).

Manufacturing. Composition and Estimated Exposure

Crystalline D-tagatose is manufactured from food grade lactose by a two-step process involving enzymatic hydrolysis (immobilized *A. oryzae* lactase) to D-galactose followed by chemical isomerization of D-galactose to D-tagatose, induced by calcium hydroxide, and recrystallization. All processing aids used in the manufacturing process are considered safe and suitable. The method of manufacture uses processes common to other food ingredients. Potential impurities such as residual D-galactose, talose, aldol condensation products and other impurities arising during the manufacturing process are removed largely by demineralization, chromatographic purification, and in the final crystallization of D-tagatose. None of the compounds present are of toxicological concern.

Appropriate food grade chemical specifications supported by analytical data from several manufactured lots have been derived for crystalline D-tagatose and adopted by the Joint Expert Committee on Food Additives (JECFA) at its fifty-fifth meeting in 2000 (WHO, 2000). Potential microbiological contaminants have been addressed by the development of appropriate internal microbiological specifications.

D-Tagatose is intended to be used as a bulk sweetener in the preparation of low- and reduced-calorie foods although it has other technical effects similar to other sugars. Intended food uses and use levels include diet and/or sugar free carbonated beverages and presweetened low calorie ready to drink teas at levels up to 1%, ready to eat breakfast cereals at levels up to 20% (up to 3 g

D-tagatose per Reference Amounts Customarily Consumed (RACC), as defined in 21 C.F.R. § 101.12), sugarless chewing gum at levels up to 60%, icings and frostings for baked goods at levels up to 30%, frozen dairy desserts including light ice creams, frozen milk desserts, low- and non-fat yogurt and frozen novelties at levels up to 3%, low/reduced fat, diet, energy, or nutrient-fortified nutrient bars at levels up to 10%, regular and dietetic hard candies at levels up to 15%, dietetic soft candies at levels up to 10%, and meal replacements at levels providing 5 g D-tagatose per serving.

The consumption of D-tagatose from most of the proposed food categories was estimated using the United States Department of Agriculture Continuing Survey of Food Intake By Individuals food consumption database (USDA, 2000). Consumption from sugarless chewing gum was estimated separately using a Market Facts survey of sugarless chewing gum consumption. Consumption from use in meal replacements was also determined separately on the basis of consumption of 5 g D-tagatose per serving and replacement of 1 to 2 meals per day. Since sugarless chewing gum intake estimates were determined in a separate survey, and meal replacements were determined separately, the contribution of sugarless chewing gum and meal replacements on the total D-tagatose consumption was determined by normalization of the sugarless chewing gum and meal replacement consumption estimates.

The estimated mean consumption of users in the total population is 6.6 g/day (0.12 g/kg body weight/day). Heavy consumers (90th percentile) may consume up to 14.9 g/day (0.27 g/kg body weight/day). Heavy users in children aged 2 to 5 years and children aged 6 to 12 years have the largest consumption on a body weight basis, 0.42 g/kg body weight/day and 0.32 g/kg body weight/day, respectively, as a result of their higher requirement for energy. Consumption of D-tagatose was evenly split between meals and snacks consumed over an entire day.

Metabolism

The available data on D-tagatose demonstrate some absorption of an ingested dose from the small intestine with the remainder passing into the large intestine where it is fermented to short chain fatty acids and gaseous products (CO_2, methane and hydrogen) by the intestinal bacteria (Saunders *et al.*, 1999a; Laerke and Jensen, 1999). Studies of D-tagatose conducted in pigs and in germ-free and conventional rats indicate that about 20 to 30% of ingested D-tagatose is absorbed from the small intestine (Jensen and Laue, 1998; Laerke and Jensen, 1999; Saunders *et al.*, 1999a). Unlike fructose, the absorption of D-tagatose appears to be passive. D-Tagatose

does not interfere with the absorption of fructose since it does not bind to the fructose transporters/carriers (Sigrist-Nelson and Hopfer, **1974;** Crouzoulon, **1978;** Tatibouet ***et ul.***, 2000).

Absorption data for D-tagatose in humans is limited to a study with ileostomic patients in which subjects ingested single **15** g doses (Normén *et* ***al.,*** 2001). About 20% of the ingested D-tagatose was recovered from the 24-hour ileal effluent. The usefulness of these data with respect to estimating absorption levels in healthy populations is questionable given that similarly high absorption results have been obtained from studies in ileostomates for other polyols including sorbitol, maltitol and isomalt that are generally found to be poorly digested and absorbed (Bar, **1990;** Langkilde, **1994).**

The D-tagatose absorbed from the small intestine is metabolized by the same biochemical pathway as fructose (Raushel and Cleland, **1977).** In the initial step in this pathway, D-tagatose is phosphorylated to D-tagatose-1-phosphate by fructokinase (now termed ketohexokinase). This intermediate is then split by aldolase B to D-glyceraldehyde and dihydroxyacetone phosphate (now termed glycerone phosphate). The rate at which D-tagatose-1-phosphate is split is only about half the rate for D-fructose-1-phosphate due to a lower affinity of aldolase B for this tagatose derivative. In spite of the lower affinity of aldolase B for D-tagatose-1-phosphate, in comparison to D-fructose-1-phosphate, the intracellular concentration of this metabolite remains low. This was evidenced by a single dose study, employing magnetic resonance spectroscopy, in healthy humans in which liver concentrations of D-tagatose-1-phosphate did not exceed 1 mM following the ingestion of **30** g D-tagatose (Buemann ***et al.,*** 2000a).

Safety Studies

Animal studies

The safety of D-tagatose has been evaluated in a number of toxicological studies including acute, subchronic (up to six months), genotoxicity and developmental toxicity studies. In addition, special mechanistic studies investigating the potential for liver enlargement associated with high dietary exposure to D-tagatose were conducted. D-Tagatose did not exhibit any genotoxic potential nor were there any indications of developmental or teratogenic effects associated with dietary exposures. The principle treatment-related effect was increased liver weights in Sprague Dawley rats administered D-tagatose at **10** to 20% in the diet for **90** days (Kruger ***et al.,*** **1999a)**

and in dams in the reproduction study given D-tagatose at levels upwards of 20% in the diet (Kruger *et al.*, 1999c). Hepatocellular hypertrophy was also observed histopathologically at dietary levels of 15 and 20% in the 90-day study. The no-observed-effect level (NOEL) determined from this study was 5%. Although chronic studies are not available, there is no expectation, given the negative results from genotoxicity assays and its structural similarity to the common sugar fructose, that D-tagatose would possess any carcinogenic potential.

The toxicity of D-tagatose was investigated in a 90-day study in Sprague Dawley rats in which groups of animals (20/sex) were administered diets containing 0, 5, 10, 15 or 20% D-tagatose (Kruger *et al.*, 1999a,c). An additional "isocaloric" control group was administered a diet containing 10% fructose plus 10% cellulose. Based on the findings, following sacrifice in the fasted condition, of increased liver weights at the 10, 15, and 20% dose levels, and minimal liver hypertrophy at the 15 and 20% dose levels, the NOEL for this study was the 5% dietary level, which corresponded to dose levels of 2.3 to 6.7 g/kg/day in males and 2.8 to 6.3 g/kg/day in females. This range of intakes is because young (6-week old) animals have a higher food intake per unit body weight compared to mature animals. As a result, young animals would receive higher doses than would mature animals.

A six-month sub-chronic oral toxicity study with D-tagatose was conducted in male Wistar rats (60 rats per group) in which treatment groups were fed diets containing 0, 5, or 10% D-tagatose, 10% tagatose and 10% fructose, or 20% fructose (Lina and de Bie, 2000d). Following overnight fasting, ten males of each group were sacrificed after 3 days (subgroup 1), 7 days (subgroup 2), 14 days (subgroup 3), 28 days (subgroup 4), 3 months (subgroup 5), and 6 months (subgroup 6) of treatment. Administration of D-tagatose in the diet at levels up to 10% was not associated with any adverse effects including changes in absolute or relative liver weights or histopathological changes at any stage.

Effects of D-tagatose on the livers of pigs were evaluated as part of an energy balance study (Mann, 1997). In this study, four groups of pigs (n=2) were fed diets containing regular pig diet (0% added sucrose/D-tagatose), 20% sucrose, 20% D-tagatose (~5 g/kg bw/day), or a mixture of 10% sucrose and 10% D-tagatose for 33 days. Animals were killed about 6 hours after their last feeding. No histopathological changes were observed in liver tissues in either of the groups receiving D-tagatose.

000060

D-Tagatose was found to be inactive in genotoxicity tests (Kruger *et* ul., 1999b), including: (i) Ames Salmonella typhimurium reverse mutation assay, (ii) Escherichiu *coli*/mammalian microsome assay, (iii) mammalian chromosomal aberration assay in Chinese hamster ovary cells, (iv) forward mutation assay in mouse lymphoma L5**178** Y cells; and, (v) in vivo mouse micronucleus assay.

In a developmental study (Kruger et ***al.,*** 1999c), four groups of 24 pregnant Sprague-Dawley rats were administered D-tagatose *via* gastric intubation at doses of 0, 4, 12, or 20 g/kg/day on Days ***6*** to 15 of gestation. No adverse effects on reproductive performance, fetal weight, sex distribution, liver weight, or on external, skeletal, or visceral malformations, were reported at any dose level. Maternal absolute liver weights were increased at the high dose and mean relative liver weights were increased in the mid- and high-dose groups.

In a series of three Sprague-Dawley rat feeding studies (Lina and de Bie, 1998a; Lina and de Bie, 1998b; Lina et ***al.,*** 1998), published in Bär et al. (1999), that investigated the characteristics of D-tagatose-induced liver enlargement, it was found that dietary administration of 5 to 20% D-tagatose for up to **28** days resulted in significant liver enlargement and glycogen deposition in rats sacrificed in the non-fasted condition. However, at the **5%** level, no significant liver enlargement was observed in rats sacrificed in the fasted condition (Lina and de Bie, 1998a,b). Further, it appears that liver enlargement in rats treated with D-tagatose at 20% in the diet for 4 weeks and not subject to fasting prior to sacrifice was partly reversible upon cessation of administration for 14 days. Glycogen deposition alone cannot explain the liver enlargement/hypertrophy in fasted Sprague-Dawley rats administered D-tagatose at concentrations greater than 5% in the diet. In Sprague-Dawley rats administered D-tagatose at **20%** in the diet for **4** weeks and not fasted prior to sacrifice, reported increases in DNA and protein per liver (expressed as mg/liver) are consistent with the conclusion that growth of the liver tissue occurred (Lina and de Bie, 1998a). Also, in the livers of rats administered D-tagatose at 15 and 20% in the diet in the 90-day subchronic toxicity study (Kruger *et al.*, 1999a), the use of periodic acid-Schiff stain demonstrated that despite depletion of liver glycogen, microscopic evidence of hepatocellular hypertrophy remained.

The results of three additional unpublished rat feeding studies (Lina and de Bie, 2000a,b,c) similarly indicate that liver enlargement can be explained only in part by glycogen deposition.

Liver enlargement is also seen in rats fed large amounts of fructose, although at about **4** times higher doses than D-tagatose (Bar, 1999). It should be noted; however, that it is not known if the liver enlargement produced by fructose ingestion is solely related to glycogen deposition.

Human data

Effects associated with dietary consumption of D-tagatose were investigated in several short duration single dose and repeated dose clinical trials (up to **28** days exposure) in which healthy and/or diabetic subjects were administered doses between 15 and 75 g/day (Buemann *et al.*, 1999a,b,c, **2000b**; Donner *et al.*, 1999; Lee and Storey, 1999; Saunders ***et al.***, 1999b; Boesch *et al.*, 2001). The predominant effects associated with excessive consumption were physiological gastrointestinal effects attributed to an osmotic effect from incomplete absorption of D-tagatose. Such effects also are commonly associated with excessive consumption of other poorly digestible sugars and polyols. At doses of up to 25 g D-tagatose per meal, flatulence was generally the only side effect reported. Other symptoms noted at higher consumption levels included nausea, borborygmi (*i.e.,* rumbling or gurgling noises in the gut), colic, and laxation. Some of these symptoms are characteristically observed following the consumption of poorly absorbed carbohydrates.

Effects of D-tagatose on the volume of the human liver and postprandial liver glycogen concentration were assessed in a clinical trial involving 12 healthy male volunteers and employing a double-blind, two-way crossover study design (Boesch ***et al.,*** 2001). D-Tagatose (test substance) or sucrose (placebo) were administered in the diet in amounts of 15 g at each meal (total of 45 g/day) for 28 days each with a washout period of 28 days. Liver volume and glycogen levels were measured using magnetic resonance imaging and magnetic resonance spectroscopy, respectively. No significant differences in clinical chemistry including plasma uric acid levels, hematological parameters, or urinary endpoints were noted between sucrose and tagatose fed subjects. Liver volumes increased significantly over time with both treatments, however, there was no difference between individuals given D-tagatose and those given sucrose. **A** comparison of liver volumes on Day 1, as measured before and after breakfast, indicated no significant changes associated with short term exposures for either D-tagatose or sucrose. Post-prandial liver glycogen concentrations after the prolonged consumption of D-tagatose or sucrose did not differ significantly from those recorded during pretreatment.

000062

Consumption of D-tagatose, as with fructose, has the potential to increase plasma concentrations of uric acid as a result of the activity of fiuctokinase, in the presence of ATP, on these substrates. Phosphorylation of D-tagatose or fructose transiently decreases the hepatic ATP and P_i pools. Since P_i is an inhibitor of adenosine deaminase, a rate-limiting enzyme in the catabolism of adenosine monophosphate, phosphorylation of D-tagatose or fructose may result in increased degradation of purine nucleotides and increased release of uric acid from the liver.

Three clinical studies including single and repeated dose studies with D-tagatose in comparison to fructose, sucrose or glucose have evaluated potential uricemic effects. Ingestion of a single 15-gram dose of D-tagatose had no effect on serum uric acid concentrations in comparison to a similar dose of sucrose (Boesch *et al.*, 2001). Ingestion of single oral doses of 30 grams of D-tagatose (in solution) by 8 fasted volunteers increased serum uric acid. However, this increase remained within the stated normal range (Buemann *et al.*, 1999b). By comparison, fructose was reported to produce smaller increases in plasma uric acid. Larger, single oral doses of **75** grams of D-tagatose in healthy volunteers and type **2** diabetics did elevate serum uric acid levels (Saunders *et al.*, 1999b).

In a repeat dosing phase in which D-tagatose (to both diabetics and normals) or glucose (normals only) was administered as 3 separate 25 gram doses at meal times for an 8-week period, no significant effects on fasting plasma uric acid levels were found compared to baseline values. Consumption of 45 grams of D-tagatose by healthy male volunteers as 3 separate 15 gram doses did not alter fasting serum uric acid concentrations in comparison to consumption of equal amounts of sucrose (Boesch *et al.*, 2001). As consumed in food, the dosing pattern of the Boesch *et al.* (2001) study is most relevant to the assessment of D-tagatose on serum uric acid in humans.

Conclusions

The estimated mean consumption of D-tagatose from the proposed food uses is approximately ***6.6*** g/day with heavy users (90th percentile) consuming up to 14.9 g/day. On a body weight basis, consumption of D-tagatose is highest in children aged **2** to ***5*** years (0.42 g/kg bw/day). The corresponding 90th percentile g/day intake estimate for this age group is **7.4** g/day.

000063

Data supporting the safety of D-tagatose include several clinical trials in healthy and diabetic volunteers, and toxicology studies including acute, subchronic, genotoxicity and developmental

toxicity studies. In animals, the only notable treatment-related effects were increased liver weights observed in Sprague Dawley rats administered 10 to 20% D-tagatose in the diet for 90 days and hepatocellular hypertrophy at dietary levels of 15 and 20%, in this same study. The NOEL determined from this study was 5% (2.3 to 6.7 g/kg/day). For liver enlargement, specifically, although the mechanism is not entirely clear, it is important to note that (i) this effect was not associated with any adverse clinical symptoms, (ii) there was no histopathological evidence of overt hepatotoxicity at any dose, and (iii) the results of the clinical trial in humans did not indicate a difference in liver weight or glycogen concentration between D-tagatose and sucrose exposures. Moreover, all effects noted in rats for D-tagatose have been reported for fructose as well, and, although D-tagatose was demonstrated to be about 4 times more potent than fructose, the anticipated consumption levels of D-tagatose are almost **6** times lower.

Other issues requiring consideration were the potential for increased plasma uric acid concentration (this effect has been associated with fructose) and the possibility of metabolic interactions between fructose and D-tagatose when co-ingested. With respect to uric acid concentrations, the results of clinical trials demonstrated that fasting plasma uric acid levels were not affected by consumption of D-tagatose in food at doses up to 45 grams per day, and that the changes observed at single doses in the range of 75 grams per day were mild and did not approach serum concentrations of 15 to 20 mg/100 ml associated with uric acid nephropathy. There was no difference in post-prandial serum uric acid levels after intake of single doses of 15 grams D-tagatose or **15** grams sucrose (Boesch ***et al.,*** 2001). With regard to metabolic interactions, enzyme kinetic considerations and the results from a liver perfusion study indicate that the levels of D-tagatose from the proposed uses would not be sufficient to interfere with the metabolism of fructose. Finally, based on the available animal studies in which fructose and D-tagatose were co-administered, there is no indication of an additive or synergistic effect of D-tagatose and fructose on clinical measurements of liver function (Saunders, 1992; Lina and de Bie, 2000d).

In summary, the safety assessment of D-tagatose, under the intended conditions of use and consequent consumption levels, was based upon the presence of a 10- to 20-fold safety factor between estimated intake levels and the 5% dietary NOEL for liver enlargement established in Sprague-Dawley rats. In addition, the results of a human clinical trial in which groups of individuals were given **45** grams of D-tagatose per day for **28** days did not indicate any significant increase in liver volume in comparison to controls given sucrose, nor did D-tagatose have any effect on post-prandial liver glycogen concentrations.

Supporting corroborative data, indicative of a possible species- and or strain-specific effect of D-tagatose on the liver, include the finding of no liver enlargement in either Wistar rats fed D-tagatose at 10% in the diet for ***6*** months or in pigs administered D-tagatose at 20% in the diet for **33** days. It should be noted, however, that differences in the durations of the studies and in the dose levels used in these studies do not permit a direct quantitative comparison of the liver effects in Wistar and Sprague-Dawley rats. Finally, although the mechanism for potential effects of D-tagatose on uric acid is conjectural, any possible effect of D-tagatose on uric acid, even of a mild nature, would occur only at exposures several fold in excess of the 90th percentile estimated daily intake.

In conclusion, the available data demonstrate that the anticipated consumption levels of D-tagatose from the proposed uses in food, even at the 90th percentile, are not associated with any adverse health effects in humans and should be considered safe.

000065

DOCUMENTATION SUPPORTING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE AS A FOOD INGREDIENT

1.0 INTRODUCTION

D-Tagatose is a stereo-isomer of D-fructose, which is a common six carbon sugar. Its relative sweetness in comparison to sucrose is about 75 and 92% based on a 1 and 10% aqueous solution, respectively. Like other sugars, it can be used as a bulk sweetener, humectant, texturizer or stabilizer (Levin et *al.*, 1995). In addition, D-tagatose has a reduced physiological energy value (Livesey and Brown, 1996), is non-cariogenic (Imfeld, 1996, 1998; Noguchi and Miihlemann, 1976), exerts a prebiotic effect (Jensen and Buemann, 1998; Jensen and Laue, 1998; Bertelsen et al., 2001), and is not associated with a glycemic response (Donner et al., 1996; Zehner, 1994). Given these properties, D-tagatose is suitable for use in dietetic and conventional foods including food substances intended for diabetics.

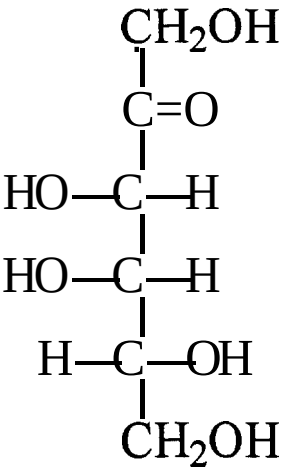
The following report presents the supporting documentation for the GRAS determination of the proposed food uses of D-tagatose. Effects observed in certain animal and clinical studies, particularly the potential for liver enlargement, increased hepatic glycogen and serum uric acid levels, are specifically addressed in this report.

2.0 MANUFACTURING AND COMPOSITION

Crystalline D-tagatose is manufactured from food grade lactose by a two-step process involving enzymatic hydrolysis (immobilized *A. oryzae* lactase) to D-galactose followed by chemical isomerization of D-galactose to D-tagatose, induced by technical grade calcium hydroxide, and recrystallization. All ion exchange resins used for chromatographic purification of the D-galactose intermediate and D-tagatose, and for demineralization, comply with 21 C.F.R. § 173.25. Food grade hydrochloric acid and technical grade sulphuric acid are used for pH adjustment. The technical grade sulphuric acid is used to release D-tagatose from the calcium salt prior to the chromatographic purification steps and recrystallization. Potential impurities such as residual D-galactose, talose, aldol condensation products and other impurities arising during the basic isomerization process are removed largely by demineralization,

000066

chromatographic purification, and in the final crystallization of D-tagatose. None of the compounds present, mostly sugars, are of toxicological concern.



D-Tagatose

Microbial sterility is maintained by the high temperature processing, and acidic and basic conditions used in the process. In addition, the immobilized enzyme preparation is sterilized every three days with solution of food grade acetic acid, potassium sorbate and sodium benzoate. Process tanks and lines are cleaned with sodium hydroxide and hydrogen peroxide following standard procedures common to the dairy industry.

The immobilized lactase preparation is prepared with inert resins and glutaraldehyde, and has been tested for safety in a 90-day rat oral gavage study. Groups of 20 male and female Sprague-Dawley rats were dosed with 0, 100,400 or 1600 mg/kg body weight/day with immobilized lactase as a suspension in corn oil. The reported no observed adverse effect level **(NOAEL)** was 1600 mg/kg body weight/day (BIBRA, 1990). The inert resin is considered GRAS (generally recognized as safe) in accordance with 21 C.F.R. § 170.30(a)(2) based on common use in food prior to January 1, 1958. Potential residuals eluting from the immobilized enzyme preparation have been measured following elution of the preparation with 10% ethanol (VVT, 1998) in accordance with FDA guidance documents (FDA, 1993). Based on the usage level of the immobilized resin and assuming 50% of the residues remain associated with D-galactose and subsequently D-tagatose, the concentration of residual extractives was calculated to be 1.4 mg/kg D-tagatose. Considering an estimated consumption of D-tagatose of **14.9** g/day for heavy

000067

consumers in the population (see section 4), a large safety factor is associated with exposure to residual materials (including glutaraldehyde) from consumption of D-tagatose.

3.0 SPECIFICATIONS

Appropriate food grade chemical specifications supported by analytical data from several manufactured lots have been derived for crystalline D-tagatose and adopted by JECFA. The specifications take into account various metallic impurities but do not recognize that the material has a small amount of residual D-galactose present (<0.6%). The proposed specifications are summarized in Table A-1, Appendix A. Appropriate internal specifications for potential microbiological contaminants supported by analytical data from several manufactured lots have also been developed.

4.0 PROPOSED USES AND ESTIMATED CONSUMPTION

D-Tagatose is intended to be used as a bulk sweetener in the preparation of low- and reduced-calorie foods although it has other technical effects similar to other sugars and can act as a humectant, texturizer and stabilizer. Intended food uses and use levels are summarized in Table B-1, Appendix B. The estimated consumption of D-tagatose from most of the proposed food categories was estimated using the United States Department of Agriculture Continuing Survey of Food Intake By Individuals (USDA CSFII, 1994-96, 1998) food consumption database (USDA, 2000; Environ, 2000). Consumption from sugarless chewing gum was estimated separately using a Market Facts survey of sugarless chewing gum consumption. Consumption estimates from use in meal replacements was also determined separately on the basis of consumption of 5g D-tagatose per serving and replacement of 1 to 2 meals per day. It was not considered appropriate to add the consumption of meal replacements to the children's and teenage categories since these products are primarily used by adults. Adult uses of meal replacers could result in a D-tagatose consumption of 0.08 to 0.16 g/kg body weight/day assuming one or two servings per day, respectively.

000068

D-Tagatose is recognized as a component of the regular diet, found in low amounts in sterilized milk products, such as sterilized cow milk and milk powder (Troyano *et al.*, 1991, 1996; Richards and Chandrasekhar, 1960), yogurt, milk-based infant formula and hot cocoa (Sanders,

1998) formed from lactose by heat treatment. It is also found in higher amounts (1%) in lactulose syrups (OTC preparations) resulting in low exposures of 0.2 to 0.3 g/day (Zehner, 1994).

From research conducted on dietary survey consumption approaches, it is known that adding intakes for different food categories will result in an overestimate of intake. Since sugarless chewing gum intake estimates were determined in a separate survey, and meal replacements were determined separately, the impact of sugarless chewing gum and meal replacements on the total D-tagatose consumption estimates can only be determined by normalization of the sugarless chewing gum and meal replacement consumption estimates (see Appendix B for detailed calculations).

As an approximation of total consumption, the consumption from the three different estimates (sugarless chewing gum, meal replacements, all other food categories) were "normalized" to obtain a projected consumption of D-tagatose. The all-users D-tagatose consumption estimates from all individual food categories were added (excluding sugarless chewing gum and meal replacements). This total is then used as the denominator to derive a theoretical percent contribution that each of the food categories which would contribute to the users intake. The all-users intake of D-tagatose from sugarless chewing gum and meal replacements is added to this corrected individual total and used to derive a normalization factor to adjust the consumption value derived from food categories, other than sugarless chewing gum and meal replacements for the additional consumption of D-tagatose from sugarless chewing gum and meal replacements.

Using the normalization methodology, the estimated consumption of D-tagatose from all proposed food uses including sugarless chewing gum and meal replacements is summarized in Tables 4-1 and 4-2. The estimated mean consumption of users in the total population is ***6.6*** g/day (see Table 4-1) (0.12 g/kg body weight/day see Table 4-2). Heavy consumers (90th percentile) may consume up to 14.9 g/day (0.27 g/kg body weight/day). Estimated consumption in the total population and in adults is higher than children and teenagers partly as a result of the consumption of meal replacement products for weight control. Heavy users in children aged **2** to 5 years and children aged 6 to 12 years have the largest consumption on a body weight basis, 0.42 g/kg body weight/day and 0.32 g/kg body weight/day, respectively, as a result of their higher requirement for energy. Consumption of D-tagatose was evenly split between meals and snacks consumed over an entire day.

000069

Table 4-1 Summary of Estimated D-tagatose Users Consumption (G/day) from All Proposed Food Uses in Different U.S. Population Groups

Population Group	% Users	Users Daily Consumption	
		Mean (g/day)	90th Percentile (g/day)
Children, 2 to 5 years	79.7	3.70	7.40
Children, 6 to 12 years	78.0	5.04	10.10
Teenagers, 13 to 19 years	59.5	6.03	12.30
Adults, 20 years and older	54.4	6.48	15.50
Total population, 2 years and older	59.0	6.55	14.85

Table 4-2 Summary of Estimated D-Tagatose Users Consumption (g/kg bw/day) from All Proposed Food Uses in Different U.S. Population Groups

Population Group	% Users	Users Daily Consumption	
		Mean (g/kg bw/day)	90th Percentile (g/kg bw/day)
Children, 2 to 5 years	79.7	0.21	0.42
Children, 6 to 12 years	78.0	0.16	0.32
Teenagers, 13 to 19 years	59.5	0.10	0.20
Adults, 20 years and older	54.4	0.09	0.21
Total population, 2 years and older	59.0	0.12	0.27

5.0 SUMMARY OF SAFETY DATA

5.1 Metabolic Data

000070

The available data on D-tagatose demonstrate some absorption of an ingested dose from the small intestine with the remainder passing into the large intestine where it is fermented to short chain fatty acids and gaseous products (CO_2, methane and hydrogen) by the intestinal bacteria (Saunders *et al.*, 1999a; Laerke and Jensen, 1999). The main short chain fatty acids produced were acetate, propionate and butyrate, with some production of formate, caproate, valerate and

lactate. The profile of short chain fatty acids produced from the fermentation of D-tagatose was similar to that from sucrose subject to fermentation (Jensen and Buemann, 1998; Laerke *et al.*, 2000).

The D-tagatose absorbed from the small intestine is metabolized by the same biochemical pathway as fructose (Raushel and Cleland, 1977). In the initial step in this pathway, D-tagatose is phosphorylated to D-tagatose-1-phosphate by fructokinase (Raushel and Cleland, 1977). This intermediate is then split by aldolase B to D-glyceraldehyde and dihydroxyacetone-phosphate. These are the same products that are formed during the breakdown of fructose; however, the rate at which D-tagatose-1-phosphate is split is only about half the rate for D-fructose-1-phosphate due to a lower affinity of aldolase B for this tagatose derivative (Rognstad, 1975, 1982). In spite of the lower affinity of aldolase B for D-tagatose-1-phosphate, in comparison to D-fructose-1-phosphate, the intracellular concentration of this metabolite remains low. This is evidenced in a single dose study, using magnetic resonance spectroscopy, and healthy humans that showed liver concentrations of D-tagatose-1-phosphate did not exceed 1 mM following the ingestion of **30** g D-tagatose (Buemann *et al.*, 2000a).

Unlike fructose, the absorption of D-tagatose appears to be passive. D-tagatose does not interfere with the absorption of fructose and there is no apparent binding of D-tagatose to the GLUT **5** and GLUT 2 transporters/carriers that are involved in the carrier-mediated diffusion of fructose through the intestinal wall (Crouzoulon, 1978; Sigrist-Nelson and Hopfer, 1974; Tatibouet *et al.*, 2000).

Studies of D-tagatose, that were conducted in germ-free and conventional rats, and in pigs, indicate that about 20 to 30% of ingested ^{14}C-radiolabeled D-tagatose is absorbed from the small intestine (Jensen and Laue, 1998; Laerke and Jensen, 1999; Saunders *et al.*, 1999a). In the rat study, the majority of the absorbed radiolabel was accounted for within 24 hours, in the expired air, urine and feces indicating that ingested D-tagatose and absorbed short-chain fatty acids would be readily metabolized (Saunders *et al.*, 1999a). Less than 10% of the radioactivity was measured in the carcass of the animals after 24 to **72** hours.

000071

In the study involving germ-free and conventional rats, groups of 2 to 4 male Sprague Dawley rats were administered a diet containing 10% radioactive D-[U-^{14}C]-tagatose. The non-adapted conventional, and the germ-free rats received a single oral dose, whereas the adapted rats were administered diets containing 10% D-tagatose for 28 days prior to testing. **A** second group of

conventional unadapted rats was administered D-[U-^{14}C]-tagatose intravenously. The difference in absorption values, as indicated by the measured radiolabel, between germ-free and unadapted conventional rats (31.8% *versus* 64.0%, respectively) show that most of an ingested dose of D-tagatose passes through to the large intestine where it is reduced to short chain fatty acids *via* fermentation. Germ-free rats have limited capacity for fermentation in the large intestine. Adaptation of rats to D-tagatose, *via* regular consumption prior to testing, resulted in a greater absorption of label (83.2%), a response that can be attributed to a greater efficiency of the intestinal bacteria.

Jensen and Laue (1998) investigated the absorption of D-tagatose from the small intestine of five male pigs. The concentration of D-tagatose in the arterial blood reached nearly 75% of that of the portal vein blood, indicating a low clearance of D-tagatose on the first passage through the liver. Adapted and unadapted animals absorbed 16 to 43% and 17 to 36% of ingested D-tagatose, respectively, with the highest absorption occurring 1 to 3 hours post-feeding. In this same study (Jensen and Laue, 1998), the formation of short-chain fatty acids (SCFAs) from D-tagatose by the intestinal microflora of pigs was also investigated. The net portal absorption of SCFA was slightly higher in pigs fed D-tagatose than in pigs fed sucrose. Another study in pigs, using chromic oxide as a digestibility marker, showed that by the distal third of the small intestine about 26% of ingested D-tagatose had been absorbed (Laerke and Jensen, 1999). A large part of the ingested D-tagatose was fermented in the cecum and proximal colon.

Absorption data of D-tagatose in humans is limited to a study in ileostomic patients in which subjects ingested single 15 g doses (Normén *et al.*, 2001). About 20% of the ingested D-tagatose was recovered from the 24-hour ileal effluent. The authors interpreted the results to indicate that 80% of the ingested dose had been absorbed higher in the gastrointestinal tract. The usefulness of these data with respect to estimating absorption levels in healthy populations is questionable given that similarly high absorption results have been obtained from studies in ileostomates for other polyols including sorbitol, maltitol and isomalt that are generally found to be poorly digested and absorbed (Bär, 1990; Langkilde, 1994).

5.2 Toxicological Studies

5.2.1 Animal Data

000072

The safety of D-tagatose has been evaluated in a number of toxicological studies including acute, subchronic (up to six months), genotoxicity and developmental toxicity studies (Trimmer, 1989;

Kruger ***et al.,*** 1999b,c). In addition, special mechanistic studies investigating the potential for liver enlargement associated with high dietary exposure to D-tagatose were conducted (Lina and de Bie, 2000a,b,c). D-Tagatose did not exhibit any genotoxic potential nor were there any indications of developmental or teratogenic effects associated with dietary exposures. The principle treatment-related effects were increased liver weights observed in Sprague Dawley rats administered 10 to 20% D-tagatose in the diet for 90 days and hepatocellular hypertrophy at dietary levels of 15 and 20%, in this same study (Kruger ***et al.,*** 1999a). The no-observed-effect level (NOEL) determined from this study was ***5%.*** No liver effects were observed in Wistar rats administered up to 10% D-tagatose in the diet for six months. However, effects at higher doses were not investigated, and thus it is not possible to determine whether the Sprague-Dawley rat has a unique sensitivity to D-tagatose compared to the Wistar rat or whether the Wistar rat is resistant to these liver effects (Lina and de Bie, 2000d). It should also be noted that differences in the durations of the studies and in the dose levels used in these studies do not permit a direct quantitative comparison of the liver effects in Wistar and Sprague-Dawley rats. Although chronic studies are not available, there is no expectation, given the negative results from genotoxicity assays and its structural similarity to the common sugar fructose, that D-tagatose would possess any carcinogenic potential.

Acute Toxicity Studies

The acute oral toxicity of D-tagatose was investigated in a gavage study in which 10 rats (5/sex) and **5** male mice were administered a single D-tagatose dose of 10 g/kg body weight (Trimmer, 1989). One female rat died immediately after dosing due to a dosing accident. No treatment related mortalities occurred, nor were any behavioural changes reported during the 14 day observation period. Although this study was not conducted according to current standards, the results do provide an indication that D-tagatose is of low acute oral toxicity.

Subchronic Toxicity Studies

Sprague-Dawley Rats

000073

The toxicity of D-tagatose was investigated in a 90 day study in Sprague Dawley rats, in which groups of animals (20/sex) were administered diets containing 0, **5**, 10, **15** or 20% D-tagatose (Kruger ***et al.,*** 1999a). An additional "isocaloric" control group was administered a diet containing 10% fructose plus 10% cellulose. Parameters evaluated included clinical signs, body

weight gain, food consumption, ophthalmic effects, temperature, and standard hematological and clinical chemistry endpoints, excluding urinary parameters. Animals were fasted overnight (12 hours) prior to sacrifice. Gross examinations were conducted on all animals. Microscopic evaluations were performed on all animals in the 0 and 20% D-tagatose groups, and the fructose/cellulose control group. In addition, the two animals in other groups that died spontaneously, as well as any organs or tissues that exhibited changes at gross necropsy, were examined microscopically.

The corresponding dose ranges of D-tagatose, on a per kg body weight basis, associated with the dietary levels, as reported by the authors, are provided in Table 5-1.

Table 5-1 Dose Levels Associated with Dietary Concentration of D-Tagatose in a 90 Day Study in Sprague Dawley Rats

Groups	D-Tagatose Dietary Level (% w/w)	D-Tagatose dose range (g/kg/day)	
		Male	Female
Control (lab chow only)	0	0	0
Fructose/Cellulose control	0	0	0
Low-dose	5	2.3 – 6.7	2.8 – 6.3
Low-mid-dose	10	4.7 – 13.3	6.0 – 12.3
High mid-dose	15	7.3 – 19.6	8.7 – 18.5
High-dose	20	9.9 – 25.6	12.2 – 24.8

Three mortalities from undetermined causes, one each in the 10% dose-group (male), 15% dose-group (male), and 20% dose-group (female), occurred prior to scheduled necropsy. These deaths were not considered to be treatment-related. The only clinical sign attributed to D-tagatose was the observation of soft stools on days 1 to 3 for most of the animals in the 15 and 20% dose groups. Compared to the lab chow control, mean body weights were significantly decreased for the **20%** dose-group males (Days 21 to 91) and females (days 63, 84, and 91), and the 15% dose-group males (Days 42 to 70 and 84). In addition, mean body weight values for the 20% dose-group males (Days 42 to 91) were significantly lower than the fructose/cellulose control group. At the end of the treatment period, body weights of the 20% dose group were about 10% lower

000074

than the lab chow control group. Mean D-tagatose consumption levels decreased over time, on a body weight basis, in both sexes for all treatment groups.

No significant hematological or clinical chemistry effects were observed in the 5% treatment group. Slight, but statistically significant reductions in hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin values were observed in both males and females of the 15 and 20% dose groups. These effects were not considered to be clinically significant. Of the clinical chemistry parameters, alanine aminotransferase was decreased in the 10, 15, and 20% females and in the 15% males; cholesterol was increased in the 15 and 20% males and females; and triglycerides were increased in the 15 and **20%** males. No gross or histopathological findings were associated with these parameters. High dietary fructose is also known to produce variable effects on cholesterol and triglycerides (Aoyama *et af.*, **1987).**

Organ weights were not significantly different from controls for the 5% dose group. Absolute and relative liver weights were increased in males and females of the 10, 15, and 20% dose groups compared to both control groups. The relative liver weight values are summarized in Table 5-2. The actual values for the absolute liver weights were not provided in the published version of the study. Relative weights but not absolute weights were statistically increased for some of the other organs (including kidney, tests, brain, heart, and spleen) in varying groups and sexes compared to either control group. These changes, which were not accompanied by any histopathological findings, were attributed to decreased body weight gain in the treatment groups. Decreased absolute weights, organ-to-brain weight, and organ-to-body weight ratios of epididymal fat pad and parametria were also observed in varying dose groups. These expected effects were not considered toxic but rather were attributed to shifts in fat stores.

Table 5-2 Relative Liver Weight Data of Sprague Dawley Rats Fed Diets Containing up to 20% D-Tagatose or 20% Fructose/Cellulose for 90 Davs

D-Tagatose (% of Diet)	Relative Liver Weight (g/kg body weight)	
	Males	Females
0 (lab chow only)	26.0 ± 2.0	25.0 ± 2.0
0 (Fructose/Cellulose)	26.0 ± 2.0	26.0 ± 2.0
5	27.0 ± 3.0	27.0 ± 3.0
10	31.0 ± 2.0"	30.0 ± 2.0"
15	34.0 ± 4.0"	33.0 ± 2.0"
20	36.3 ± 3.0'	36.0 ± 4.0[a]

'result was significantly different from both the lab chow and fructose/cellulose control (P #0.01)

Thickening and discoloration of the liver was observed in both sexes in the 15 and 20% dose groups. Thickening of the liver also was observed in one male in the 10% dose-group. It is expected that the visual appearance of greater depth (*i.e.,* thickening) of the liver was due to the enlargement of the hepatocytes. No other gross pathological findings were observed. Treatment related microscopic changes were limited to hepatocellular hypertropy in the 15% (4/20 males and 4/20 females) and 20% (7/20 males and 11/20 females) dose-groups. Overnight fasting is known to deplete glycogen in the liver. Based on the findings of increased liver weights at the 10, 15, and 20% dose levels, and minimal liver hypertrophy at the 15 and 20% dose levels, the NOEL for this study was the 5% dietary level, which corresponded to dose levels of 2.3 to 6.7 g/kg/day in males and **2.8** to **6.3** g/kg/day in females.

Wistar Rats

000076

A six-month sub-chronic oral toxicity study with D-tagatose was conducted in male Wistar rats (Lina and de Bie, 2000d). Treatment groups of 60 rats each were as follows: (i) Group **A:** control (20% pregelatinized potato starch); (ii) Group B: 5% tagatose + 15% pregelatinized potato starch; (iii) Group C: 10% tagatose + 10% pregelatinized potato starch; (iv) Group D: 20% fructose; and (v) Group E: 10% tagatose + 10% fructose. Following overnight fasting, ten males of each group were sacrificed after **3** days (subgroup 1), 7 days (subgroup 2), 14 days

(subgroup 3), 28 days (subgroup 4), 3 months (subgroup 5), and 6 months (subgroup 6) of treatment.

No treatment-related clinical signs were observed in any of the groups. One rat in group C was found dead in week 20 of the study. The cause of death could not be established due to autolysis. Mean body weights were slightly decreased in groups C and E (*i.e.*, those receiving 10% tagatose). However, food intake was also decreased in these same groups over the first week of the study. Also, there were no consistent changes in clinical chemistry parameters including alkaline phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), or gamma glutamyl transferase (GGT) activities or in plasma concentrations of total protein, albumin, bilirubin, cholesterol or inorganic phosphate among the groups. On Days 3 and 7, phospholipid concentrations were decreased in groups C and E, but this finding was not observed at the subsequent stages. Triglyceride levels tended to be lower in these same groups at several stages, but generally were not statistically significant.

There were no changes in absolute or relative liver weights at any stage. Microscopic examination of the livers of rats in subgroup 6 did not reveal any treatment-related histopathological changes. There were no significant differences in bromodeoxyuridine labeling index or in nuclear density of hepatocytes between groups. There were no significant changes in lipid or DNA levels per total liver in any treatment group. Protein levels per total liver were significantly increased in groups D and E (*i.e.*, those receiving fructose) at several stages. No consistent differences were observed in glycogen or glucose levels between the treatment groups. Glycogen levels were low in all groups, as may be expected following overnight fasting.

The percentage of fat and moisture was decreased, while the percentage of protein and ash was increased in the carcasses of groups C and E (*i.e.*, those receiving 10% tagatose). A similar, but less marked, shift was observed in group B (*i.e.*, those receiving 5% tagatose). The results of group D (20% fructose) were similar to those of group A (20% starch).

000077

The effects on body weight and carcass weight were attributed to tagatose. However, these were not considered to represent adverse effects. D-tagatose did not significantly affect clinical chemistry parameters related to liver function, liver weight, liver pathology, or content of lipid, protein, DNA, glycogen, or glucose in the liver at any stage. Administration of D-tagatose in the diet at levels up to 10% was not associated with any adverse effects.

Pigs

Effects of D-tagatose on the livers of pigs were evaluated as part of an energy balance study (Mann, 1997). In this study, four groups of pigs (n=2) were fed diets containing regular pig diet (0%), 20% sucrose, **20%** D-tagatose (–5 g/kg/day), or a mixture of 10% sucrose and 10% D-tagatose for **33** days. Animals were killed about ***6*** hours after their last feeding. No histopathological changes were observed in liver tissues in either of the groups receiving D-tagatose. A few necrotic cells were observed in the control group.

Genotoxicitv

The genotoxic potential of D-tagatose was evaluated in five standard assays which included (i) the Ames Salmonella typhimuriurn reverse mutation assay, (ii) the Escherichia *coli*/mammalian microsome assay, (iii) the mammalian chromosomal aberration assay in Chinese hamster ovary cells, (iv) the mammalian cell forward mutation assay in mouse lymphoma L5178Y cells; and (v) the in ***vivo*** mouse micronucleus assay. D-Tagatose was not found to be genotoxic under the conditions of any of these tests (Kruger ***et*** al., 1999b). Details of the studies are provided in Table 5-3.

Table 5-3 Summary of Genotoxicity Studies with D-Tagatose

Test	Test System	Concentration/ Dose	Result	References
In Vitro Bacterial Assays				
Ames ***Salmonella typhimurium*** reverse mutation assay	***S. typhimurium*** strains ***TA*** **1535,** TA **1537,** TA **1538,** TA 98, TA 100	100-5000:g/plate **(±S9)**	Negative	Lawlor, 1993; Kruger ***et al.,*** 1999b
Escherichia *coli*/mammalian microsome assay	***E. coli*** strain **WP2*uvr*A**	100-5000:g/plate **(±S9)**	Negative	Lawlor, 1993; Kruger ***et al.,*** 1999b
In Vitro Mammalian Assays				
Chromosomal aberration assay"	Chinese hamster ovary cells	***1250-5000*** :g/ml **(±S9)**	Negative	Murli, 1994a; Kruger ***et al.***, 1999b
Cell forward mutation assay[b]	Mouse lymphoma L5**178Y** cells	***500-5000*** :g/ml **(±S9)**	Negative	Cifone, 1994; Kruger ***et al.***, 1999b

000078

Table 5-3 Summary of Genotoxicity Studies with D-Tagatose

Test	Test System	Concentration/ Dose	Result	References
In Vivo *Assays*				
Mouse micronucleus assay[c]	CD-1 mouse bone marrow	1250-5000 mg/kg body weight (p.o.)	Negative	Murli, 1994b; Kruger *et al.*, 1999b

[a] treatment time, 7.4 h (without activation), 2 h (with activation); harvest time, 10 h
[b] treatment time, 4 h
[c] animals were terminated at 24, 48 and 72 h after dosing

Develoumental Toxicity

A developmental toxicity study of D-tagatose in Sprague-Dawley rats showed no adverse effects on reproductive performance, no adverse treatment-related effects on fetal weight, sex distribution, liver weight, or external, skeletal, or visceral malformations at any dose level (Kruger *et al.*, 1999c). In this study, four groups of 24 pregnant rats were orally administered D-tagatose *via* gastric intubation at doses of 0, 4, 12, or 20 g/kg/day on Days 6 to 15 of gestation. These doses were based on the results of a dose-range finding study in which the highest dose level tested (20 g/kg) showed no adverse embryotoxic/teratogenic effects (Schroeder, 1994a,b). The only clinical signs observed in dams, both the range finding and full-scale studies, were laxative effects (stool softening, unformed or watery stools) at doses at or greater than 12 g/kg/day. Maternal absolute liver weights were increased at the high dose (20 g/kg/day) and mean relative liver weights were increased in the mid- and high-dose groups (12 g/kg/day, at least 10% in the diet, and 20 g/kg/day, at least 20% in the diet) in the full-scale study but not in the range finding study. The observed increased liver weights were not accompanied by any microscopic changes.

5.2.2 Human Data

000079

Potential effects associated with dietary consumption of D-tagatose were investigated in several short duration clinical trials (up to 28 days exposure), in which healthy and/or diabetic subjects were administered doses between 15 and 75 g/day (Buemann *et al.*, 1999a,b,c, 2000b; Donner *et al.*, 1999; Lee and Storey, 1999; Saunders *et al.*, 1999b; Boesch *et al.*, 2001). The predominant effects associated with excessive consumption were physiological gastrointestinal effects

attributed to an osmotic effect from incomplete absorption of D-tagatose. Such effects also are commonly associated with excessive consumption of other sugars and polyols (Beaugerie *et al.*, 1991; Koutsou *et al.*, 1996; Rambaud and Flourie, 1994).

Single Dose Studies

In a screening test, 73 male volunteers were administered a single **30** g dose of D-tagatose in cake in the afternoon (Buemann *et al.*, 1999a). Side-effects included nausea and diarrhea, reported by 11 and **23** subjects, respectively. Of the subjects that reported diarrhea, the severity was rated as mild, moderate and strong by 11, 10 and 2 individuals, respectively.

Gastrointestinal tolerance was assessed in a single dose, double-blind, cross-over study in which **34** subjects **(23** women and 11 men) were administered 29 g of D-tagatose or sucrose with breakfast (Buemann *et al.*, 1999c). Symptoms were assessed on the day of the test and for the following two days. Subjects rated the symptoms on a scale of 0 to **4** (none to very strong), and reported the number of stools. Twenty one of 33 subjects reported a higher score from D-tagatose use than sucrose for one or more of the gastrointestinal symptoms. Scores were significantly higher for D-tagatose compared to sucrose for the following effects: rumbling in the stomach and gut; distention; nausea; flatulence; and diarrhea. Of the ***6*** subjects reporting diarrhea, 3 described it as "watery stool" and 3 as "loose stool." With the exception of distention, increased scores of the symptoms were reported only on the day of treatment but not on the 2 days following the test. All reported symptoms, except nausea, are typically observed with the consumption of low-digestible sugars and polyols. The nausea was usually scored as mild, and was attributed, by the authors, to the osmotic effects of unabsorbed D-tagatose in the small intestine. In another single dose study, by these researchers, 20 male medical students, previously found to tolerate 30 g of D-tagatose, were administered 29 g of either sucrose or D-tagatose with breakfast (Buemann *et al.*, 2000b). Noteworthy symptoms were limited to two cases **of** moderate nausea and one case of strong flatulence after consumption of D-tagatose.

Tolerance to D-tagatose was compared to sucrose and lactitol in 50 healthy male and female subjects that were administered the substances with chocolate (administered **40** g in two equal portions between 1 p.m. and 4 p.m.) (Lee and Storey, 1999). In comparison to sucrose, more subjects reported nausea, bloating, borborygmi (rumbling or gurgling in the gut), colic and flatulence, **as** well as thirst and loss of appetite, following D-tagatose ingestion. D-tagatose did

not increase the frequency of any symptoms, with the exception of thirst, when compared to lactitol.

The potential effect of a single 30 g oral dose of D-tagatose on plasma uric acid levels was assessed in a study involving **8** male volunteers (Buemann *et* ***al.,*** 1999b). Solutions containing 30 g of D-tagatose, 30 g fructose, or pure water were administered to **8** male fasted volunteers. Arterial blood samples were collected prior to dosing, and at regular intervals thereafter for a period of 7 hours. Parameters assessed included D-tagatose, uric acid, inorganic phosphate, glucose, lactate, insulin, glucagon, glucagon-like peptide (GLP-1), cholecystokinin, gastric inhibitory peptide (GIP), adrenaline and noradrenaline. D-tagatose appeared in the arterial blood shortly after dosing and reached a maximum concentration of about 0.17 mMol/L after 50 minutes.

tagatose were slightly lower. No effects of either sugar were recorded on plasma epinephrine or norepinephrine levels.

In another single dose study, **8** healthy and **8** diabetic subjects were administered 75 g of D-tagatose or glucose (Saunders ***et al.,*** 1999b). This very high dose of D-tagatose increased plasma uric acid concentrations by about 1.5 mg/100 ml (range -0.6 to 2.9 mg/100) over the first hour

after dosing. Levels declined after the first hour but had not reached pretreatment levels within three hours after dosing. Glucose treatment did not increase plasma uric acid levels.

Repeated Dose Studies

Selected subjects (3 men, 5 women) that tolerated a single 30 g dose of D-tagatose without nausea and diarrhea were included in a second tolerance study, in which D-tagatose or sucrose was consumed daily for 15 days (1 dose of 30 g per day) (Buemann et *al.*, 1999a). Since the subjects had been prescreened, the symptoms reported following D-tagatose consumption occurred only slightly more frequently or with higher scores than those reported following sucrose intake.

In a study assessing potential effects of D-tagatose on glucose tolerance, 8 healthy and 8 type-2 diabetic subjects were administered increasing doses of D-tagatose with each meal, starting with 5 g per meal on Day 1 (Donner *et al.*, 1999). Gastrointestinal symptoms were associated with intakes of 10 to 25 g/meal. The incidence of symptoms was not significantly different between healthy and diabetic subjects. With regard to glucose tolerance, D-tagatose significantly attenuated the rise in plasma glucose following oral glucose loading in the type-2 diabetics. Insulin levels were not affected.

In a cross-over tolerance study involving eight healthy and eight diabetic volunteers, D-tagatose or sucrose was administered at doses of 75 g/day (25 g/meal) for 8 weeks (Saunders *et al.*, 1999b). Gastrointestinal symptoms reported included flatulence in 7 subjects from each group, and diarrhea of varying degrees in 6 subjects from each group. These side-effects did not improve over the course of the study. Plasma samples were collected at the beginning and end of each treatment period following overnight fasting. Clinical chemistry parameters assessed included liver enzymes, bilirubin, uric acid, insulin, glucose and lipids. No significant treatment related changes to these parameters were observed in healthy or diabetic volunteers.

Potential effects of D-tagatose on the volume of the human liver and postprandial liver glycogen concentration were assessed in a clinical trial involving 12 healthy male volunteers (21 to 30 years, 59 to 93 kg, body mass index # 25 kg/m^2, non-smokers) and employing a double-blind, two-way crossover study design (Boesch *et al.*, 2001). Half of the subjects randomly were given D-tagatose in the first period with the remainder receiving sucrose first. This study was

restricted to male volunteers since there was no evidence from toxicological studies of a sex-related difference associated with liver-enlargement. D-Tagatose (test substance) or sucrose (placebo) was administered in the diet in portions of 15 g at each meal (total of **45** g/day) for **28** days each with a washout period of **28** days. The 45 g/day dose of sucrose was reported to be about half of the average total daily intake of sucrose whereas, for D-tagatose, this dose level was three times greater than the projected daily consumption by 90th percentile consumers.

Liver volume and glycogen levels were measured using magnetic resonance imaging and magnetic resonance spectroscopy, respectively. Both techniques are widely used diagnostic and analytical methods in clinical routine and clinical trials (Boesch, 1999). Based on experience from a comparable study (Slotboom ***et al.,*** 1999), it was determined that 12 subjects would be sufficient to detect a 10% difference in liver volume at a level of significance of p=0.05 (two sided) with a power of **80%.** Magnetic resonance imaging and spectroscopy investigations were conducted in the morning on Day 1 (prior to breakfast), in the afternoon on Day 1 after a standardized breakfast with 15 grams of D-tagatose or 15 grams of sucrose, and in the morning on Day **29** for each of the treatment periods. After dinner on Day **28,** subjects were allowed only water or tea until completion of the MR the next day. At these same intervals, routine medical examinations (body weight, blood pressure and heart rate) and questionnaires were conducted, and blood (collected before and every hour up to **7** hours after breakfast) samples were collected to determine hematological (hemoglobin, hematocrit, erythrocytes, leukocytes, thrombocytes) and clinical parameters (glucose, insulin, triglycerides, cholesterol, bilirubin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), (glutamyl transferase (GGT), creatinine, urea, uric acid). Urinary protein, glucose, leucocytes, erythrocytes, nitrite, pH, ketones, bilirubin, and urobilinogen were evaluated by the dipstick method.

The D-tagatose treatment was well tolerated by all subjects. Gastrointestinal side-effects including flatulence, bloating and laxative effects were reported more frequently with D-tagatose than sucrose treatment but did not result in withdrawals or decreased compliance. Compared to pre-treatment values, D-tagatose had no effect on the serum chemistry and hematological parameters measured. In contrast, sucrose intake significantly increased fasting levels of **AP,** HDL-cholesterol, insulin, hemoglobin, erythrocytes and leukocytes compared to pre-treatment levels. Neither treatment resulted in any changes to the urinary endpoints **as** measured with the dipstick method. For D-tagatose, plasma uric acid levels were slightly but significantly elevated above pretreatment values at 1 hour after intake of breakfast on Day 1, and slightly but

significantly decreased at 5, 6, and 7 hours. For sucrose, plasma uric acid levels were slightly but significantly decreased at 3, 4, 5, 6, and 7 hours after dosing. Plasma uric acid levels were not significantly different between the two treatments. Liver volumes increased significantly over time with both treatments, however, the increase over time with D-tagatose was not statistically different from the increase over time associated with sucrose. A comparison of liver volumes on Day 1, as measured before and after breakfast, indicated no significant changes associated with short term exposures for either D-tagatose or sucrose. The authors suggested that seasonal effects (November to December) or the more regular food intake under supervision may have played a role in the gradual increase in liver volume over the course of the study. Following D-tagatose consumption, liver glycogen concentrations were not significantly different from those present before treatment or following sucrose consumption. In contrast to the results of toxicological studies in Sprague-Dawley rats administered higher dietary levels of D-tagatose, there was no evidence in this clinical trial that D-tagatose would change liver volumes or glycogen concentrations in healthy male subjects in comparison with sucrose.

5.3 Special Studies

5.3.1 Special Studies on Liver Enlargement

In the first of these studies (Lina and de Bie, 1998a; Bär *et al.*, 1999), four groups of male Sprague-Dawley rats received Purina diet (group A, 30 rats), Purina diet with 20% D-tagatose (group B, 30 rats), SDS diet (group C, 10 rats), and SDS diet with 20% D-tagatose (group D, 10 rats) for up to 28 days. An interim sacrifice was conducted on 10 rats in each of groups A and B on Days 14 and 28. Animals in Groups C and D were terminated on Day 28. The remaining 10 rats in each of groups A and B were terminated on Day 42 (*i.e.*, after a 2-week recovery period for group B). There was no fasting period prior to sacrifice for any of the groups.

Relative wet liver weights in group B were increased by 23 and 38% by Day 14 and 28, respectively, as compared to group A. At the end of the recovery period, the increase was lowered to 14% in group B. Relative wet liver weights in group D were increased by 44% by Day 28, as compared to group C. In group B, total amounts of glycogen, protein, DNA, and lipid (expressed as mg/liver) were significantly increased by 94, 26, 23, and 11% respectively, on Day 28, as compared to group A. These results indicate that dietary administration of 20% D-tagatose produced significant liver enlargement in rats sacrificed under non-fasted conditions.

The increase in glycogen, DNA, and protein per liver supports the conclusion that increased glycogen deposition as well as growth of the liver tissue occurred.

The second study (Lina ***et al.***, 1998; Bar ***et al.***, 1999) showed a dose-dependent liver enlargement in rats fed an SDS diet with 0, 5, 10, or 20% D-tagatose (20 rats/group) for 29 to 31 days. **As** in the study described above, these rats also were sacrificed in the non-fasted condition. The study included blood chemistry analyses and analysis of the livers of ***6*** rats/group for acyl-CoA oxidase and laurate-12-hydroxylase activity, DNA synthesis, and numbers of nuclei/fixed area of liver tissue. In the high-dose group, serum transaminases, albumin, phosphorus and inorganic phosphate were elevated. Relative liver weights were increased in all dose groups. In the mid- and high-dose groups the mean number of nuclei/fixed area of liver tissue was significantly decreased, indicative of hypertrophy. With the exception of glycogen accumulation in the livers of D-tagatose-treated rats, no other ultrastructural changes were observed in the control or 20% dose groups.

The results of the third study led to the conclusion that the 5% dose level for dietary administration of D-tagatose was a no-effect level with regard to liver enlargement when rats were sacrificed in the fasted condition (Lina and de Bie, 1998b; Bär ***et al.***, 1999), the normal condition for sacrifice **of** rats in the conduct of standard subchronic toxicity studies. In contrast, rats fed 5% D-tagatose and sacrificed in the non-fasted condition showed glycogen accumulation in the liver and correspondingly increased liver weights.

An additional three unpublished rat feeding studies (Lina and de Bie, 2000a,b,c) were conducted to determine the effects **of** separate and simultaneous administration of D-tagatose and glycogen precursors on liver weight and glycogen levels. Polycose (a glucose polymer) was used as a glycogen precursor in each **of** these studies. In the first of these studies, D-tagatose was administered to Wistar rats (Lina and de Bie, 2000a) as shown in Table 5-4.

Table 5-4 Treatment Groups (Lina and de Bie, 2000a)

Group	Number of Animals	Treatment 1 dark/feeding period	Treatment 2[3] light/gavage treatment
A	20	Polycose *via* diet'	Carrier *via* gavage
B	*20*	D-Tagatose *via* diet'	Polycose *via* gavage
C	20	Polycose +tagatose *via* diet'	Carrier *via* gavage
D	*20*	Polycose + tagatose *via* diet'	Carrier *via* gavage

[1] 18 g feed/rat was administered at the start of the dark period. The remaining feed (if any) was removed the next morning at –900 h. When D-tagatose was included in the diet, it was present at concentrations of approximately 14.5%.

[2] 2 equal portions (9 g/rat each) were administered at the start and towards the end of the dark period. The remaining feed was removed the next day at ~1100 h.

[3] treatments were administered as 2 separate doses 5 and 8 hours into the light cycle.

After 7 days of treatment, 10 rats/group were sacrificed at the end of the dark/feeding period (subgroup 1), and 10 rats/group were sacrificed at the end of the light/gavage treatment period (subgroup 2).

There were no mortalities or treatment-related clinical signs in any of the groups. Weight loss occurred in all rats during the treatment period, with the most marked weight loss observed in groups B and D. These latter groups also had a decreased food intake. Results of liver weights and liver glycogen levels are presented in Table 5-5.

Table 5-5 Mean Liver Weights and Liver Glycogen Levels (Lina and de Bie, 2000a)

Croup	Subgroup	Mean Liver Weight (g)	Mean Liver Glycogen Levels (g/liver/100 g body weight)
A	1	11.14 ± 0.58	0.14± 0.01
	2	9.44± 0.79	<0.01*
B	1	9.75± 0.44	0.04 ± 0.02
	2	10.37 ± 1.14	0.10 ± 0.03
C	1	12.71 ± 0.60	0.26 ± 0.03
	2	10.39 ± 0.59	<0.01*
D	1	11.50 ± 0.90	0.14 ± 0.04
	2	10.71 ± 1.05	0.02± 0.02

* limit of detection

000086

The results from subgroup 1 indicate that D-tagatose may lead to an increase in liver glycogen deposition and increased liver mass when co-ingested with a source of glucose (groups ***C*** and D), whereas administration of D-tagatose and glucose separated by a time interval (group B) does not (Bär, 2000). Similarly, subgroup 2 data indicates that glycogen precursors also increase glycogen deposition and that necropsy following an interval where no D-tagatose is ingested results in a partial reversal of glycogen deposition and liver weight increase.

The second Lina and de Bie (2000b) study was conducted in male Sprague-Dawley rats (rather than Wistar) according to the study design presented in Table 5-6. Again, animals in a given group received both treatment **1** and 2 in the manner described.

Table 5-6 Treatment Groups (Lina and de Bie, 2000b)

Group	Number of Animals		Treatment 1 dark/feeding period	Treatment 2 light/gavage treatment
	Subgroup 1'	Subgroup 2[2]		
A	12	**18**	Polycose ***via*** diet"	2 g peptone/rat/day ***via*** gavage
B	**12**	**18**	D-Tagatose ***via*** diet[3]	6 g polycose + 2 g pepton/rat/day ***via*** gavage
C	12	**18**	Polycose + tagatose ***via*** diet[3]	2 g pepton/rat/day ***via*** gavage

'Subgroup **1** was aborted on day 10 of the study.

'Animals in subgroup 2 were sacrificed at different stages (6 rats/stage) after treatment for ***5*** days:

Subgroup 2a: from 0600h during the dark/feeding period

Subgroup **2b:** from 1200h during the light period prior to gavage dosing

Subgroup 2c: from 1800h during the light period after the last gavage dosing

[3] During the treatment period, 16 g feed/rat/day was given at the start of the dark period. Remaining feed (if any) was removed from the cages in the morning.-When D-tagatose was included in the diet, it was present at concentrations of approximately 16.5%.

There were no mortalities or treatment-related clinical signs in any of the groups, with the exception of occasional salivation and grunting in rats of group B. Weight loss occurred in all groups during the first few days of treatment. There were no significant differences in body weights among the groups, either during the treatment period, or at necropsy at all three stages. The results of this study, similar to the previously described study, further indicate that glycogen deposition is associated with liver enlargement and that these effects are associated with high

intakes of glycogen precursors and appear to be accentuated by high-dose administration of D-tagatose.

The last of the 3 studies evaluating the effects of co-administration of glycogen precursors on D-tagatose induced liver enlargement was a 28-day study with male Sprague-Dawley rats (Lina and de Bie, 2000c). The study was conducted according to the design presented in Table 5-7.

Table 5-7 Treatment Groups (Lina and de Bie, 2000c)

Group	Number of Animals	Treatment 1 dark/feeding period	Treatment 2 light/gavage treatment
A	12	Polycose *via* diet'	Tap water *via* gavage
B[5]	12	D-Tagatose *via* diet?	Polycose in tap water *via* gavage[4]
C[5]	12	Polycose + D-tagatose *via* diet'	Tap water *via* gavage
D[5]	12	Polycose + D-tagatose *via* diet"	In order not to interfere with the feeding schedule, rats of group D did not receive placebo gavage treatment

[1] 18 g (day 0-8) or 19.2 g (from day 8) feed/rat/day was administered at the start of the dark period. D-tagatose was present at dietary concentrations of approximately 14.5%.

'12 g (day 0-8) or 12.8 g (from day 8) feed/rat/day was administered at the start of the dark period. D-tagatose was present at dietary concentrations of approximately 22%.

4 x 4.5 g (day 0-8) or 4 x 4.8 g (from day 8) was administered in equal portions, equally spread over the 24 hour period. D-tagatose was present at dietary concentrations of approximately 14.5%.

[4] 6 g (day 0-8) or 6.4 g (from day 8) polycose/rat/day

Intake of tagatose was ~ 7 g/kg body weight/day

There were no treatment-related clinical signs in any of the groups, although one rat in group B died on Day 12 following gavage dosing. Weight loss occurred in all groups during the first 10 days of treatment. Results of liver weights and liver analysis, including total protein, total lipid, residual moisture, glycogen, and glucose levels are presented in Table 5-8.

000088

Table 5-8 Absolute Values for Liver Parameters (Lina and de Bie, 2000c)

Group	Liver weight (g)	Total lipid (g/liver)	Glycogen (g/liver)	Glucose (g/liver)	Protein (g/liver)	DNA (mg/liver)	Total moisture (g/liver)
A	9.88	0.58	0.091	0.109	1.68	22.2	6.88
B	10.87	0.67	0.078	0.122	1.83	22.8	7.54
C	11.16	0.60	0.115	0.108	1.88	24.6	7.74
D	11.52	0.64	0.089	0.127	2.00	23.8	8.00

Relative liver weights were increased in groups B, C, and D and absolute liver weights were also increased in these groups (Table 5-8), but only to statistical significance in groups C and D. Because relative liver weights were increased even in group B, high-doses of D-tagatose, at least to some degree, can produce liver weight increases in the absence of glucose co-ingestion. Groups C and D [*i.e.*, administered polycose and D-tagatose in the diet followed by gavage with tap water (group C) or no further treatment (group D) during the "light" phase] showed the highest liver weights.

The results of three additional unpublished rat feeding studies (Lina and de Bie, 2000a,b,c) similarly indicate that liver enlargement can be explained only in part by glycogen deposition.

6.0 CRITICAL ENDPOINTS FOR THE SAFETY OF D-TAGATOSE

6.1 GI Effects

It is well established that incompletely absorbed sugars (*e.g.*, lactose) and polyols (*e.g.*, sorbitol), occurring as a result of excessive consumption, can induce uncomfortable gastrointestinal effects

laxation. These latter symptoms suggest the presence in the gut of an osmotically active substance (Buemann ***et al.***, 1999c; Saunders ***et al.,*** 1999b).

It is anticipated that heavy consumption (90th percentile) of D-tagatose by adults would be up to 14.9 g/day. This level of exposure is lower than dose levels that were reasonably well tolerated in clinical trials. In addition, anticipated exposures from the proposed use of D-tagatose would likely be spread out over the day, rather than consumed in single large doses as was the case for the clinical trials. Therefore, D-tagatose consumption, at the levels associated with the proposed uses, would not likely be associated with gastrointestinal physiological effects other than perhaps flatulence.

6.2 Liver Effects

Liver enlargement was the only notable effect in Sprague-Dawley rats consuming diets containing 10 to 20% D-tagatose for 90 days (Kruger ***et al.,*** 1999a). **A** NOEL of 5% was established from this study. The exact mechanism that leads to increased liver weight is not entirely understood, however, this effect is not unique to D-tagatose as liver enlargement, without overt signs of organ damage, has been observed with many other common food substances, including fructose.

A series of studies in rats was conducted to elucidate possible mechanisms for liver enlargement reported in rats fed high-doses of D-tagatose. Following review of these studies, Bar ***et al.*** (1999) hypothesized that the increase in liver weight may have been associated with an increase in glycogen storage due to D-tagatose that then triggered a compensatory (adaptive) growth of the liver in Sprague-Dawley rats. While glycogen deposition in concert with intake of glycogen precursors may be related to the liver enlargement, in certain instances residual liver enlargement remained following cessation of D-tagatose treatment or following a fasting period.

Based on the results of the 90-day study, the NOEL for liver enlargement in fasted Sprague-Dawley rats was **5%** (males: **2.3** to 6.7 g/kg/day; females: **2.8** to **6.3** g/kg body weight/day). Liver enlargement was not seen in pigs dosed with D-tagatose at **20%** in the diet or at 10% in combination with 10% sucrose for a period of **33** days (Mann, 1997).

000090

Liver enlargement is also seen in rats with high consumption of fructose (Kazdova *et al.*, 1976; Poulsom, 1986), although at about 4 times higher doses than D-tagatose (Bar, 1999,2000).

In any case, liver enlargement seen at high doses in Sprague-Dawley rats (*i.e.*, NOEL of 2.3 to 6.7 g/kg body weight/day) is not considered to be of toxicological significance to potential human exposures anticipated to occur under the intended conditions of use. The anticipated exposures to D-tagatose from heavy consumption are at least 10-fold lower than the NOEL (5% in the diet) for liver enlargement reported in the rat studies. More importantly, the results of a repeated-dose clinical trial where D-tagatose was administered with food at a dose rate of 45 g/day in humans, showed, by use of magnetic resonance imaging technology, that there was no differences in liver volume or in liver glycogen content between subjects consuming D-tagatose and those consuming equivalent amounts of sucrose.

On the basis of the NOEL in Sprague-Dawley rats (5% in the diet), the lack of histopathological evidence of overt hepatotoxicity at any dose, the lack of effects on the livers of Wistar rats or of pigs, the results of the human clinical trial, and the estimated daily intake associated with the intended uses of D-tagatose, liver enlargement is considered not to be a safety concern for humans.

6.3 Plasma Uric Acid Effect

Consumption of D-tagatose, as with fructose, has the potential to increase plasma concentrations of uric acid as a result of the activity of fructokinase, in the presence of ATP, on these substrates. Phosphorylation of D-tagatose or fructose transiently decreases the hepatic ATP and P_i pools. Since P_i is an inhibitor of adenosine deaminase, a rate-limiting enzyme in the catabolism of adenosine monophosphate, phosphorylation of D-tagatose or fructose may result in increased degradation of purine nucleotides and increased release of uric acid from the liver. Fructose, xylitol, and sorbitol have all been reported to produce hyperuricemia in humans at doses in the range of 10 g/kg body weight/day, equivalent to 60 to 80 g/day (reviewed in Saunders *et al.*, 1999b).

000091

Three clinical studies with D-tagatose have evaluated potential uricemic effects. Ingestion of single oral doses of 30 grams of D-tagatose (in solution) by 8 fasted volunteers was found to increase serum uric acid from 6.1 to 7.2 mg/100 ml within 50 minutes of dosing. This increase

remained within the stated normal range of less than 7.6 mg/100 ml. Similarly, as expected, inorganic phosphate values were also decreased 50 minutes post-dosing (Buemann *et al.*, 1999b). By comparison, fructose was reported to produce smaller increases in plasma uric acid and did not change inorganic phosphate levels.

The results of a second clinical study in which 8 healthy and 8 type-2 diabetic subjects consumed single 75 gram doses of D-tagatose or glucose showed increased plasma uric acid after dosing with D-tagatose, but not with glucose. Three hours after dosing, plasma uric acid levels had declined, but not to baseline values (Saunders *et al.*, 1999b). In a repeat dosing phase of the study in which D-tagatose (to both diabetics and normals) or glucose (normals only) was administered as 3 separate 25 gram doses at meal times for an 8-week period, no significant effects on fasting plasma uric acid levels were found compared to baseline values (Saunders *et al.*, 1999b). These data demonstrate that D-tagatose does not produce a chronic cumulative effect on uric acid production (*i.e.*, plasma uric acid levels did not increase over time).

A third study, the one evaluating the effects of D-tagatose on liver volume and liver glycogen content, found that consumption of 45 grams of D-tagatose as 3 separate 15 gram doses produced no increases in fasting serum uric acid and did not alter post-prandial serum uric acid concentrations in comparison to consumption of equal amounts of sucrose (Boesch *et al.*, 2001). As consumed in food, the dosing pattern of the Boesch *et al.* (2001) study is most relevant to the assessment of D-tagatose on serum uric acid in humans.

Based on the information indicating that plasma uric acid levels would not be affected by consumption in D-tagatose in food at doses up to 15 grams 3 times per day (Boesch *et al.*, 2001), and noting that changes even at single doses in the range of 30 to 75 grams per day are mild and do not approach serum concentrations of 15 to 20 mg/dl associated with uric acid nephropathy (Brady *et al.*, 1996), increased uric acid is not a major concern for D-tagatose at its intended level of use in foods (*i.e.*, up to 14.9 g/day).

6.4 D-Tagatose and Fructose Interaction

000092

Since fructose and D-tagatose have a similar chemical structure and share a common metabolic pathway, involving fructokinase and aldolase B, the potential for effects associated with an interaction between these two sweeteners, if consumed at the same time, requires consideration.

The possibility of metabolic interactions between fructose and D-tagatose has been discussed in detail by Bar (2000). The key issues raised in this review were the following:

- With respect to intestinal absorption, D-tagatose does not promote nor inhibit the carrier-mediated diffusion of fructose through the intestinal mucosa (Crouzoulon, 1978; Sigrist-Nelson and Hopfer, 1974). The interaction of D-tagatose with one of the carriers for fructose (GLUTS) was demonstrated to be weak (Km=59 mM) (Tatibouet *et al.*, 2000).

- The phosphorylation rate of fructose and D-tagatose on first hepatic passage is determined by the rate of cellular uptake (Sestoft and Fleron, 1974; Baur and Heldt, 1977; Okuno and Glieman, 1986). **As** the GLUT2 transporter appears to be responsible for the uptake of fructose by the hepatocytes, D-tagatose is not expected to interfere with the hepatocellular uptake of fructose.

- Fructokinase has a higher affinity to fructose than D-tagatose (Sanchez *et al.*, 1971; Raushel and Cleland, 1973, 1977). ***In vitro,*** the presence of D-tagatose at a concentration of **0.35** mM reduced the phosphorylation of fructose (0.05 mM) by only 10% (Malaisse *et al.*, 1989). Under physiological conditions, the intracellular concentrations of D-tagatose would not be sufficient to inhibit fructokinase.

- Consumption of a single 30 g dose of D-tagatose was shown to decrease ATP levels in human liver by about 10% (Buemann *et al.*, 2000a). At the lower levels of intake from the proposed uses of D-tagatose (90th percentile intake of 14.9 g spread over the day), this decrease in **ATP** levels would be much lower. Thus, the corresponding increase in ADP, a known inhibitor of fructokinase, would not be sufficient to produce any significant inhibition of the enzyme.

- Fructokinase is inhibited by fructose-1-phosphate only at relatively high concentrations (Ki = 12 mM) (Raushel and Cleland, 1977). In the perfused rat liver, fructose (0.5 mM) was rapidly phosphorylated even in the presence of D-tagatose (0.5 mM) (Quistorff and Therkelsen, 1999). The maximum D-tagatose-1-phosphate concentration detected in the human liver after consumption of a 30 g dose of D-tagatose was 1 mM (Buemann *et al.*, 2000a).

000093

- < There was no indication of an additive or synergistic effect of fructose plus D-tagatose on liver enlargement in rats administered diets containing 15% sucrose, 15% D-tagatose, 5% D-tagatose plus 10% fructose, or 15% fructose for 90 days (Saunders, 1992). Although liver weights were increased in the group receiving 15% D-tagatose alone, such was not the case for the 5% D-tagatose plus 10% fructose; *i.e.,* liver weights of rats receiving the combined sweeteners were not significantly different from the two controls (15% sucrose and 15% fructose). Additive or synergistic effects on liver weights also were not observed in groups of rats administered 10% tagatose + 10% fructose for six months compared to rats fed a control starch diet (Lina and de Bie, 2000d).

- < Enzyme kinetic considerations and the results from a liver perfusion study indicate that the levels of D-tagatose from the proposed uses would not be sufficient to interfere with the metabolism of fructose (Quistorff and Therkelsen, 1999; Bar, 2000).

- < With respect to uric acid production, the results of clinical trials demonstrated that fasting plasma uric acid levels were not affected by consumption of D-tagatose in food at doses up to 45 grams per day, and that the changes observed at single doses in the range of 30 to 75 grams per day were mild and did not approach serum concentrations of 15 to 20 mg/100 ml associated with uric acid nephropathy (Buemann ***et al.,*** 1999b; Saunders ***et al.,*** 1999b; Boesch ***et al.***, 2001).

The above data provide reasonable evidence that ingested D-tagatose, based on the proposed use levels, will not interfere with the metabolism of co-ingested fructose.

6.5 Glycation of Proteins

Reducing sugars are known to react in a non-enzymatic (Maillard reaction) fashion with amino groups, including those of peptides and proteins. This reaction occurs in poorly controlled diabetics resulting in the formation of glycosylated hemoglobin (Hb**1A,)** during hyperglycemic episodes. The reactivity of various aldose and ketose sugars, including D-tagatose, has been evaluated in **an** ***in vitro*** assay in which the sugars were incubated with myofibrillar protein for **48** hours followed by measurement of the degree of glycation (Syrovy, 1994). The results demonstrated that compared to other sugars, the reactivity of D-tagatose was low. Similarly, in a short-term, 4-hour study, **Bunn** and Higgins (1981) report that rhamnose, glucose, and D-

tagatose were the least reactive sugars. These data support the conclusion that D-tagatose does not pose any unique concern to the diabetic population.

7.0 DISCUSSION AND CONCLUSION

D-Tagatose, a stereoisomer of fructose, has been proposed for use as a bulk sweetener with prebiotic activity in the preparation of low- and reduced-calorie foods including diet beverages, ready to eat cereals, sugar free chewing gum, icings or glazes, light ice cream and yogurt, diet nutrient bars, dietetic candy, and diet meal replacements. Currently, trace levels of D-tagatose are consumed from the diet, as a result of its presence in sterilized milk products (formed from lactose by heat treatment). Additionally, consumption of lactulose syrups (OTC preparations) result in exposures of 0.2 to **0.3** g/day.

The estimated mean consumption of D-tagatose from the proposed food uses is approximately ***6.6*** g/day with heavy users (**90**th percentile) consuming up to 14.9 g/day. Estimated consumption in the total population and in adults is higher than children and teenagers partly as a result of the consumption of meal replacements for weight control. However, on a body weight basis, consumption of D-tagatose is highest in children aged 2 to 5 years (0.42 g/kg bw/day). The corresponding 90th percentile g/day intake estimate for this age group is 7.4 g/day. The average intake is 3.7 g/day. As a comparison, average fructose consumption (based on the 1977-79 USDA Nationwide Food Survey) ranged from 15 g for infants to 54 g for males aged 15 to 18 years (Park and Yetley, 1993).

Data supporting the safety of D-tagatose include several clinical trials in healthy and diabetic volunteers, and toxicology studies including acute, subchronic, genotoxicity and developmental toxicity studies (Trimmer, 1989; Buemann ***et af.,*** 1999a,b,c, 2000b; Donner ***et af.,*** 1999; Kruger ***et af.,*** 1999b,c; Lee and Storey, 1999; Saunders *et* ***af.,*** 1999b; Boesch *et al.*, 2001). In animals, the only notable treatment-related effects were increased liver weights observed in Sprague-Dawley rats administered 10 to 20% D-tagatose in the diet for 90 days and hepatocellular hypertrophy at dietary levels of 15 and 20%, in this same study (Kruger ***et af.,*** 1999a). The NOEL determined from this study was 5% (**2.3** to **6.7** g/kg/day). No liver effects were observed in Wistar rats administered up to 10% D-tagatose in the diet (Lina and de Bie, 2000d) or in pigs administered D-tagatose at up to 20% in the diet (Mann, 1997). Also, in a double-blind, two-

way cross-over clinical trial, specifically conducted to assess potential effects on liver volume and liver glycogen concentration, no significant differences in these parameters were found between D-tagatose and sucrose at doses of 45 g/day (Boesch ***et al.,*** 2001).

The predominant effects associated with excessive consumption of D-tagatose (up to 75 g/day), noted in the clinical trials, were physiological gastrointestinal effects (Buemann ***et al.***, 1999a,b,c, 2000b; Donner ***et al.,*** 1999; Lee and Storey, 1999; Saunders ***et al.,*** 1999b; Boesch ***et al.***, 2001). Such effects also are commonly associated with excessive consumption of other sugars and polyols (Beaugerie ***et al.,*** 1991; Rambaud and Flourie, 1994; Koutsou ***et al.,*** 1996). At doses of up to 25 g D-tagatose per eating occasion, flatulence was generally the only side-effect reported (Donner ***et al.,*** 1999; Lee and Storey, 1999; Boesch ***et al.,*** 2001).

In summary, the safety assessment of D-tagatose, under the intended conditions of use and consequent consumption levels, was based upon the presence of at least a 10-fold safety factor between estimated intake levels and the **5%** dietary NOEL established in Sprague-Dawley rats based on the absence of liver enlargement and on the results of a human clinical trial that demonstrated D-tagatose produced no signs of liver enlargement or increased glycogen deposition, at exposure levels of 45 grams/day. Supporting data, indicative of a possible species- and or strain-specific effect of D-tagatose on the liver, include the finding of no liver enlargement either in Wistar rats fed D-tagatose at 10% in the diet for ***6*** months or in pigs administered D-tagatose at 20% in the diet for **33** days. It should be noted, however, that differences in the durations of the studies and in the dose levels used in these studies do not permit a direct quantitative comparison of the liver effects in Wistar and Sprague-Dawley rats. Finally, although the mechanism for potential effects of D-tagatose on uric acid is conjectural, any possible effect of D-tagatose on uric acid, even of a mild nature, would occur only at exposures several fold in excess of the 90th percentile estimated daily intake.

In conclusion, the available data demonstrate that the anticipated consumption levels of D-tagatose from the proposed uses in food, even **at** the 90th percentile, are not likely to be associated with any adverse health effects in humans and should be considered safe.

000096

8.0 REFERENCES

Aoyama, Y. Otaki, R., Yoshino, K., Yoshida, A., and Ashida, K. 1987. Effects of dietary fructose, glucose, fibers, and hypolipidemic drugs on serum and liver lipids in rats. Agric Biol Chem 51(3):671-682.

Bär, A. 1990. Factorial calculation model for the estimation of the physiological caloric value of polyols. Proc. Int. Symposium on Caloric Evaluation of Carbohydrates. Hosoya, N. (Ed.), The Japan Assoc. Dietetic & Enriched Foods, Tokyo, pp. 209-257.

Bar, A. 1999. Characteristics and significance of D-tagatose-induced liver enlargement in rats: An interpretative review. Regul Toxicol Pharmacol 29(2, 2/2):S83-S93.

Bär, A. 2000. Dossier prepared on behalf of Arla Food Ingredients amba, Viby, Denmark for safety assessment of D-tagatose by a panel of independent experts.

Bär, **A.,** Lina, B.A.R., de Groot, D.M.G., de Bie, B., and Appel, M.J. 1999. Effects of D-tagatose on liver weight and glycogen content of rats. Regul Toxicol Pharmacol 29 (2, 2/2):S11-S28.

Baur, H., and Heldt, H.W. 1977. Transport of hexose across the liver-cell membrane. Eur J Biochem 74:397-403.

Beaugerie, L., Fourie, B., Pellier, P., Achour, L., Franchisseur, C., and Rambaud, J.C. 1991. Tolerance clinique, absorption intestinale et valuer energetique de quatre polyols pris a jeun. Gastroenterol Clin Biol 15:929-932.

Bertelsen, H., Andersen, H., and Tvede, M. 2001. Fermentation of D-tagatose by human intestinal bacteria and dairy lactic acid bacteria. Microbial Ecology Health Disease (accepted for publication).

BIBRA 1990. A 90-day range in rats with immobilised lactase enzyme system administered daily by gavage. Unpublished report to the British Industrial Biological Research Association, Carshalton, Surrey (U.K.).

Boesch, C. 1999. Molecular aspects of magnetic resonance imaging and spectroscopy. Mol Aspects Med 20:185-138. Cited In: Boesch *et al.*, 2001.

000097

Boesch, C., Diamantis, I., and Bar, A. 2001. Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. Regul Toxicol Pharmacol, accepted for publication.

Brady, H.R., Brenner, B.M., and Lieberthal, W. 1996. Acute Renal Failure In: B.M. Brenner (Ed.) Brenner and Rector's: The Kidney, Fifth Edition, Saunders, Philadelphia, p. 1207. ~~Cited In~~: Saunders *et al.*, 1999b.

Buemann, B., Toubro S., and Astrup, A. 1999a. Human gastrointestinal tolerance to D-tagatose. Regul Toxicol Pharmacol 29(2, 2/2):S71-S77.

Buemann, B., Toubro, S., Holst, J.J., Rehfeld, J., and Astrup, A. 1999b. D-Tagatose, a stereoisomer of D-fructose, increases blood uric acid concentration. Metabolism 49(8):969-976.

Buemann, B., Toubro, S., Raben, A., and Astrup, A. 1999c. Human tolerance to single, high dose of D-tagatose. Regul Toxicol Pharmacol 29(2, 2/2):S66-S70.

Buemann, B., Gesmar, H., Astrup, A., and Quistorff, B. 2000a. Effects of oral D-tagatose, a stereoisomer of D-fructose, on liver metabolism in man as examined by ^{31}PMRS. Metabolism 49(10):1335-1339.

Buemann, B., Toubro, S., Raben, A., Blundell, J., and Astrup, A. 2000b. The acute effect of D-tagatose on food intake in human subjects. Br J Nutr 84:227-231.

Bunn, H.F., and Higgins, P.J. 1981. Reaction of monosaccharides with proteins: Possible evolutionary significance. Science 213(7):222-224.

Cifone, M.A. 1994. Mutagenicity test on D-tagatose in the L5178YTK+/- mouse lymphoma forward mutation assay. Report of Hazleton Washington, Inc., Vienna, VA for Biospherics Inc., Beltsville, MD.

Crouzoulon, G. 1978. Les propriétés cinétiques du flux d'entrée du fructose a travers la bordure en brosse du jéjunum du rat. Arch Int Physiol. Biochim 86:725-740.

Donner, T., Wilber, J., and Ostrowski, D. 1996. D-Tagatose: A novel therapeutic adjunct for non-insulin-dependent diabetes. Diabetes 45(Suppl. 2):125A.

000098

Donner, T.W., Wilber, J.F., and Ostrowski, D. **1999.** D-Tagatose, a novel hexose: Acute effects on carbohydrate tolerance in subjects with and without type 2 diabetes. Diabetes, Obesity and Metabolism 1:285-291.

Elia, M. and Livesey, G. **1988.** Theory and validity of indirect colorimetry during net lipid synthesis. Am J Clin Nutr 47:591-607.

Environ. 2000. Estimated Intake of D-Tagatose from Proposed Uses in Foods and Chewing Gum. Prepared for Arla Foods Ingredients Amba, Denmark. Environ International Corporation, Arlington, VA.

FDA **1993.** Enzyme Preparations: Chemistry Recommendations for Food Additives and GRAS Affirmation Petitions. Version 1.1. Center for Food Safety and Applied Nutrition, Office of Premarket Approval, Chemistry Review Branch, Washington, D.C.

Imfeld, T. **1996.** Telemetric evaluation of D-tagatose provided by MD foods Ingredients Amba, Denmark, with regard to the product's qualification as being "safe for teeth". Dental Institute, University of Zurich, Switzerland.

Imfeld, T. **1998.** Telemetric evaluation of D-tagatose provided by MD Foods Ingredients Amba, Denmark, with regard to the products's qualification as being "safe for teeth". Study performed after different plaque-adaptation periods. Dental Institute, University of Zurich, Switzerland.

Jensen, B.B., and Buemann, B. **1998.** D-Tagatose. The influence of D-tagatose on bacterial composition and fermentation capacity of faecal samples from human volunteers. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Jensen, B.B., and Laue, A. **1998.** D-Tagatose. Absorption of tagatose and fermentation products of tagatose from the gastrointestinal tract of pigs. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Kazdova, L., Vrána, **A.,** and Fabry, P. **1976.** Effect of dietary fructose on the nucleic acid content and DNA synthesis in rat liver and kidneys. Physiol Bohemoslov 25:264.

Koutsou, G.A., Lee, **A.,** Zumbe, A., Flourie, B., and Storey, D.M. **1996.** Dose-related gastrointestinal response to the ingestion of either isomalt, lactitol or maltitol in chocolate. Eur J Clin Nutr 50:17-21.

000099

Kruger, C.L., Whittaker, M.H., Frankos, V.H., and Trimmer, G.W. 1999a. 90-Day oral toxicity study of D-tagatose in rats. Regul Toxicol Pharmacol 29(2, 2/2):S1-S10.

Kruger, C.L., Whittaker, M.H., and Frankos, W.H. 1999b. Genotoxicity tests on D-tagatose. Regul Toxicol Pharmacol 29(2, 2/2):S36-S42.

Kruger, C.L., Whittaker, M.H., Frankos, V.H., and Schroeder, R.E. 1999c. Developmental toxicity study of D-tagatose in rats. Regul Toxicol Pharmacol 29(2, 2/2):S29-S35.

Laerke, H.N., and Jensen, B.B. 1999. D-Tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. J Nutr 129:1002-1009.

Laerke, H.N., Jensen, B.B., and Hojsgaard, S. 2000. The in vitro fermentation pattern of D-tagatose is affected by adaptation microbiota form the gastrointestinal tract of pigs. J Nutr 130:1771-1779.

Langkilde, A.M. 1994. Digestion and absorption of sorbitol, maltitol and isomalt from the small bowel. A study in ileostomy subjects. Eur J Clin Nutr 48:768-775.

Lawlor, T.E. 1993. Mutagenicity test with D-tagatose in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay. Study report from Hazleton Washington, Inc., Vienna, VA for Biospherics, Inc., Beltsville, MD.

Lee, A., and Storey, D.M. 1999. Comparative gastrointestinal tolerance of sucrose, lactitol, or D-tagatose in chocolate. Regul Toxicol Pharmacol 29(2, 2/2):S78-S82.

Levin, G.V., Zehner, L.R., Saunders, J.P., and Beadle, J.R. 1995. Sugar substitutes: Their energy values, bulk characteristics, and potential health benefits. Am J Clin Nutr 62(Suppl.):1161S-1168S.

Lina, B.A.R., Appel, M.J., de Groot, D.M.G., and Salmon, F.G.Ch. 1998. Oral toxicity study with D-tagatose in rats. Unpublished report No. V98.728 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 1998a. Short-term oral feeding study with tagatose in rats. Unpublished report No. V98.500 of TNO Nutrition and Food Research institute, Zeist, The Netherlands for MD Foods amba, Denmark,

000100

Lina, B.A.R., and de Bie, A.Th.H.J. 1998b. Oral toxicity study with D-tagatose in rats. Unpublished report No. V98.927 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000a. Investigation into the consequences of administering D-tagatose spaced from, and simultaneously with, the ingestion of precursors of glycogen in Wistar rats (7-day study). Unpublished report No. V99.1094 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000b. Investigation into the consequences of administering D-tagatose spaced from, and simultaneously with, the ingestion of precursors of glycogen in male Sprague Dawley rats. Unpublished report No. V2529 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000c. Investigation into the consequences of administering D-tagatose spaced from, and simultaneously with, the ingestion of precursors of glycogen in male Sprague-Dawley rats (28-day study). Unpublished report No. V2678 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina, B.A.R., and de Bie, A.Th.H.J. 2000d. Investigation into the consequences of feeding D-tagatose and fructose on liver parameters in Wistar rats. Unpublished report No. V99.1123 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Livesey, G., and Brown, J.C. 1996. D-tagatose is a bulk sweetener with zero energy determined in rats. J Nutr 126:1601-1609.

Malaisse, W.J., Malaisse-Lagae, F., Davies, D.R., and Van Schaftingen, E. 1989. Presence of fructokinase in pancreatic islets. FEBS Lett 255(1): 175-178.

Mann, P.C. 1997. Light microscopic and ultrastructural analysis of livers of pigs fed tagatose. Unpublished pathology report of Experimental Pathology Laboratories Inc., Research Triangle Park, NC, USA.

Murli, H. 1994a. Mutagenicity test on D-tagatose measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells. Study report from Hazleton Washington, Inc., Vienna, VA for Biospherics Inc., Beltsville, MD.

000101

Murli, H. 1994b. Mutagenicity test on D-tagatose ***in vivo*** mouse micronucleus assay. Study report from Hazleton Washington, Inc., Vienna, VA for Biospherics lnc., Beltsville, MD.

Noguchi, T., and Muhlemann, H.R. 1976. The effects of some carbohydrates on in vitro growth of Streptococcus mutans and Actinomyces viscosus. Schweiz Mschr Zahnheilk 86:1361/45.

Normén, L., Laerke, H.N., Langkilde, A.-M., and Anderson, H. 2001. Small bowel absorption **of** D-tagatose and related effects on carbohydrate digestibility. An ileostomy study. Am J Clin Nutr 73(1):105-110.

Okuno, Y., and Gliemann, J. 1986. Transport of glucose and fructose in rat hepatocytes at 37EC. Biochim Biophys Acta 862:329-334.

Park, Y.K., and Yetley, E.A. 1993. Intakes and food sources of fructose in the United States. Am J Clin Nutr 58(Suppl.):S737-S747.

Poulsom, R. 1986. Morphological changes of organs after sucrose or fructose feeding. Prog Biochem Pharmacol 21:104-134.

Quistorff, B., and Therkelsen, I. 1999. Effects of combined D-tagatose and D-fructose in the perfused rat liver. Unpublished report.

Rambaud, **J.C.,** and Flourie, B. 1994. Mechanisms of Carbohydrate-induced Diarrhoea. In: Binder, H.J., Cummings, **J.H.,** and Soergel, K. (Eds.) Short Chain Fatty Acids. Kluwer Academic, Boston, MA, pp.232-239.

Raushel, F.M., and Cleland, W.W. 1973. The substrate and anomeric specificity of fructokinase. J Biol Chem 248(23):8174-8177.

Raushel, F.M., and Cleland, W.W. 1977. Bovine liver fructokinase: Purification and kinetic properties. Biochem 16(10):2169-2175.

Richards, E.L., and Chandrasekhara, M.R. 1960. Chemical changes in dried skim-milk during storage. J Dairy Res 27:59-66.

Rognstad, R. 1975. Gluconeogenesis from D-tagatose by isolated rat and hamster liver cells. FEBS Letters 52(2):292-294.

000102

Rognstad, R. 1982. Pathway of gluconeogenesis from tagatose in rat hepatocytes. Arch Biochem Biophys 218(2):488-491.

Sanchez, J.J., Gonzalez, N.S., and Pontis, H.G. 1971. Fructokinase from rat liver. I. Purification and properties. Biochim Biophys Acta 227:67-78.

Saunders, J.P. 1998. Analysis of selected foods for D-tagatose. Unpublished Report of Biospherics Inc., Beltsville, MD.

Saunders, J.P. 1992. Multiple fat rat study. Unpublished report of Biospherics, Inc., Beltsville, MD.

Saunders, J.P., Zehner, L.R., and Levin, G.V. 1999a. Disposition of D-[U-^{14}C] tagatose in the rat. Regul Toxicol Pharmacol 29(2, 2/2):S46-S56.

Saunders, J.P., Donner, T.W., Sadler, J.H., Levin, G.V., and Makris, N.G. 1999b. Effects of acute and repeated oral doses of D-tagatose on plasma uric acid in normal and diabetic humans. Regul Toxicol Pharmacol 29(2,2/2):S57-S65.

Schroeder, R.E. 1994a. A range-finding teratology study in rats with D-tagatose. Study report from Pharmaco LSR Inc., East Millstone, N.J. for Biospherics Inc., Beltsville, MD.

Schroeder, R.E. 1994b. A teratology study in rats with D-tagatose. Study report from Pharmaco LSR Inc. East Millstone, N.J. for Biospherics, Inc., Beltsville, MD.

Sestoft, L., and Fleron, P. 1974. Determination of the kinetic constants of fructose transport and phosphorylation in the perfused rat liver. Biochim Biophys Acta 345:27-38.

Sigrist-Nelson, K., and Hopfer, U. 1974. A distinct D-fructose transport system in isolated brush border membrane. Biochim Biophys Acta 367:247-254.

Slotboom, J., Fluck, C.E., Nuoffer, J.M., Kreis, R., Mullis, P.E., and Boesch, C. 1999. Hepatic glycogen metabolism in children and adolescents with IDDM compared to normals: a 13C magnetic resonance spectroscopy study. MAGMA 8 (Suppl. 1):225-226.

Syrovy, I. 1994. Glycation of myofibrillar proteins and ATPase activity after incubation with eleven sugars. Physiol Res 43:61-64.

000103

Tatibouet, A., Yang, J., Morin, C., and Holman, G.D. 2000. Synthesis and evaluation of fructose analogues as inhibitors of the D-fructose transporter GLUT5. Bioorg Med Chem 8:1825-1833.

Trimmer, G.W. 1989. Acute oral toxicity study (MRD-89-393). Study report by Exxon Biomedical Science, Inc., East Millstone, N.J. for Biospherics Inc., Beltsville, MD.

Troyano, E., Olano, A., Fernández-Díaz, M., Sanz, J., and Martinez-Castro, I. 1991, Gas chromatographic analysis of free monosaccharides in milk. Chromatographia 32(7/8):379-382.

Troyano, E., Villamiel, M., Olano, A., Sanz, J., and Martinez-Castro, I. 1996. Monosaccharides and myo-inositol in commercial milks. J Agric Food Chem 44:815-817.

USDA 2000. 1994-96, 98 Continuing Survey of Food Intakes by Individuals [CD-ROM], data and documentation. Agricultural Research Service, United States Department of Agriculture. National Technical Information Service, Accession No. PB2000-500027.

VVT 1998. Research Report No. BEL399/98 of VTT Biotechnology and Food Research, Espoo, Finland for Valio Oy.

WHO 2000. D-Tagatose. Safety evaluation of certain food additives and contaminants. Fifty-fifth Meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additive Series. World Health Organization, Geneva.

Zehner, L.R. 1994. Human exposure to D-tagatose in two lactulose-based drugs. Internal memorandum of Biospherics Inc., Beltsville, MD.

APPENDIX A

SPECIFICATIONS

Table A-1 Specifications for D-Tagatose

Parameter	Specification
Identification	
Solubility	Very soluble in water, very slightly soluble in ethanol
Specific rotation	$[\alpha]^{20}_{D}$ -4 to -5.6° (1% aqueous solution)
Melting range	133-137°C
Reaction with alkaline cupric tartrate	Formation of red cuprous oxide
Chromatography	Retention time of major peak same as standard in chromatographic assay
Purity	
Assay	Not less than 98% on dry basis
Loss on drying	Not more than 0.5% (102oC, 2hr)
Total ash	Not more than 0.1%
Lead	Not more than .5 mg/kg*

* According to FDA's Advanced Notice of Proposed Rulemaking on Reducing Lead Levels in Foods, D-Tagatose is a moderately high-poundage ingredient (estimated 20 million pounds per year). See 59 Fed. Reg. 5363,5368 (Feb. 4,1994).

000105

APPENDIX B

PROPOSED FOOD USE CATEGORIES AND NORMALIZED CONSUMPTION ESTIMATES

Table B-2 Proposed Food Uses and Use-Levels for D-Tagatose

Regulatory Category	Food Type	Use Level (%)
Beverages and beverage bases	Diet and/or sugar free carbonated beverages	1
Breakfast cereals	Ready to eat cereals	5.45-20 (serving size dependent)
Chewing gum	Sugar less and sugar free chewing gum	60
Coffee and tea	Ready to drink teas presweetened with low-calorie sweeteners	1
Confections and frostings	icings or glazes used on baked goods (cookies, pastries, brownies, and angel food, chiffon and pound cakes)	30
Frozen dairy desserts	Light ice cream (ice milk), frozen milk desserts, low-fat and non-fat frozen yogurt and related frozen novelties	3
Grain products	Low-fat, reduced-fat, diet, energy or nutrient fortified bars	10
Hard candy and cough drops	Regular and dietetic hard candies	15
Soft candy	Dietetic soft candies	10
Meal replacements	Powdered products prepared with milk	5 g/serving

000106

Normalization of D-Tagatose Intakes from the USDA CSFII Food Consumption Database to Combine Consumption Estimates from All Other Proposed Food Categories with Those from Chewing Gum/Meal Replacements

I. Users-only average, total population, 2 years and over.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex 6, Table 1; Environ, 2000) is 0.35 grams per kilogram body weight per day (g/day). Mean users D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 4.5 g/day or 0.08 g/kg body weightlday and from meal replacers is 5.0 g/day or 0.09 g/kg body weight/day assuming 1 serving per day. The sum of the intake from all 7 food categories, sugarless chewing gum and meal replacements (1 serving) is 0.52 g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(0.52)}{0.35} \times 0.08 \text{ g/kg body weightlday} = 0.12 \text{ g/kg body weightlday}$$

11. Users-only 90th percentile, total population, 2 years and over.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex 6, Table 1; Environ, 2000) is 0.71 grams per kilogram body weight per day (g/kg body weightlday). Heavy users (90th percentile) D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 9.0 g/day or 0.16 g/kg body weight/day and from meal replacers is 10.0 g/day or 0.18 g/kg body weight/day assuming 2 servings per day. The sum of the intake from all 7 food categories, chewing gum and meal replacers is 1.05 g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(1.05)}{0.71} \times (0.18) \text{ g/kg body weight/day} = 0.27 \text{ g/kg body weight/day}$$

000107

Normalization of D-Tagatose Intakes from the USDA CSFII Food Consumption Database to Combine Consumption Estimates from All Other Proposed Food Categories with Those from Chewing Gum

I. Users-only average, children aged 2 to **5** years.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex 6, Table 1; Environ, 2000) is 0.67 grams per kilogram body weight per day (g/day). Mean users D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 2.8 g/day or 0.16 g/kg body weightlday. The sum of the intake from all 7 food categories and chewing gum is 0.83 g/kg body weight/day.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(0.83)}{0.67} \times (0.17) \text{ g/kg body weightlday} = 0.21 \text{ g/kg body weight/day}$$

11. Users-only 90th percentile, children aged 2 to **5** years.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex 6, Table 1; Environ, 2000) is 1.2 grams per kilogram body weight per day (g/kg body weightlday). Heavy users (90th percentile) D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 5.6 g/day or 0.32 g/kg body weightlday. The sum of the intake from all 7 food categories and chewing gum is 1.52 g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(1.52)}{1.20} \times (0.33) \text{ g/kg body weight/day} = 0.42 \text{ g/kg body weightlday}$$

000108

Normalization of D-Tagatose Intakes from the USDA CSFII Food Consumption Database to Combine Consumption Estimates from All Other Proposed Food Categories with Those from Chewing **Gum**

I. Users-only average, children aged 6 to 12 years.

Sum of separate average D-tagatose intakes for **7** food groups (see Annex 6, Table 1; Environ, 2000) is 0.48 grams per kilogram body weight per day (g/day). Mean users D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 3.9 g/day or 0.12 g/kg body weightlday. The sum of the intake from all 7 food categories and chewing gum is 0.60 g/kg body weight/day.

The normalized total D-tagatose consumption from all proposed food categories is

$$\left(\frac{0.60}{0.48}\right) \times (0.13) \text{ g/kg body weightlday} = 0.16 \text{ g/kg body weightlday}$$

11. Users-only 90th percentile, children 6 to 12 years.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex **6**, Table **1**; Environ, 2000) is **0.87** grams per kilogram body weight per day (g/kg body weightlday). Heavy users (90th percentile) D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is **7.8** g/day or 0.25 g/kg body weightlday. The sum of the intake from all 7 food categories and chewing gum is 1.12 g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\left(\frac{1.12}{0.87}\right) \times (0.25) \text{ g/kg body weightlday} = 0.32 \text{ g/kg body weightlday}$$

000109

Normalization of D-Tagatose Intakes from the USDA CSFII Food Consumption Database to Combine Consumption Estimates from All Other Proposed Food Categories with Those from Chewing Gum

I. Users-only average, teenagers aged **13** to **19** years.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex 6, Table 1; Environ, 2000) is **0.41** grams per kilogram body weight per day (g/day). Mean users D-tagatose consumption from the Market Facts chewing gum survey (see Table **10;** Environ, 2000) is 5.6 g/day or **0.09** g/kg body weightlday. The sum of the intake from all 7 food categories and chewing gum is 0.50 g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(0.50)}{\mathbf{0.41}} \times (0.08) \text{ g/kg body weightlday} = 0.10 \text{ g/kg body weightlday}$$

11. Users-only 90th percentile, total population, teenagers aged 13 to **19** years.

Sum of separate average D-tagatose intakes for **7** food groups (see Annex 6, Table 1; Environ, 2000) is 0.60 grams per kilogram body weight per day (g/kg body weightlday). Heavy users (90th percentile) D-tagatose consumption from the Market Facts chewing gum survey (see Table **10;** Environ, 2000) is 11.2 g/day or 0.18 g/kg body weight/day. The sum of the intake from all 7 food categories and chewing gum is **0.78** g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(0.78)}{0.60} \times (0.15) \text{ g/kg body weightlday} = 0.20 \text{ g/kg body weightlday}$$

000110

Normalization of D-Tagatose Intakes from the USDA CSFII Food Consumption Database to Combine Consumption Estimates from All Other Proposed Food Categories with Those from Chewing Gum/meal Replacements

I. Users-only average, adults 20 years and older.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex **6,** Table 1; Environ, 2000) is 0.33 grams per kilogram body weight per day (g/day). Mean users D-tagatose consumption from the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 4.7 g/day or 0.06 g/kg body weightlday and from meal replacers is 5.0 g/day or 0.09 g/kg body weight/day assuming 1 serving per day. The sum of the intake from all **7** food categories, chewing gum and meal replacers is 0.48 g/kg body weightlday.

The normalized total D-tagatose consumption fiom all proposed food categories is

$$\frac{(0.48)}{0.33} \times (0.06) \text{ g/kg body weightlday} = 0.09 \text{ g/kg body weight/day}$$

11. Users-only 90th percentile, adults 20 years and older.

Sum of separate average D-tagatose intakes for 7 food groups (see Annex **6,** Table 1; Environ, 2000) is 0.52 grams per kilogram body weight per day (g/kg body weightlday). Heavy users (90th percentile) D-tagatose consumption fiom the Market Facts chewing gum survey (see Table 10; Environ, 2000) is 9.4 g/day or 0.13 g/kg body weight/day and from meal replacers is 10.0 g or 0.1**8** g/kg body weight/day assuming **2** servings per day. The sum of the intake from all 7 food categories and chewing gum is 0.83 g/kg body weightlday.

The normalized total D-tagatose consumption from all proposed food categories is

$$\frac{(0.83)}{0.52} \times (0.13) \text{ g/kg body weight/day} = 0.21 \text{ g/kg body weightlday}$$

000111

Use By
Hyperuricemics

000112

Supplementary considerations in relation to the hyper-uricemic potential of D-tagatose

Author: Albert Bar, Ph.D.

Date: May 11,2001

1. Introduction

The ingestion of single high bolus doses of D-tagatose (≥30 g), fructose (≥50 g) and sucrose (≥100 g) is associated with a mild, transient increase of plasma uric acid concentrations in healthy human subjects. At lower doses, none of these sugars has a significant effect on plasma uric levels (Table 1). In addition, repeated daily doses of 3 x 15 g D-tagatose, ingested with the main meals for a period of 28 days, also produced no effect on fasting plasma uric acid levels (Boesch et al., 2001).

Although D-tagatose has a somewhat higher uricemic potential than fructose, it should be noted that intake of D-tagatose under the conditions of intended use is several fold lower than normal daily consumption of fructose. The reason for the higher uricemic potential of D-tagatose is due to the fact that the intracellular inorganic phosphate (P_i) concentration is restored at a slower rate after exposure to D-tagatose than fructose because tagatose-1-phosphate is cleaved by aldolase B at a slower rate than fructose-1-phosphate (Vincent et al., 1986). In addition, the intake of a high dose of D-tagatose has a small hemo-concentration effect because water is secreted in the intestinal lumen in order to restore the isotonicity of the D-tagatose-containing chyme (Buemann et al., 1999).

In the context of the safety assessment of D-tagatose under the conditions of its intended use (i.e., 90th percentile consumption of about 15 grams/day), the question arose as to whether hyperuricemic persons would be more susceptible than normal individuals to the uricemic effects of D-tagatose.

2. Expected consequences of D-tagatose and fructose ingestion on plasma uric acid concentrations of hyperuricemic individuals

Hyperuricemia is caused by either an increased production or a decreased urinary excretion of uric acid.

An overproduction of uric acid may be the result of an increased de-novo synthesis of purines, an increased intake of purines with the diet, an increased DNA breakdown from cell damage, or an increased catabolism of purines (e.g., ATP, ADP, AMP).

A reduced renal elimination of uric acid may result from an insufficient intake of liquids, an excessive intake of sodium or alcohol, intense exercise, the use of certain drugs (e.g., aspirin, thiazide diuretics), or nephropathy.

Except for a higher uric acid production due to increased adenosine (ATP, ADP, AMP) degradation, none of these mechanisms is related to the mechanism by which fructose or D-tagatose increase plasma uric acid concentrations.

The mechanism by which D-tagatose and fructose elicit a uricemic response has been identified (van den Berghe, 1986). In-vivo studies and in-vitro experiments with isolated hepatocytes revealed that the initial rapid phosphorylation of absorbed D-tagatose and fructose to their corresponding 1-phosphates leads to a partial depletion of intracellular ATP and inorganic P_i. Since P_i is an inhibitor of adenosine deaminase, the rate-limiting enzyme in the catabolism of adenosine monophosphate (AMP), lowering of the intracellular P_i concentration promotes the deamination of AMP to inosine monophosphate (IMP) and thereby its further degradation to uric acid (Fig. 1).

Consequently, fructose or D-tagatose at oral doses which *per se* do not elicit a uricemic response will not enhance uricemia caused by mechanisms biochemically unrelated to depletion of

intracellular ATP and P_i. In other words, the doses of D-tagatose or fructose required to increase plasma uric acid would be the same in normouricemic and hyperuricemic individuals. This would also be true if the hyperuricemia is caused by an impaired renal excretion of uric acid.

Evidence that ingested D-tagatose at proposed use levels will not interfere with the metabolism of co-ingested fructose has been reviewed in the "Documentation Supporting the Generally Recognized as Safe (GRAS) Status of D-Tagatose as a Food Ingredient (6.4, D-Tagatose and Fructose Interaction)," which is also provided with this GRAS notification.

At doses of D-tagatose or fructose which elicit a uricemic effect, this effect will be additive to the hyper-production of uric acid through biochemically unrelated mechanisms. Direct evidence for this expectation is provided by a study in which 10 metabolically normal subjects and 5 hyperuricemic subjects received fructose (0.5 g/kg bw) by rapid infusion. The fructose-induced increment of plasma uric acid concentrations did not differ between the normal and hyperuricemic subjects (Morozzi et al., 1982).

3. Expected consequences of D-tagatose and fructose ingestion by individuals suffering from gout

Gout is the syndrome caused by the crystallization of monosodium urate monohydrate from supersaturated body fluids. Favored sites for crystallization are the synovial membranes of joints, bursae and tendon sheaths. Between 10-25% of gouty patients develop uric acid urinary stones and nephropathy. The etiology of gout is multifactorial but hyperuricemia is generally considered to be a necessary though not sufficient precondition for gout (Lin et al., 2000).

For the mechanistic reasons explained previously, fructose and D-tagatose ingested at doses not associated with uricemic effects are not expected to increase plasma uric acid concentrations in gouty individuals.

At intakes that are known to increase the production of uric acid, fructose (i.e., bolus doses of ≥50 grams) and D-tagatose (i.e., bolus doses of ≥30 grams) may elicit a somewhat higher and longer lasting uricemic response in gouty individuals. This has been demonstrated by two studies which included 6 and 8 gouty subjects, respectively (fructose was ingested in both studies at a dose of 1 g/kg bw) (Menghini & Della Corte, 1987; Stirpe et al., 1970). However, at proposed use levels (90'" percentile consumption of about 15 grams per day), no increase in plasma uric acid would be anticipated in either normal persons or gouty individuals.

4. Conclusions

Single doses of 15 g D-tagatose given **3** times daily for a total of 45 grams have no effect on plasma uric acid concentrations of healthy subjects. Based on biochemical considerations about the etiology **of** hyperuricemia, it is not expected that D-tagatose, under the conditions of its intended use (i.e., 15 grams/day), would increase plasma uric acid in either hyperuricemic individuals or persons suffering from gout.

5. References

Boesch C., Ith M., Jung B., Bruegger K., Erban S., Diamantis I., Kreis R. and Bär A. (2001). Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. Reg. Toxicol. Pharmacol. 33: 257-267.

Buemann B., Toubro S., Holst J.J., Rehfeld J. and Astrup A. (1999). D-Tagatose, a stereoisomer of D-fructose, increases blood uric acid concentration. Metabolism 49 (8): 969-976.

Lin K.-C., Lin H.-Y. and Chou P. (2000). The interaction between uric acid level and other risk factors on the development of gout among asymptomatic hyperuricemic men in a prospective study. J. Rheumatol. 27: 1501-1505.

Menghini S. and Della Corte E. (1987). Valutazioni sull'iperuricemia da carico di fruttosio in condizioni di alterato metabolismo dell'acido urico. Quad. Sclavo Diagn. 23 (4): 441-446.

Morozzi G., Berti C., Pellegrini R., Lalumera M. and Marcolongo R. (1982). Inibizione dell'iperuricemia da fruttoso mediante pre-trattamento con fosfati. Boll. Soc. Ital. Biol. Sper. 58 (8): 491-496.

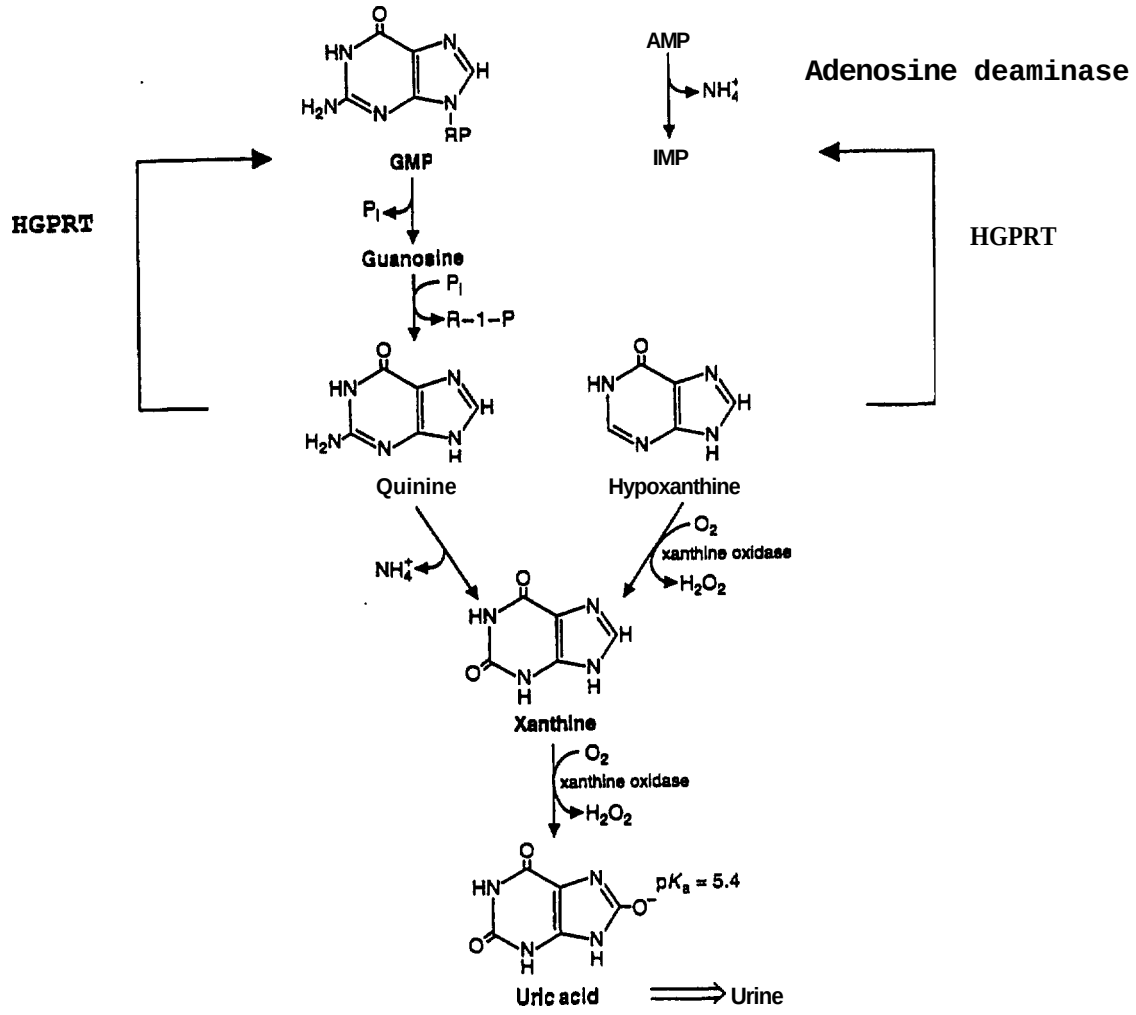
Stirpe F., Della Corte E., Sonetti E., Abbondanza A., Abbati A. and de Stefano F. (1970). Fructose-induced hyperuricemia. Lancet 2: 1310-1311.

Van den Berghe G. (1986). Fructose: metabolism and short-term effects on carbohydrate and purine metabolic pathways. Prog. Biochem. Pharmacol. 21: 1-32.

Vincent M.F., Van den Berghe G. and Hers H.G. (1986). Effect of fructose on the concentration of phosphoribosylpyrophosphate in isolated hepatocytes. Adv. Exp. Med. Biol. 195: 615-621.

000119

Fag. 1 Degradation of the purine bases



Abbreviations:

HGPRT	Hypoxanthine phosphoribosyltransferase (EC 2.4.2.8)
AMP	Adenosine-5'-phosphate
IMP	Inosine-5'-phosphate
GMP	Guanosine-5'-phosphate

000120

Table 1. Effects of acute oral doses of D-tagatose, fructose or sucrose on human blood uric acid levels[a]

Reference	Subjects	n	Sugar	Dose (g)	Relative dose (g/kg bw)	Peak Response (min.)	Normal Range (mg/dL)	Baseline Level (mg/dL)	Peak Level (mg/dL)	Increase Over Baseline (at peak)(mg/dL)
Boesch et al., 2001	Normal males	12	D-Tagatose	15	0.19	60	--	5.6	5.9[b]	0.4
Macdonald et al. 1978	Normal males	9	Fructose	~18[c]	0.25	30	--	7.0	7.6	0.6
Förster et al. 1972	Normalmales	6	Fructose	25	~0.36'	60	--	5.4	5.8	0.4
Buernann et al. 1999	Normal males	8	Fructose	30	0.39	50	<7.6	6.1	6.5	0.4
	Normalmales	8	D-Tagatose	30	0.39	50	<7.6	6.0	7.2	1.2
Macdonald et al. 1978	Normal males	9	Fructose	~35'	0.50	15	--	7.0	7.5	0.5
Förster and Ziege 1971	Normal males	10	Fructose	50	~0.71[c]	30	--	6.5	7.6	1.1
Förster et al. 1972	Normal males	9	Fructose	50	~0.71'	30	--	6.4	7.2	0.8
Oberhaensli et al. 1987	Normal subjects	8	Fructose	50	~0.71[c]	60	3.4-7.6	--[d]	--[d]	0.4
Macdonald et al. 1978	Normal males	9	Fructose	~52'	0.75	30	--	7.6	8.0	0.4
Saunders et al. 1999	Normal males	4	D-Tagatose	75	0.74	60	3.5-8.5	8.0	9.8	1.8
	Diabetic males	4	D-Tagatose	75	0.87	30	3.5-8.5	7.2	8.2	1.0
	Diabetic females	4	D-Tagatose	75	0.87	60	2.5-7.5	5.2	6.5	1.3
	Normal females	4	D-Tagatose	75	0.93	60	2.5-7.0	3.8	5.7	1.9
Reiser et al. 1984	Normal males and females	15 M 8 F	Fructose	69	0.93	30	--	5.1	6.1	1.0
Macdonald et al. 1978	Normal males	9	Fructose	~70'	1.00	30	--	7.8	9.0	1.2
			Sucrose	~70[c]	1.00	60		6.1	6.4	0.3
Stirpe et al. 1970	Normal males	6	Fructose	~70'	1.00	60	--	4.8	5.3	0.5
Davies et al. 1998	Normal males	19	Fructose	75	~1.07'	120	--	5.2	6.2	1.0
Menghini & Della Corte 1987	Normal males and females	9	Fructose	~70	1.0	30	--	4.3	5.3	1.0
Forster et al. 1972	Normal males	9	Sucrose	100	~1.43'	120	--	6.1	6.5	0.4

[a] Note: None of the values exceeded 10 mg/dl, which is generally regarded as the level above which plasma uric acid becomes clinically relevant (Fessel 1979).
[b] Not significantly different from values observed after intake of 15 g sucrose
[c] Assumed body weight is approximately 70 kg.
[d] No data were available.

080121

References for Table 1.

Boesch C., Ith M., Bruegger K., Erban S., Diamantis I., Kreis R. and Bär A. (2001). Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. Reg. Toxicol. Pharmacol. 33: 257-267.

Buemann B., Toubro S., Raben A. and Astrup A. (1999). Human tolerance to single, high dose of D-tagatose. Regul. Toxicol. Pharmacol. 29 (2,2/2): S66-S70.

Davies P.M., Simmonds H.A., Singer B., Mant T.G., Allen E.M., Vassos A.B. and Hounslow N.J. (1998). Plasma uridine as well as uric acid is elevated following fructose loading. Adv. Exp. Med. Biol. 431: 31-35.

Fessel W. (1979). Renal outcomes of gout and hyperuricemia. Am. J. Med. 67: 74-82.

Forster H., Boecker S. and Ziege M. (1972). Anstieg der Konzentration der Serumharnsaure nach akuter und chronischer Zufuhr von Saccharose, Fructose, Sorbit und Xylit. Medizin und Ernährung 13: 193-196.

Förster H. and Ziege M. (1971). Anstieg der Senunharnsaurekonzentration nach oraler Zufuhr von Fructose, Sorbit und Xylit. Z. Ernährungswiss. 10: 394-396.

Macdonald I., Keyser A. and Pacy D. (1978). Some effects, in man, of varying the load of glucose, sucrose, fructose, or sorbitol on various metabolites in blood. Am. J. Clin. Nutr. 31: 1305-1311.

Menghini S. and Della Corte E. (1987). Valutazioni sull'iperuricemia da carico di fruttosio in condizioni di alterato metabolismo dell'acido urico. Quad. Sclavo Diagn. 23 (4): 441-446.

Oberhaensli R.D., Taylor D.J., Rajogopalan B., Radda G.K., Collins J.E., Leonard J.V., Schwarz H. and Herschkowitz N. (1987). Study of hereditary fructose intolerance by use of ^{31}P magnetic resonance spectroscopy. Lancet i: 931-934.

Reiser S., Scholfield D., Trout D., Wilson A. and Aparicio P. (1984). Effect of glucose and fructose on the absorption of leucine in humans. Nutr. Rep. Int. 30: 151-162.

Saunders J.P., Donner T.W., Sadler J.H., Levin G.V.. and Makris N.G. (1999). Effects of acute and repeated oral doses of D-tagatose on plasma uric acid in normal and diabetic humans. Regul. Toxicol. Pharmacol., 29 (2,2/2): S57-S65.

Stirpe F., Della Corte E., Bonetti E., Abbondanza A., Abbati A. and de Stefano F. (1970). Fructose-induced hyperuricaemia. Lancet ii: 1310-1311.

End Submission

AM

Jones. Kathleen

From:
Sent: Monday, July 02, 2001 4:03 PM
To: kristian.eriknauer@arlafoods.com; Jones, Kathleen
Subject: Re: Meeting for GRN 78 - tagatose

Dear Dr. McAveney-Jones:

Accompanying me to the meeting on August 3 will be Albert Bar, Ph.D., scientific consultant to the notifier, Arla Foods Ingredients amba, and three representative members of the Expert Panel convened by Arla to review the safety of tagatose, Walter Glinsmann, M.D., Ian Munro, Ph.D. and Ron Thurman, Ph.D. A tentative agenda will be forwarded to you in the next week or so. When you have confirmed which Agency scientists will attend the meeting, I would appreciate receiving a list of the FDA attendees.

Thank you,
Diane McColl

****
This e-mail is sent by a law firm and may contain information that is privileged or confidential. If you are not the intended recipient, please delete the e-mail and any attachments and notify us immediately.
****

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth St. N.W.
Washington, D.C. 20005

>>> "Jones, Kathleen" <Kathleen.Jones@cfsan.fda.gov> 07/02/01 03:02PM >>>
Ms. McColl,

I have scheduled the meeting you requested to discuss tagatose on August 3, 2001, 9AM-11AM at the Office of Food Additive Safety (OFAS) located at 1110 Vermont Ave., NW, Suite 1295, Washington, DC 20005. If this is not convenient for you, please contact me as soon as possible so that I may reschedule the meeting.

Thank you,

Kathleen McAveney Jones, Ph.D.
Division of Biotechnology and GRAS Notice Review, HFS-206
Office of Food Additive Safety
Center for Food Safety
and Applied Nutrition
phone (202)418-3097
fax (202)418-3131

000146

AM

Jones. Kathleen

From:
Sent: Tuesday, July 17,2001 2:30 PM
To: Jones, Kathleen
cc: kristian.eriknauer@arlafoods.com
Subject: August 3 meeting re tagatose GRAS notice (GRN 78)

Dear Dr. McAveney-Jones:

As promised, attached is a tentative agenda for the upcoming meeting on tagatose. I have just learned that a member of the Expert Panel, Ron Thurman, Ph.D., School of Medicine, University of North Carolina at Chapel Hill, suffered a massive heart attack and died last Saturday night. Professor Thurman was a fine pharmacologist and will be sorely missed.

Consequently, joining me at the meeting will be Hans Bertelsen, Ph.D., Arla Foods Ingredients amba; Albert Bar, Ph.D., Bioresco; Walter Glinsmann, M.D., Glinsmann Inc.; and Ian Munro, Ph.D., FACT, FRCPath, Cantox Health Sciences International. We are compiling the supporting documentation for the estimated intake analysis and hope to forward the material to you for review prior to the meeting.

If any further questions arise before the meeting, please let me know as soon as possible so we can add them to the agenda.

Sincerely,
Diane McColl
Counsel to Arla Foods Ingredients amba

****
This e-mail is sent by a law firm and may contain information that is privileged or confidential. If you are not the intended recipient, please delete the e-mail and any attachments and notify us immediately.
****

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth St. N.W.
Washington, D.C. 20005

000151

AM

Jones. Kathleen

From:
Sent: Friday, July 27, 2001 4:52 PM
To: Jones, Kathleen
Subject: RE: August 3 meeting re tagatose GRAS notice (GRN 78)

Dear Dr. Jones:

Thank you for the list of FDA attendees. As requested, we will be prepared to discuss the estimated intake calculations at the beginning of the meeting.
If you need any additional information or have any further questions prior to the meeting, please do not hesitate to ask.

Sincerely,
Diane McColl

****
This e-mail is sent by a law firm and may contain information that is privileged or confidential. If you are not the intended recipient, please delete the e-mail and any attachments and notify us immediately.
****

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth St. N.W.
Washington, D.C. 20005

>>> "Jones, Kathleen" <Kathleen.Jones@cfsan.fda.gov> 07/27/01 10:40AM >>>
Ms. McColl,

You had asked me for a list of FDA employees that would be attending next Friday's meeting on Arla Food's GRN 78 tagatose. Currently, FDA attendees for the meeting include: Drs. Linda Kahl, Antonia Mattia, D. Charles Thompson, David Hattan, Paulette Gaynor, Rebecca Edelstein, Michael DiNovi, and myself. The meeting is scheduled for Friday, August 3, 9 - 11 AM, Suite 1295, 1110 Vermont Ave NW, Washington, DC.

Because of the concern of Drs. DiNovi and Edelstein regarding exposure estimate calculations, I would ask if we could address this issue first in our meeting. Dr. DiNovi assures me that it will take a maximum of 15-20 minutes. Then we can have the rest of the meeting to discuss any implications of the JECFA report.

If any further questions arise before next Friday, please feel free to contact me.

Sincerely,
Kathleen McAveney Jones, Ph.D.

Division of Biotechnology and GRAS Notice Review, HFS-206
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Phone (202)418-3097
FAX (202)418-3131

-----Original Messa e---

Sent: Tuesday, July 17, 2001 2:30 PM
To: Jones, Kathleen
Cc: kristian.eriknauer@arlafoods.com
Subject: August 3 meeting re tagatose GRAS notice (GRN 78)

Dear Dr. McAveney-Jones:

As promised, attached is a tentative agenda for the upcoming meeting on

000152

tagatose. I have just learned that a member of the Expert Panel, Ron Thurman, Ph.D., School of Medicine, University of North Carolina at Chapel Hill, suffered a massive heart attack and died last Saturday night. Professor Thurman was a fine pharmacologist and will be sorely missed.

Consequently, joining me at the meeting will be Hans Bertelsen, Ph.D., Arla Foods Ingredients amba; Albert Bar, Ph.D., Bioresco; Walter Glinsmann, M.D., Glinsmann Inc.; and Ian Munro, Ph.D., FACT, FRCPath, Cantox Health Sciences International. We are compiling the supporting documentation for the estimated intake analysis and hope to forward the material to you for review prior to the meeting.

If any further questions arise before the meeting, please let me know as soon as possible so we can add them to the agenda.

Sincerely,
Diane McColl
Counsel to Arla Foods Ingredients amba

****
This e-mail is sent by a law firm and may contain information that is privileged or confidential. If you are not the intended recipient, please delete the e-mail and any attachments and notify us immediately.

****

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth St. N.W.
Washington, D.C. 20005

000153

AM

Jones, Kathleen

From:
Sent: Monday, July 30, 2001 5:12 PM
To: Jones, Kathleen
Subject: RE: August 3 meeting re tagatose GRAS notice (GRN 78)

Dear Dr. Jones:

I have just learned that Henrik Andersen, Executive Director, Arla Foods Ingredients amba, will also join us for the tagatose meeting on Friday. Please add him to your list of attendees.

Thank you,
Diane McColl

****
This e-mail is sent by a law firm and may contain information that is privileged or confidential. If you are not the intended recipient, please delete the e-mail and any attachments and notify us immediately.
****

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth St. N.W.
Washington, D.C. 20005

>>> "Jones, Kathleen"<Kathleen.Jones@cfsan.fda.gov> 07/27/01 10:40AM >>>
Ms. McColl,

You had asked me for a list of FDA employees that would be attending next Friday's meeting on Arla Food's GRN 78 tagatose. Currently, FDA attendees for the meeting include: Drs. Linda Kahl, Antonia Mattia, D. Charles Thompson, David Hattan, Paulette Gaynor, Rebecca Edelstein, Michael DiNovi, and myself. The meeting is scheduled for Friday, August 3, 9 - 11 AM, Suite 1295, 1110 Vermont Ave NW, Washington, DC.

Because of the concern of Drs. DiNovi and Edelstein regarding exposure estimate calculations, I would ask if we could address this issue first in our meeting. Dr. DiNovi assures me that it will take a maximum of 15-20 minutes. Then we can have the rest of the meeting to discuss any implications of the JECFA report.

If any further questions arise before next Friday, please feel free to contact me.

Sincerely,
Kathleen McAveney Jones, Ph.D.

Division of Biotechnology and GRAS Notice Review, HFS-206
Office of Food Additive Safety
Center for **Food** Safety and Applied Nutrition
Phone (202)418-3097
FAX (202)418-3131

-----Original Message-----
From:
Sent: Tuesday, July 17, 2001 2:30 PM
To: Jones, Kathleen

Subject: August 3 meeting re tagatose GRAS notice (GRN 78)

Dear Dr. McAveney-Jones:

As promised, attached is a tentative agenda for the upcoming meeting on tagatose. I have just learned that a member of the Expert Panel, Ron

000154

Thurman, Ph.D., School of Medicine, University of North Carolina at Chapel Hill, suffered a massive heart attack and died last Saturday night. Professor Thurman was a fine pharmacologist and will be sorely missed.

Consequently, joining me at the meeting will be Hans Bertelsen, Ph.D., Arla Foods Ingredients amba; Albert Bar, Ph.D., Bioresco; Walter Glinsmann, M.D., Glinsmann Inc.; and Ian Munro, Ph.D., FACT, FRCPath, Cantox Health Sciences International. We are compiling the supporting documentation for the estimated intake analysis and hope to forward the material to you for review prior to the meeting.

If any further questions arise before the meeting, please let me know as soon as possible so we can add them to the agenda.

Sincerely,
Diane McColl
Counsel to Arla Foods Ingredients amba

****
This e-mail is sent by a law firm and may contain information that is privileged or confidential. If you are not the intended recipient, please delete the e-mail and any attachments and notify us immediately.

****

Diane B. McColl
Hyman, Phelps & McNamara, P.C.
700 Thirteenth St. N.W.
Washington, D.C. 20005

000155

AM

LAW OFFICES

HYMAN, PHELPS 8 MCNAMARA, P.C.

700 THIRTEENTH STREET. N.W.
SUITE 1200
WASHINGTON, D.C. 20005-5929
(202) 737-5600
FACSIMILE
(202) 737-9329
M.hDm.COm

JAMES R PHELPS
PAUL M HYMAN
ROBERT A DORMER
STEPHEN H McNAMARA
ROGER C THIES
THOMAS SCARLETT
JEFFREY N GIBBS
BRIAN J DONATO
FRANK J SASINOWSKI
DIANE B MCCOLL
A WES SIEGNER JR
ALAN M KIRSCHENBAUM
DOUGLAS O FAROUHAR
JOHN A GILBERT JR
JOHN R FLEDER
MARCH SHAPIRO

ROBERT T ANGAROLA
11945-19961

MARY KATE WHALEN
OF COUNSEL

JENNIFER B. DAVIS
FRANCES K. WU
DAVID B. CLISSOLD
CASSANDRA A. SOLTIS
JOSEPHINE M. TORRENTE
MICHELLE L. BUTLER
PATRICIA A.A. VANSTORY
THOMAS R. GIBSON
LEIGH E. KENNEDY.
ANNE MARIE MURPHY
PAUL L. FERRARI
JEFFREY N. WASSERSTEIN
BRIAN J. MALKIN

*NOT ADMITTED IN DC

DIRECT DIAL (202) 737-4291

September 19,2001

RECEIVED
SEP 20 2001
OFFICE OF
FOOD ADDITIVE SAFETY

BY FEDERAL EXPRESS

Linda S. Kahl, Ph.D.
Division of Biotech & GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street S.W.
Washington, D.C. 20204

Re: Supplemental Submission to Tagatose GRAS Notice (GRN **78)**

Dear Dr. Kahl:

On behalf of Arla Foods Ingredients amba ("Arla Foods"), we submit the enclosed comment from the Expert Panel accompanied by a detailed description of the uric acid data and information that was considered by the Panel in its evaluation of the "generally recognized as safe" ("GRAS") status of tagatose. Also included are English translations of two Italian studies cited in the uric acid data description. For your convenience, three copies of the enclosures are provided.

In April 2001, after critical and independent evaluation of all available data and information pertinent to the safety of tagatose, the Expert Panel concluded that under the conditions of intended use in foods, an intake of up to 15 grams per day of tagatose (90th percentile) is GRAS based on scientific procedures. In June 2001, the FAO/WHO Joint Expert Committee on Food Additives ("JECFA") established an "acceptable daily intake" ("ADI") of 4.5 g for tagatose. As explained below, Arla Foods and the Expert Panel believe that their GRAS determination for tagatose is unaffected by JECFA's ADI decision.

000156

2603 MAIN STREET
SUITE 760
IRVINE. CALIFORNIA 92614
(949) 553-7400
FAX. (9491553-7433

4819 EMPEROR BOULEVARD
SUITE 400
DURHAM, NORTH CAROLINA 27703
19191 313-4750
FAX. 19191313-4751

As you may know, an ADI is defined as an estimate of the amount of a substance, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk.[1] The process of establishing an ADI involves examination and evaluation of available animal toxicity data on the substance in question, with a view to establishing the highest daily dose, expressed on a body weight basis, that is without any toxicological effect. This value is described as the no observed effect level ("NOEL"). The NOEL is then divided by an arbitrary safety factor, usually a factor of **100,** to establish an ADI for humans. In cases where human data are available, a lower safety factor, typically **10,** may be used in establishing the ADI. The ADI is not a lower bound of toxicity; rather, the ADI defines an exposure below which no harmful effects would be expected.

In contrast, a determination of GRAS status based on scientific procedures involves the same critical examination of available toxicological data on the substance in question, but the process of evaluation is different. In the GRAS process, the amount of daily intake of the substance is calculated from food intake data for foods proposed to contain the substance. This is accrued across all food categories to provide a mean and **90th** percentile intake for the general population and specific age and gender groups within the population. The intakes estimated in this manner are then compared with doses used in the animal toxicity studies and human clinical trials, especially those that induce no demonstrable effects ("NOELs") and/or no observed adverse effects ("NOAELs"), and those that may be associated with physiological or toxicological response. In this way a margin of safety ("MOS") can be calculated between the estimated human dose and the doses used in the toxicity studies. There is no standard MOS that must be achieved; however, some MOS is usually considered essential. This means that a decision can be made that the substance, used in accordance with current good manufacturing practices, will meet the appropriate standard of "reasonable certainty in the minds of experts, qualified by training and experience to evaluate the safety of food ingredients, that the substance is not harmful under the conditions of intended use." See 21 U.S.C. § 321(s); 21 C.F.R. § 170.3(i). The extent of the MOS that is desirable will depend on the nature of effects the substance produces. Substances that produce clear evidence of high-dose toxicity should have a larger margin of safety, usually in the range of 100, while those such as common food ingredients (*e.g.*, sugars, starches, gums) typically have MOSs of 2 to **5** fold.

Conceptually, there is a significant difference in how a substance may be evaluated depending on whether an ADI is established or a GRAS determination is made, An ADI is based on a NOEL to which an arbitrary safety factor is applied, typically a factor of 10 if clinical data are available, and establishes a level below which use of the substance in food is

---

1 **1987. WHO. Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization (WHO), International Programme on Chemical Safety (IPCS); Geneva, Switzerland. Environmental Health Criteria,** No. **70.**

of no toxicological concern. An ADI is silent as to safety of use of the substance in foods under specific conditions at higher levels. By contrast, a GRAS determination does not rely on the mechanical application of an arbitrary safety factor, but instead is based on a margin of safety, typically a 2-5 fold margin for poorly-absorbed carbohydrates, and defines specific conditions of use that qualified food safety experts would agree are safe. A GRAS determination requires a consensus among qualified experts that a substance is safe under the specified conditions of intended use. As stated by the Food and Drug Administration ("FDA"), "[t]o establish [general] recognition, the proponent must show that there is a consensus of expert opinion regarding the safety of the use of the substance." 62 Fed. Reg. 18937, 18939 (Apr. 17, 1997) (preamble to proposed rule for GRAS notices). FDA also acknowledges that "such consensus does not require unanimity among qualified experts." *62* Fed. Reg. at 18941 (emphasis added). This long-standing principle has been upheld repeatedly by the courts. See e.g., United States v. Articles of Drug . . . 5906 Boxes, 745 F.2d 105, 120 n.22 (1st Cir. 1984) ("It is by now clear that unanimity among experts **is** not required to demonstrate "general" recognition"); United States v. Article of Drug. . . 4680 Pails, 725 F.2d 976, 990 (5th Cir. 1984) ("general recognition does not require unanimous recognition"); United States v. Articles of Food and Drug. . . Coli-Trol, 518 F.2d 743,746 (5th Cir. 1975) ("It is not enough to show merely a conflict in the evidence of general recognition, for even properly conducted studies may produce disagreement. What is required is not unanimous recognition but general recognition"); United States v. Articles of Drug . . . Promise Toothpaste, 624 F. Supp. 776, 782 (N.D. Ill. 1985) (same).

As evidenced by the "Expert Panel Consensus Statement" provided in the GRAS notice, there exists a consensus among qualified experts that, under the conditions of intended use in food, tagatose is GRAS based on scientific procedures.

If you have any questions concerning this matter, please do not hesitate to contact me.

Sincerely,

Diane B. McColl
Counsel to Arla Foods Ingredients amba

DBM/dmh
Enclosure

Expert Panel Comments on Uric Acid Issue

August 22,2001

EXPERT PANEL COMMENT ON THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF D-TAGATOSE WITH RESPECT TO EFFECTS ON URIC ACID METABOLISM

The Expert Panel, specially convened at the request of Arla Food Ingredients amba (Arla) to evaluate the GRAS status of specific food uses of the sugar D-tagatose, considered in detail the effects of dietary exposure to the sugar, alone and in combination with dietary fructose, its stereoisomer. Based on scientific procedures involving critical review of the pertinent data and information, the Panel concluded that D-tagatose is GRAS at an estimated intake level of 15 g/day (90th percentile) for the specified food uses. Arla submitted a GRAS Notification for these specified uses to the **U.S.** Food and Drug Administration (FDA) supported by the Panel's decision.

Following submission of the GRAS Notification, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) considered D-tagatose at its June 2001 meeting and established an Acceptable Daily Intake (ADI) of 4.5 g/day. The Panel is not aware of the all of the circumstances leading to JECFA's ADI designation. It is aware that one issue considered in the JECFA assessment involved the potential for D-tagatose to elevate plasma uric acid levels. In order to more fully understand the issues involving uric acid metabolism that may be of concern to the FDA, members of the Expert Panel with representatives of Arla present, reviewed with the FDA the Panel's analysis of the effects of D-tagatose on uric acid metabolism. This led to identification of specific questions for which the Panel agreed to provide further explanation. These questions are:

1. Does D-tagatose, under its intended conditions of use, have the potential for increasing serum uric acid levels in:
   - Healthy and diabetic persons, thereby increasing their risk of developing gout?
   - Persons with asymptomatic elevations of plasma uric acid (i.e., persons with asymptomatic hyperuricemia)?
   - Persons with manifest gout, thereby increasing their risk of future gouty attacks?

2. Could the combined intake of D-tagatose and fructose produce a relatively greater than anticipated (synergistic) effect?

3. Could the chronic ingestion of D-tagatose lead to an adaptive increase in intestinal absorption and thereby to a higher uricemic response?

000160

Data and information originally considered by the Expert Panel, and now reconsidered as a result of the JECFA decision and discussions with the FDA, are presented in greater detail in the accompanying attachment. The attachment addresses the above questions and expands upon the discussion of data and the analytical logic used by the Panel to arrive at its original GRAS decision.

Following critical review and discussion of the data and information provided in the attachment and additional discussion of approaches to analysis, we, the members of the Expert Panel conclude that the available scientific evidence supports our consensus opinion that the potential for a uricemic effect of D-tagatose does not present a risk to safety related to hyperuricemia under the conditions of its intended use in food as specified in the GRAS Notification submitted by Arla. We conclude that our April, 2001 Consensus Statement confirming the GRAS status of D-tagatose remains valid.

B

Herbert Blumenthal, Ph.D.	September 5, 2001 Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date

000161

Data and information originally considered by the Expert Panel, and now reconsidered as a result of the JECFA decision and discussions with the FDA, are presented in greater detail in the accompanying attachment. The attachment addresses the above questions and expands upon the discussion of data and the analytical logic used by the Panel to arrive at its original GRAS decision.

Following critical review and discussion of the data and information provided in the attachment and additional discussion of approaches to analysis, **we,** the members of the Expert Panel conclude that the available scientific evidence supports our consensus opinion that the potential for a uricemic effect of D-tagatose does not present a risk to safety related to hyperuricemia under the conditions of its intended use in food as specified in the GRAS Notification submitted by Arla. We conclude that our April, 2001 Consensus Statement confirming the GRAS status of D-tagatose remains valid.

BY:

Herbert Blumenthal. Ph.D.	Date 9/3/2001.
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date

000162

Data and information originally considered by the Expert Panel, and now reconsidered as a result of the JECFA decision and discussions with the FDA, are presented in greater detail in the accompanying attachment. The attachment addresses the above questions and expands upon the discussion of data and the analytical logic used by the Panel to arrive at its original **GRAS** decision.

Following critical review and discussion of the data and information provided in the attachment and additional discussion of approaches to analysis, we, the members of the Expert Panel conclude that the available scientific evidence supports our consensus opinion that the potential for a uricemic effect of D-tagatose does not present a risk to safety related to hyperuricemia under the conditions of its intended use in food as specified in the **GRAS** Notification submitted by Arla. We conclude that our April, 2001 Consensus Statement confirming the GRAS status of D-tagatose remains valid.

BY:

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date Aug. 24, 2001
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date

000163

Data and information originally considered by the Expert Panel, and now reconsidered as a result of the JECFA decision and discussions with the FDA, are presented in greater detail in the accompanying attachment. The attachment addresses the above questions and expands upon the discussion of data and the analytical logic used by the Panel to arrive at its original GRAS decision.

Following critical review and discussion of the data and information provided in the attachment and additional discussion of approaches to analysis, we, the members of the Expert Panel conclude that the available scientific evidence supports our consensus opinion that the potential for a uricemic effect of D-tagatose does not present a risk to safety related to hyperuricemia under the conditions of its intended use in food as specified in the GRAS Notification submitted by Arla. We conclude that our April, **2001** Consensus Statement confirming the GRAS status of D-tagatose remains valid.

BY:

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date' 9/6/01
Ian C. Munro, Ph.D., F.R.C.Path.	Date

000164

Data and information originally considered by the Expert Panel, and now reconsidered as a result of the JECFA decision and discussions with the FDA, are presented in greater detail in the accompanying attachment. The attachment addresses the above questions and expands upon the discussion of data and the analytical logic used by the Panel to arrive at its original GRAS decision.

Following critical review and discussion of the data and information provided in the attachment and additional discussion of approaches to analysis, we, the members of the Expert Panel conclude that the available scientific evidence supports our consensus opinion that the potential for a uricemic effect of D-tagatose does not present a risk to safety related to hyperuricemia under the conditions of its intended use in food as specified in the GRAS Notification submitted by Arla. We conclude that our April, 2001 Consensus Statement confirming the GRAS status of D-tagatose remains valid.

BY:

Herbert Blumenthal, Ph.D.	Date
John Doull, M.D., Ph.D.	Date
Walter H. Glinsmann, M.D.	Date
Michael D. Levitt, M.D.	Date
Ian C. Munro, Ph.D., F.R.C.Path.	Date Aug 24, 01

000165

Uric Acid Data and Information

000166

Bioresco

Bioresearch Management and Consulting Ltd.

Questions and answers in relation to the uri potential of · tose

Author: Dr. Albert Bar

Date: August 20, 2001

000167

CONTENTS

1. Introduction.....4

2. Relevant aspects of the absorption and metabolism of D-tagatose 5

2.1 Absorption.....5
2.2. Metabolism 8
2.2.1. Metabolism of absorbed D-tagatose.....8
2.2.2. Fate of unabsorbed D-tagatose.....10
2.3 Mechanism of the uricemic effect of fructose and D-tagatose.....11

3. Does D-tagatose, under the conditions of intended use, have the potential to increase serum uric acid levels?.....12

3.1. Uricemic effect of D-tagatose in healthy and diabetic subjects..... 12
3.1.1. Effects of a single dose on postprandial serum acid concentrations.....12
3.1.2. Effects of repeated doses on fasting serum uric acid concentrations.....14
3.2 Uricemic effects in subjects with asymptomatic hyperuricemia.....15

3.3 Uricemic effect in subjects suffering from gout.....17

4. Could the combined intake of D-tagatose and fructose produce a higher uricemic response (synergistic effect)?...18

5. Could the chronic ingestion of D-tagatose lead to an increased intestinal absorption and thereby to a higher uricemic response? 19

6. Conclusions..... 20
6.1 Absence of uricemic effects of fructose and D-tagatose at intakes resulting from the intended uses..... 20
6.2 Uricemic effects of fructose and D-tagatose at levels which will not be reached under the intended conditions of use..... 20
6.3 Overall conclusion..... 21

7. References 22

000168

FIGURES

la. Plasma uric acid before and after intake of a standard breakfast containing about 99 g starch and 15 g D-tagatose or sucrose on day 1 of each treatment period (mean ± SD)

1b. Plasma uric acid before and after intake of a standard breakfast containing about 99 g starch and 15 g D-tagatose or sucrose on day 1 of each treatment period (individual values)

2. Effect of low single doses of fructose on serum uric acid in male healthy subjects

3. Initial steps of fructose and D-tagatose metabolism and mechanism of uric acid formation

4. Catabolism in humans of adenine nucleotides to oxypurines (hypoxanthine, xanthine) and uric acid

5. Effect of single doses of fructose or D-tagatose (30 g in 400 ml water) or plain water (400 ml) on postprandial plasma uric acid concentrations

6. Effect of single doses of fructose or D-tagatose (30 g in 400 ml water) or plain water on postprandial plasma phosphate concentrations

7. Effect of serum uric acid of the ingestion of a low-purine diet (day 1-7) followed by a corresponding diet with calf liver (150 g/d, day 8-15)

8 Acute effect of protein ingestion (80 g) on serum and urinary uric acid levels in healthy subjects

9. Serum uric acid after ingestion of native, de-alcoholized, or PVPP-treated red wine (5 ml/kg bw)

10. Effect of single oral doses of D-tagatose, fructose or sucrose on serum uric acid (maximum postprandial increase above baseline)

11. Effect of repeated oral doses of D-tagatose, fructose or sucrose on fasting serum uric acid concentrations

12. Plasma uric acid concentrations after a single oral dose of fructose (1 g/kg bw in 250 ml water) in healthy subjects and patients suffering from gout (untreated) or non-gouty arthropathy

13. Effect of a single oral dose of fructose (1 g/kg bw) on serum uric acid concentrations in healthy and untreated gouty subjects

14. Plasma uric acid concentration in healthy and untreated gouty subjects after infusion (i.v.) of fructose (0.5 g/kg bw within 10 min)

15. Hypothetical (schematic) dose-response relationship of the uricemic effect of single doses of fructose and D-tagatose in healthy, hyperuricemic and gouty subjects

QUESTIONS AND ANSWERS IN RELATION TO THE URICEMIC POTENTIAL OF D-TAGATOSE

1. Introduction

The ingestion of single high doses of D-tagatose (≥30 g) is associated with a mild transient increase of serum uric acid concentrations in fasted healthy male volunteers (Buemann, 2000 a, b). Single doses of fructose (≥50 g), sucrose (≥100 g) and xylitol (≥12.5 g) produce similar increases of serum uric levels in healthy subjects (Forster et al., 1972, Macdonald et al., 1978; Davies et al., 1998).

At lower doses, these carbohydrates have no uricemic effect in healthy subjects. The ingestion of single doses of 15 g D-tagatose did not result in an increase of serum uric acid (Boesch et al., 2001) (Figs. 1a,b). Doses of 25 - 30 g fructose produced only a marginal, if any, increase of serum uric acid in fasted subjects Fig. 2). Thus, a threshold dose exists for the uricemic effects of hese sugars in healthy subjects. This threshold dose is higher than the intakes which result from the actual (fructose) or projected (D-tagatose) uses of these sugars.

Chronic hyperuricemia is a well-known risk factor of gout (Campion et al., 1987; Hall et al., 1967; Roubenoff et al. 1991, Roubenoff, 1990; Lin et al., 2000). Furthermore, it is known that sudden changes of serum uric acid concentrations (up or down) may trigger the occurrence of gouty attacks in persons suffering from gout (Maclachlan & Rodnan, 1967).

000170

The question therefore arose as to whether hyperuricemic subjects or patients suffering from gout might be more susceptible than normal individuals, i.e., whether the threshold dose may be lower in hyperuricemic or gouty subjects. Since fructose and D-tagatose share metabolic pathways and mediate an increase of serum uric acid by the same mechanism, it also had to be considered whether the simultaneous ingestion of both sugars might produce an increased uricemic response. Finally, it was necessary to determine whether the chronic ingestion of D-tagatose might have consequences on serum uric acid levels that could not be predicted from acute studies with ingestion of a single dose.

2. Relevant aspects of the absorption and metabolism of D-tagatose

2.1 Absorption

D-Tagatose is absorbed by passive diffusion. Since D-tagatose does not interfere with the absorption of fructose, there appears to be no binding of D-tagatose to the carriers (GLUT 5 and GLUT 2) that account for the facilitated diffusion of fructose through the intestinal mucosa (Crouzoulon, 1978; Sigrist-Nelson & Hopfer, 1974; Tatibouet et al., 2000).

There are no methods available for measuring the absorption of D-tagatose directly and non-invasively in humans. However, numerous studies on the absorption of L-rhamnose in humans provide a good basis for an estimate. L-Rhamnose (6-deoxy-D-mannose) which is also absorbed by passive diffusion, has a slightly lower molecular weight

and is slightly more lipophilic than D-tagatose. Since the rate of passive diffusion of a substance through the intestinal mucosa is determined mainly by its molecular volume and lipophilicity (Hamilton et al., 1987), the intestinal absorption of L-rhamnose is expected to be slightly higher than that of D-tagatose. The absorption of ingested L-rhamnose can be determined quantitatively from its urinary excretion because L-rhamnose is metabolized only slowly and thus incompletely. In humans and dogs, about 70% of an intravenous dose of L-rhamnose is excreted unchanged with the urine (Jenkins et al., 1994; Bjarnason et al., 1994; Hall & Batt, 1996). Studies in humans show that not more than about 12 - 17% of ingested L-rhamnose (single doses of 0.5 -5 g) is excreted with the urine within 5 to 10 hours *of* ingestion (Delahunty & Hollander, 1987; Maxton et al., 1986; Howden et al., 1991; Bjarnason et al., 1991, 1994; Mooradian et al., 1986; Menzies et al., 1990). AS this represents 70% of the absorbed L-rhamnose, it follows that not more than about 17 - 24% of ingested L-rhamnose is absorbed. Since the absorption of D-tagatose most probably is somewhat lower than that of L-rhamnose, the fractional absorption of D-tagatose in humans will hardly exceed 20%.

Indirect evidence for the incomplete absorption of D-tagatose in humans is provided by the results of studies on the intestinal tolerance of this sugar. A single dose of 20 g D-tagatose produced the same mild intestinal symptoms (e.g., flatulence) as a single dose of 20 g lactitol, a not absorbed disaccharide sugar alcohol (Lee & Storey, 1999).

The rate at which molecules permeate through the intestinal mucosa is determined mainly by their molecular size and lipophilicity. However, host-related parameters, such as the "pore size" of the intestinal mucosa (more precisely, the width of the so-called "tight junctions"

000172

between the epithelial cells), the direction of the water flux (absorption or secretion, so-called "solvent drag effect"), the thickness of the mucus layer covering the mucosa, the viscosity of the chyme, and the small intestinal transit time, influence the extent of absorption (fractional absorption in percent of the ingested dose). None of these modulating factors is expected to change in response to the duration of D-tagatose intake or the magnitude of the applied dose (except at very high (laxative) doses at which water flux will turn from absorption to secretion whereby D-tagatose absorption is reduced). Therefore, the absorption in the small intestine would not change in response to chronic consumption of D-tagatose.

Direct evidence for the absence of an adaptive effect of continued D-tagatose consumption on its small intestinal absorption is provided by a study in pigs. The absorption of D-tagatose was determined by measuring D-tagatose in the portal vein blood after administration of a portion of feed containing 20% D-tagatose. Measurements were made in five pigs before and after a 7-day adaption period during which the animals received a diet supplemented with 20% D-tagatose. Before and after adaption, the absorption rate was highest 1 – 3 hours after feeding. Adapted animals absorbed 32 to 85 grams of D-tagatose per kilogram of ingested feed, equal to 16% to 43% of the ingested dose (mean ± SD: 27.6 ± 10.3%). Not adapted animals absorbed 34 to 68 grams of D-tagatose per kilogram of feed, equal to 17% to 36% of the ingested dose (mean ± SD: 26.3 ± 7.0%). The urinary excretion of D-tagatose ranged between 3% and 7% of the ingested dose in adapted and not adapted animals (Jensen & Laue, 1998). While the data on small intestinal absorption show considerable variation, the mean values of adapted and unadapted pigs are nearly identical. They also are in line with a 26% fractional absorption that has been found in a pig study in which disappearance of D-tagatose from the small intestine

was determined (using chromium oxide as an inert marker) (Laerke & Jensen, 1999).

2.2 Metabolism

2.2.1 Metabolism of absorbed D-tagatose

The metabolism of absorbed D-tagatose proceeds along well established biochemical pathways (Fig. 3).

In vitro studies with enzyme preparations and isolated hepatocytes demonstrate that, in the first step, D-tagatose is phosphorylated to D-tagatose-1-phosphate by fructokinase in the presence of ATP. The formation of D-tagatose-1-phosphate occurs mainly in the liver and kidney, and to a smaller extent the intestinal mucosa and the pancreatic islet cells, because fructokinase is found only in these organs (Mayes, 1993; Malaisse et al., 1989; van den Berghe, 1986). The formation of D-tagatose-l-phosphate was observed directly and non-invasively by magnetic resonance spectroscopy (MRS) of the human liver (Buemann et al., 2000b). Following ingestion of a single 30-g dose of D-tagatose, ^{31}P-MRS revealed the formation of D-tagatose-1-phosphate reaching a maximum concentration of about 1 mM within 30-60 minutes. After about 150 minutes, the D-tagatose-1-phosphate had disappeared. As a consequence of the phosphorylation of D-tagatose, the hepatic concentration of ATP decreased transiently by about 11%. This decrease is small in comparison with the diurnal fluctuations of ATP in human blood (Chagoya de Sanchez et al., 1996).

0001'74

Fructokinase has a higher affinity for fructose than to D-tagatose (Sanchez et al., 1971; Raushel & Cleland, 1973, 1977). However, the difference of the K_m for phosphorylation has probably no practical consequence for the intracellular formation of fructose-1-phosphate or tagatose-1-phosphate in vivo because the K_m for the cellular uptake of fructose (and presumably also D-tagatose) is substantially higher [i.e., the cellular uptake is rate-limiting for the phosphorylation at physiological concentrations of fructose and D-tagatose (<1 mM)] (Sestoft & Fleron, 1974; Baur & Heldt, 1977; Okuno & Glieman, 1986).

In the second step, D-tagatose-1-phosphate is split to D-glyceraldehyde and dihydroxyacetone-phosphate, i.e., the same intermediate products that result from the catabolism of fructose. A comparison of the gluconeogenesis from D-fructose and D-tagatose in isolated hepatocytes indicates that the cleavage of D-tagatose-1-phosphate occurs at about half the rate of that of D-fructose-1-phosphate (Rognstad, 1975, 1982). The lower affinity of aldolase B for tagatose-1-phosphate is also evidenced by a longer lasting reduction of ATP and P_i levels in hepatocytes incubated in with D-tagatose (as compared to fructose), and by a lower glycolytic rate for D-tagatose (about 25-60% that of fructose) as measured by the formation of lactate and pyruvate from these two sugars (Vincent et al., 1989; Martinez et al., 1982, 1987; Zeid et al., 1997).

Despite the lower affinity of aldolase B for tagatose-1-phosphate, the intracellular concentration of this metabolite remains small in comparison to the ingested and absorbed dose. After ingestion of 30 g (166 mMol) D-tagatose, the liver concentration of D-tagatose-1-phosphate did not exceed 1 mM in healthy human volunteers (Buemann et al., 2000b).

2.2.2 Fate of unabsorbed D-tagatose

The unabsorbed portion of ingested D-tagatose is fermented nearly completely in the large intestine by intestinal microorganisms, mainly to acetate, propionate and butyrate which are then almost completely absorbed. After ingestion of 29 g D-tagatose, an increased expiration of H_2, an indicator of colonic fermentation of poorly absorbed carbohydrates, was observed (Buemann et al., 1998).

Upon ingestion of D-tagatose for several days, the intestinal microflora adapts to the availability of this substrate as evidenced by a faster fermentation of D-tagatose and a somewhat higher formation of butyrate in humans and pigs (Jensen & Buemann, 1998; Laerke & et al., 2000).

It is this adaptation of the intestinal microflora that accounts for an improved intestinal tolerance of low-digestible carbohydrates upon chronic consumption.

2.3 Mechanism of the uricemic effect of fructose and D-tagatose

The mechanism by which D-tagatose and fructose elicit a uricemic response, has been identified (Fox & Kelly, 1972; Smith et al., 1977; van den Berghe et al., 1977; Brosh et al., 1982; van den Berghe, 1986; Thoma & Ugurbil, 1989). In vivo studies and in-vitro experiments with isolated hepatocytes revealed that the initial rapid phosphorylation of absorbed D-tagatose and fructose to their corresponding 1-phosphates leads to a partial depletion of intracellular ATP and inorganic phosphorus (Pi)(Vincent et al., 1986; van den Berghe, 1986; Buemann et al., 2000b; Masson et al., 1994; Oberhaensli et al., 1986). Since P_i is a strong competitive inhibitor of AMP deaminase (EC 3.5.4.6.), the rate-limiting enzyme in the catabolism of adenosine monophosphate (AMP), lowering of the intracellular P_i concentration promotes the deamination of AMP to inosine monophosphate (IMP) (Moss, 1977; Itoh, 1981). IMP then is further degraded via inosine to hypoxanthine, xanthine, and finally uric acid (Fig. 4).

The importance of phosphate depletion as the driving force for the degradation of AMP, and thus uric acid formation, is demonstrated by a study in which the uricemic effect of fructose infusions (0.5 g/kg bw; 10 min) was measured in healthy and gouty persons. It was found that co-administration of a phosphate buffer for two hours before and during fructose infusion not only abolished the uricemic effect of fructose completely but even lowered the serum uric acid levels in the gouty subjects below pretreatment values (Morozzi et al., 1982).

000177

3. Does D-tagatose, under the conditions of intended use, have the potential to increase serum uric acid levels?

3.1 Uricemic effect of D-tagatose in healthy and diabetic subjects

3.1.1 Effects of a single dose on postprandial serum acid concentrations

Consumption of a single dose of 15 g D-tagatose or 15 g sucrose (corresponding to about 0.2 g/kg bw), given together with a standardized breakfast to fasted male healthy volunteers, does not increase serum uric acid levels (Boesch et al., 2001) (Figs. la, b).

Under the intended conditions of use, the intake of D-tagatose per eating occasion (5 - 6 g for the average consumer) will remain substantially below the level that has been found to not produce a uricemic response (15g single dose).

In two separate studies, the acute bolus ingestion of 30 g D-tagatose was associated with a transient postprandial increase of serum uric acid concentrations of 0.9 and 1.2 mg/dl in fasted healthy male volunteers (Buemann et al., 2000a, b). Fructose which served as a comparison treatment in these studies, produced no significant uricemic effect in one study (Buemann et al., 2000b) and a small increase of serum uric acid of about 0.4 mg/dl in the other study (Buemann et al., 2000a) (Fig. 5). The increases of serum uric acid were associated with transient decreases of serum phosphate concentrations (Fig. 6).

The increase of plasma uric acid concentrations which was seen after administration of 30 g D-tagatose to fasted human volunteers, may overemphasize the uricemic potential of D-tagatose under normal dietary conditions because the depletion of ATP and P_i. Thus, the uric acid formation, may be more marked in the fasted condition in which liver glycogen is depleted and intracellular ATP and P, levels may be at the lower end of their physiological range. (For diurnal fluctuations of plasma ATP levels, see Chagoya de Sánchez et al., 1996; for effects of food intake on plasma P, concentrations, see Fig. 6). In this context it also may be noted that serum uric acid exhibits a diurnal rhythm with a zenith of 5.8 mg/dl at 9 am (i.e., after overnight fasting) and a nadir of about 5.3 mg/dl at 5 pm (Devgun & Dhillon, 1992).

The ingestion of 30 g D-tagatose increased serum uric acid by 0.9 – 1.2 mg/dl at peak levels corresponding to an increase of about 15 – 20 % above baseline values. Transient increases of serum uric acid of this magnitude are not unusual as changes of similar magnitude have been observed after ingestion of ordinary foods such as liver (150 g), soy protein (80 g), or red wine with or without alcohol (5 ml/kg bw) (Colling & Wolfram, 1987; Garrel et al., 1991; Abu-Amsha et al., 2000 (Figs. 7-9).

Single doses of 75 g D-tagatose produced transient, postprandial serum uric acid increases of 1.8, 1.9, 1.0 and 1.3 mg/dl in healthy male, healthy female, diabetic male, and diabetic female subjects, respectively (Saunders et al., 1999). A comparison of the uricemic effect of acute bolus doses of D-tagatose, fructose and sucrose is shown in Figure 10.

000179

3.1.2 *Effects of repeated doses on fasting serum uric acid concentrations*

The chronic ingestion of D-tagatose at doses of 45 and 75 g/day for periods of 4 and 8 weeks, respectively, did not influence fasting uric acid levels in healthy human volunteers (Boesch et al., 2001; Saunders et al., 1999). Subjects with type-2 diabetes who ingested D-tagatose for 8 weeks (75 g/day) or 12 months (45 g day) also did not exhibit changes of fasting serum uric acid concentrations that could be attributed to the treatment (Saunders et al., 1999; Makris, 1999).

Similar observations were made for sucrose, fructose and xylitol. (Fig. 11). The chronic ingestion of these carbohydrates at daily doses of up to 1 g/kg bw (sucrose, fructose) and 0.7 g/kg bw (xylitol) for up to 22 months did not affect fasting serum uric acid concentrations in healthy and diabetic subjects (Huttunen et al., 1975; Osei et al., 1987; Anderson et al., 1989).

The absence of effects on fasting serum uric acid levels of the chronic ingestion of D-tagatose at levels several times above the estimated daily intake indicates that D-tagatose, under the conditions of intended use, will not increase the risk of gout in healthy subjects.

Similarly, the ingestion of fructose and/or sucrose with a regular diet has not been identified as a factor that would contribute to in-

creases of serum uric acid levels in elderly subjects. Only alcohol intake (men), meat and fish consumption (women), diuretic therapy and body weight were found to be positively associated with serum uric acid levels (Loenen et al., 1990).

3.2 Uricemic effects in subjects with asymptomatic hyperuricemia

Asymptomatic hyperuricemia is the consequence of an imbalance between uric acid production and excretion. In the majority of cases ($\geq$75%), persistent hyperuricemia is the result of a decreased uric acid clearance. Hyperuricemia due to a genetically determined overproduction of uric acid is rare ($<$1% of cases). Hyperuricemia due to excessive ingestion of alcohol or a purine rich diet with and without decreased uric acid clearance accounts for about 15% and 10% of cases, respectively.

Hyperuricemia occurs often in association with essential hypertension, impaired glucose tolerance, hyperlipidemia and obesity ("Syndrome X"). However, hyperuricemia is only a marker, not a cause of this syndrome. In other words, asymptomatic hyperuricemia is a laboratory finding, not a disease.

The available evidence indicates that a uricemic effect of D-tagatose would not occur in hyperuricemic subjects as long as its intake does not exceed 15 g per eating occasion (0.2 g/kg bw). Two lines of reasoning lead to this conclusion.

First, it has been shown that single doses of 15 g D-tagatose do not produce a uricemic effect in healthy subjects. If a 15-g dose of D-tagatose does not increase uric acid production, it is irrelevant whether uric acid excretion is reduced, or not. It also is irrelevant whether uric acid production is increased by mechanisms which are unrelated to that mediating the fructose or tagatose-induced effect (e.g., consumption of purine rich foods).

Second, it has been shown experimentally that the ingestion of fructose, at a dose which elicits a transient increase of serum uric acid (1 g/kg bw), produces the same uricemic effect in healthy subjects and in individuals exhibiting asymptomatic hyperuricemia (Menghini & Della Corte, 1987) (Fig. 12).

The coexistence of diabetes with hyperuricemia is not expected to have any effect in this regard because the metabolic aberrations associated with diabetes have no relationship to the mechanism by which D-tagatose or fructose produce a uricemic response. This notion is supported by the observation of smaller rather than larger increases of serum uric acid levels after ingestion of 75 g D-tagatose in type-2 diabetic compared to healthy subjects (Saunders et al., 1999) (Fig. 10).

3.3 Uricemic effect in subjects suffering from gout

The considerations applicable for hyperuricemic subjects are equally valid for individuals with manifest gout.

At intakes below the threshold at which D-tagatose starts to develop a uricemic effect, no increase of serum acid levels can be expected in gouty subjects for the reasons explained in section 3.2.

At exposures which produce a uricemic response, fructose (1 g/kg bw) was found to either not cause a bigger uricemic effect, or to increase serum uric acid levels only slightly more in gouty subjects (without allopurinol treatment) than in healthy subjects (Morozzi et al., 1982, Stirpe et al., 1970) (Figs. 13,14). Even the intravenous infusion of 50 g fructose produced only a slightly bigger increase, if any, of serum uric acid levels in gouty than normal subjects (Al-Hujaj et al., 1972; Narins et al., 1975). Only in one study with the ingestion of a single oral fructose dose of 1 g/kg bw, subjects with untreated gout (of undefined degree) exhibited a clearly higher uricemic response (Menghini & Della Corte, 1987) (Fig. 12). It would appear that renal urate excretion was markedly impaired in these subjects.

From a practical point of view, it is relevant that the intakes of D-tagatose, under the conditions of intended use, will at all times remain below levels that would trigger a uricemic response (Fig. 15). Therefore, it cannot be expected that the use of D-tagatose in foods would increase the risk of attacks of gout in gouty persons. Moreover,

the standard treatment of gouty persons with allopurinol or uricosuric drugs provides an additional safeguard because these drugs would attenuate an increase of serum uric acid levels following the ingestion of excessive amounts of D-tagatose or fructose (Al-Hujaj et al., 1972).

4. Could the combined intake of D-taaatose and fructose produce a higher uricemic response (synergistic effect)?

Fructose is ingested in substantial amounts with a regular American diet. Mean and 90th percentile intakes have been estimated to range from 36 - 47 g/d (0.7 g/kg bw) and from 64 - 80 g/d (1.1 g/kg bw), respectively (Park & Yetley, 1993).

Since D-tagatose is metabolized by the same enzymes as fructose (fructokinase and aldolase B), the question arises as to whether the uricemic effect might be enhanced if the two sugars are ingested together.

As explained in section 2.3, uric acid formation is triggered by a depletion of intracellular phosphate below usual levels. Phosphate depletion is the direct consequence of the sequestration of phosphate in tagatose-1-phosphate and fructose-1-phosphate. The concentration of these two metabolites have been determined in the rat liver after perfusion with D-tagatose (0.5 mM), fructose (2 mM) and a combination of the two sugars (Quistorff & Therkelsen, 1999). It was found that the phosphorylation of D-tagatose is inhibited in the presence of fructose. This finding may be explained by the higher affinity of fructo-

kinase for fructose than D-tagatose. The rate of disappearance of tagatose-l-phosphate and fructose-l-phosphate was not affected by the presence of fructose and D-tagatose, respectively.

Based on the consideration that fructose, not D-tagatose, is the preferred substrate for fructokinase, it may be concluded that the simultaneous ingestion of D-tagatose with fructose leads to a lower, rather than higher, production of uric acid.

5. Could the chronic ingestion of D-tagatose lead to anincreased intestinal absorption and thereby to a higher uricemic response?

As explained in section 2.1, the intestinal absorption of D-tagatose does not increase with duration of the treatment. Therefore, no increase of the uricemic effect is to be expected from long-term intake of D-tagatose.

6. Conclusions

6.1 Absence of uricemic effects of fructose and D-tagatose at intakes resulting from the intended uses

Fructose, at actual levels of consumption (<25 - 30 g per eating occasion for the mean consumer), does not increase postprandial serum uric acid levels to a clinically relevant extent in healthy, hyperuricemic and gouty subjects.

D-Tagatose (15 g single dose) does not increase postprandial serum uric acid levels in healthy subjects. On biochemical grounds and in analogy to fructose, no uricemic effect is to be expected for D-tagatose at the projected levels of intake (5 - 6 g per eating occasion) in healthy, hyperuricemic and gouty subjects. The duration of D-tagatose intake or the simultaneous ingestion of fructose does not play a role in this regard.

6.2 Uricemic effects of fructose and D-tagatose at levels which will not be reached under realistic conditions of use

At intakes which are substantially higher than those resulting from the actual uses of fructose or the intended uses of D-tagatose (single doses of >50 g fructose or >30 g D-tagatose), either sugar produces a transient, postprandial increase of serum uric acid in healthy subjects. Studies with fructose demonstrate that this increase is not greater in hyperuricemic subjects. In gouty people who receive standard treatment, the therapeutic drugs will attenuate the uricemic effect of fructose and D-tagatose. Only a small number of patients with untreated gout and markedly reduced renal clearance of uric acid might experience a greater uricemic response than healthy

subjects after the intake of high doses of fructose or D-tagatose. However, this is a hypothetical consideration because such levels of intake will not be reached under normal conditions of use. For D-tagatose, the self-limitation of its intake (due to intestinal effects) provides additional assurance that such high levels of intake will not be reached.

No increases in fasting serum uric acid levels were noted in a number of studies in which healthy and diabetic subjects consumed fructose and D-tagatose at daily doses of up to about 1 g/kg bw for periods of weeks to months.

6.3 Overall conclusion

There exists no reason to expect a postprandial increase of plasma uric acid concentrations in response to the ingestion of single 15-g doses of D-tagatose in healthy, diabetic, hyperuricemic or gouty subjects. The difference between this dose and the projected mean intake of D-tagatose per eating occasion provides a three-fold margin of safety. Studies with chronic intake of D-tagatose (75 g/d for 8 weeks, 45 g/d for 12 months) have shown that even at intakes which exceed the estimated mean daily intake by a factor of more than ten, there is no cumulative effect on fasting serum uric acid levels.

7. References

Abu-Amsha C., Croft K.D., Beilin L.J. and Puddey I.B. (2000). Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vitro lipoprotein oxidizability. Am. J. Clin. Nutr., 71: 67-74.

Al-Hujaj M., Schonthal H. and Elbrechter J. (1972). Differential diagnosis of gout. The Lancet, ii: 606.

Anderson J.W., Story L.J., Zettwoch N.C., Gustafson N.J. and Jefferson B.S. (1989). Metabolic effects of fructose supplementation in diabetic individuals. Diabetes Care, 12: 337-344.

Baur H. and Heldt H.W. (1977). Transport of hexose across the liver-cell membrane. Eur. J. Biochem., 74: 397-403.

Bjarnason, I., Fehilly, B., Smethurst, P., Menzies, I.S. and Levi, A.J. (1991). Importance of local versus systemic effects of non-steroidal anti-inflammatory drugs in increasing small intestinal permeability in man. Gut, 32: 275-277.

Bjarnason, I., Maxton, D., Reynolds, A.P., Catt, S., Peters, T.J. and Menzies, I.S. (1994). Comparison of four markers of intestinal permeability in control subjects and patients with coeliac disease. Scand. J. Gastroenterol., 29: 630-639.

000188

Boesch Ch., Ith M., Jung B., Bruegger K., Erban S., Diamantis I., Kreis R. and Bar A. (2001). Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. Reg. Tox. Pharmacol., 33: 257-267.

Brosh S., Boer P. and Sperling O. (1982). Effects of fructose on synthesis and degradation of purine nucleotides in isolated rat hepatocates. Biochim. Biophys. Acta, 717: 459-464.

Buemann B., Gesmar H., Astrup A. and Quistorff B. (2000b). Effects of oral D-tagatose, a stereoisomer of D-fructose, on liver metabolism in man as examined by ^{31}P-magnetic resonance spectroscopy. Metabolism, 49: 1335-1339.

Buemann B., Toubro S., Holst J.J., Rehfeld J.F., Bibby B.M. and Astrup A. (2000a). D-Tagatose, a stereoisomer of D-fructose, increased blood uric acid concentration. Metabolism, 49: 969-976.

Buemann, B., Toubro, S. and Astrup, A. (1998). D-Tagatose, a stereoisomer of D-fructose, increases hydrogen production without affecting 24 hours energy expenditure, or respiratory exchange ratio. J. Nutr., 128: 1481-1486.

Caccetta A.-A., Croft K.D., Beilin L.J. and Puddey I.B. (2000). Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability. Am. J. Clin. Nutr., 71: 67-74.

Campion E.W., Glynn R.J. and DeLabry G.R. (1987). Asymptomatic hyperuricemia. Risks and consequences in the normative aging study. Am. J. Med., 82: 421-426.

Chagoya de Sanchez V., Hernández-Muñoz R., Suarez J., Vidrio S., Yáñez L., Aguilar-Roblero R., Oksenberg A., Vega-Gonzalez A., Villalobos L., Rosenthal L., Fernandez-Cancino F., Drucker-Colin R. and Díaz-Muñoz M. (1996). Temporal variations of adenosine metabolism in human blood. Chronobiol. Int., 13(3): 163-177.

Colling M. and Wolfram G. (1987). Zum Einfluss von DNS und RNS in Lebensmitteln auf die Harnsaurekonzentration im Serum des Menschen. Z. Ernahrungswiss., 26: 171-178.

Crouzoulon G. (1978). Les proprietes cinétiques du flux d'entrée du fructose a travers la bordure en brosse du jéjunum du rat. Arch. Int. Physiol. Biochim., 86: 725-740.

Davies P.M., Simmonds H.A., Singer B., Mant T.G., Allen E.M., Vassos A.B. and Hounslow N.J. (1998). Plasma uridine as well as uric acid is elevated following fructose loading. Adv. Exp. Med. Biol., 431: 31-35.

Delahunty, T.J. and Hollander, D. (1987). A comparison of intestinal permeability between humans and three common laboratory animals. Comp. Biochem. Physiol., 86A: 565-567.

Devugn M.S. and Dhillon H.S. (1992). Importance of diurnal variations on clinical value and interpretation of serum urate measurements. J. Clin. Pathol., 45: 110-113.

000190

Forster H. and Ziege M. (1971). Anstieg der Serumharnsaurekonzentration nach oraler Zufuhr von Fructose, Sorbit und Xylit. Z. Ernahrungswiss., 10: 394-396.

Forster H., Boecker S. and Ziege M. (1972). Anstieg der Konzentration der Serumharnsaure nach akuter und chronischer Zufuhr von Saccharose, Fructose, Sorbit und Xylit. Medizin und Ernahrung, 13: 193-196.

Fox I.H. and Kelley W.N. (1972). Studies on the mechanism of fructose-induced hyperuricemia in man. Metabolism, 21: 713-721.

Garrel D.R., Verdy M., PetitClerc C., Martin Ch., Brulé D. and Hamet P. (1991). Milk- and soy-protein ingestion: acute effect on serum uric acid concentration. Am. J. Clin. Nutr., 53: 665-669.

Hall A.P., Barry P.E., Dawber T.R. and McNamara P.M. (1967). Epidemiology of gout hyperuricemia. Am. J. Med., 42: 27-37.

Hall, E.J. and Batt, R.M. (1996). Urinary excretion by dogs of intravenously administered simple sugars. Res. Vet. Sci., 60: 280-282.

Hamilton, I., Rothwell, J., Archer, D. and Axon, A.T.R. (1987). Permeability of the rat small intestine to carbohydrate probe molecules. Clin. Sci., 73: 189-196.

Howden, C.W., Robertson, C., Duncan, A., Morris, A.J. and Russell, R.I. (1991). Comparison of different measurements of intestinal permeability in inflammatory bowel disease. Am. J. Gastroenterol., 86: 1445-1449.

Huttunen J.K., Mäkinen K.K. and Scheinin A. (1975). Turku sugar studies XI. Effect of sucrose, fructose and xylitol diets on glucase, lipid and urate metabolism. Acta Odont. Scand., 33, Suppl. 70: 239-245.

Itoh R. (1981). Regulation of cytosol 5' nucleotidase EC-3.1.3.5 by adenylate energy charge. Biochim. Biophys. Acta, 659: 31-37.

Jenkins, A.P., Menzies, I.S., Nukajam, W.S. and Creamer, B. (1994). The effect of ingested lactulose on absorption of L-rhamnose, D-xylose, and 3-O-methyl-D-glucose in subjects with ileostomies. Scan. J. Gastroenterol., 29: 820-825.

Jensen B.B. and Buemann B. (1998). D-Tagatose. The influence of D-tagatose on bacterial composition and fermentation capacity of faecal samples from human volunteers. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Jensen B.B. and Laue A. (1998). D-Tagatose. Absorption of tagatose and fermentation products of tagatose from the gastrointestinal tract of pigs. Internal report of the Research Centre Foulum, Danish Institute of Agricultural Science, for MD Foods amba.

Kelsay J.L., Behall K.M., Moser Ph.B. and Prather E.S. (1977). The effect of kind of carbohydrate in the diet and use of oral contraceptives on metabolism of young women. I. Blood and urinary lactate, uric acid, and phosphorus. Am. J. Clin. Nutr., 30: 2016-2022.

000132

Laerke H.N. and Jensen B.B. (1999). D-Tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. J. Nutr., 129: 1002-1009.

Laerke H.N., Jensen B.B. and Hojsgaard S. (2000). In vitro fermentation pattern of D-tagatose is affected by adaption of the microbiota from the gastrointestinal tract of pigs. J. Nutr., 130: 1772-1779.

Lee A. and Storey D.M. (1999). Comparative gastrointestinal tolerance of sucrose, lactitol, or D-tagatose in chocolate. Regul. Toxicol. Pharmacol., 29 (2,2/2): S78-S82.

Lin K.-Ch., Lin H.-Y. and Chou P. (2000). Community based epidemiological study on hyperuricemia and gout in kin-hu, kinmen. J. Rheumatol., 27: 1045-1050.

Loenen H.M.J.A., Eshius H., Lowik M.R.H., Schouten E.G., Hulshof K.F.A.M., Odink J. and Kok F.J. (1990). Serum uric acid correlates in elderly men and women with special reference to body composition and dietary intake (Dutch nutrition surveillance system). J. Clin. Epidemiol., 43: 1297-1303.

Macdonald I., Keyser A. and Pacy D. (1978). Some effects, in man, of varying the load of glucose, sucrose, fructose, or sorbitol on various metabolites in blood. Am. J. Clin. Nutr., 31: 1305-1311.

Maclachlan M.J. and Rodnan G.P. (1967). Effects of food, fast and alcohol on serum uric acid and acute attacks of gout. Am. J. Med., 42: 38-57.

000193

Makris N. (1999). Tagatose University of Maryland Clinical Study, Phase 2 Study, Statistical Analyses, for Biospherics, Inc., Beltsville, MD.

Malaisse W. J., Malaisse-Lagae F., Davies D.R. and Van Schaftingen E. (1989). Presence of fructokinase in pancreatic islets. FEBS Lett., 255 (1): 175-178.

Martinez P., Carrascosa J.M. and Núñez de Castro I. (1982). Efecto Crabtree causado por cetosas en hepatocitos aislados de rata. Rev. Españ. Fisiol., 38 (suppl.): 181-184.

Martinez P., Carrascosa J.M. and Núñez de Castro I. (1987). Ketose induced respiratory inhibition in isolated hepatocytes. Rev. Espafi. Fisiol., 43 (2): 163-172.

Masson S., Henriksen O., Stengaard A., Thomsen C. and Quistorff B. (1994). Hepatic metabolism during constant infusion of fructose; comparative studies with P-magnetic resonance spectroscope in man and rats. Biochim. Biophys. Acta, 1199: 166-174.

Maxton, D.G., Bjarnason, I., Reynolds, A.P., Catt, S.D., Peters, T.J. and Menzies, I.S. (1986). Lactulose ^{51}Cr-labelled ethylenediaminetetra-acetate, L-rhamnose and polyethylene-eglycol 500 as probe markers for assessment in vivo of human intestinal permeability. Clin. Sci., 71: 71-80.

Mayes P.A. (1993). Intermediary metabolism of fructose. Am. J. Clin. Nutr., 58 (suppl.) :754S-765S.

000194

Menghini S. and Della Corte E. (1987). Valutazioni sull''iperuricemia da carico di fruttosio in condizioni di alterato metabolismo dell'acido urico. Quad. Sclavo Diagn., 23 (4): 441-446.

Menzies, I.S., Jenkins, A.P., Heduan, E., Catt, S.D., Segal, M.B. and Creamer, B. (1990). The effect of poorly absorbed solute on intestinal absorption. Scand. J. Gastroenterol., 25: 1257-1264.

Mooradian, A.D., Morley, J.E., Levine, A.S., Prigge, W.F. and Gebhard, R.L. (1986). Abnormal intestinal permeability to sugars in diabetes mellitus. Diabetologia, 29: 221-224.

Morozzi G., Berti C., Pellegrini R., Lalumera M. and Marcolongo R. (1982). Inibizione dell'iperuricemia da fruttoso mediante pretrattamento con fosfati. Boll. Soc. It. Biol. Sper., 58 (8): 491-496.

Moss K.M. (1977). The effect of purine nucleotides and phosphate on activity of AMP deaminase in a cytosol extract of rat liver. FEBS Lett., 75: 141-144.

Narins R.G., Weisberg J. and Meyers A.R. (1975). Fructose and hyperuricemia. Metabolism, 24: 899-900.

Oberhaensli R.D., Galloway M.D., Taylor D.J., Phil D., Bore P.J. and Radda G.K. (1986). Assessment of human liver metabolism by phosphorus-31 magnetic resonance spectroscopy. Brit. J. Radiol., 59: 695-699.

Okuno Y. and Gliemann J. (1986). Transport of glucose and fructose in rat hepatocytes at 37°C. Biochim. Biophys. Acta, 862: 329-334.

Osei K., Falko J., Bossetti B.M. and Holland G.C. (1987). Metabolic effects of fructose as a natural sweetener in the physiological meals of ambulatory obese patients with type II diabetes. Am. J. Med., 83: 249-255.

Park Y.K. and Yetley E.A. (1993). Intakes and food sources of fructose in the United States. Am. J. Clin. Nutr., 58 (Suppl.): S737-S747.

Quistorff B. and Therkelsen I. (1999). Effects of combined D-tagatose and D-fructose in the perfused rat liver. Unpublished report of The Panum NMR Center, Institute of Medical Biochemistry and Genetics, University *of* Copenhagen, Denmark.

Raushel F.M. and Cleland W.W. (1973). The substrate and anomeric specificity of fructokinase. J. Biol. Chem., 248 (23): 8174-8177.

Raushel F.M. and Cleland W.W. (1977). Bovine liver fructokinase: purification and kinetic properties. Biochem., 16 (10): 2169-2175.

Reiser S., Scholfield D., Trout D., Wilson A. and Aparicio P. (1984). Effect of glucose and fructose on the absorption of leucine in humans. Nutr. Rep. Int., 30: 151-162.

Rognstad R. (1975). Gluconeogenesis from D-tagatose by isolated rat and hamster liver cells. FEBS Lett., 52 (2): 292-294.

Rognstad R. (1982). Pathway of gluconeogenesis from tagatose in rat hepatocytes. Arch. Biochem. Biophys., 218 (2): 488-491.

000196

000196

Roubenoff R. (1990). Gout and hyperuricemia. Rheumatic Dis. Clin. North America, 16: 539-550.

Roubenoff R., Klag M., Mead L., Liang K.-Y., Seidler A.J. and Hochberg M. (1991). Incidence and risk factors for gout in white men. JAMA, 266: 3004-3007.

Sanchez J.J., González N.S. and Pontis H.G. (1971). Fructokinase from rat liver. I. Purification and properties. Biochim. Biophys. Acta, 227: 67-78.

Saunders J.P., Donner T.W., Sadler J.H., Levin G.V. and Makris N.G. (1999a). Effects of acute and repeated oral doses of D-tagatose on plasma uric acid in normal and diabetic humans. Regul. Toxicol. Pharmacol., 29 (2,2/2): S57-S65.

Seegmiller E., Dixon R.M., Kemp G.J., Angus P.W., McAlindon T.E., Dieppe P., Rajagopalan B. and Radda G.K. (1990). Fructose-induced aberration of metabolism in familial gout identified by ^{31}P magnetic resonance spectroscopy. Proc. Natl. Acad. Sci., 87: 8326-8330.

Sestoft L. and Fleron P. (1974). Determination of the kinetic constants of fructose transport and phosphorylation in the perfused rat liver. Biochim. Biophys. Acta, 345: 27-38.

Sigrist-Nelson K. and Hopfer U. (1974). A distinct D-fructose transport system in isolated brush border membrane. Biochim. Biophys. Acta, 367: 247-254.

000197

Smith C.M., Rovamo L.M. and Raivio K.O. (1977). Fructose induced adenine nucleotide catabolism in isolated rat hepatocytes. Can. J. Biochem., 55: 1237-1240.

Stirpe F., Della Corte E., Sonetti E., Abbondanza A., Abbati A. and de Stefano F. (1970). Fructose-induced hyperuricemia. Lancet, ii: 1310-1311.

Tatibouet A., Yang J., Morin C. and Holman G.D. (2000). Synthesis and evaluation of fructose analogues as inhibitors of the D-fructose transporter GLUT5. Bioorg. Med. Chem., 8: 1825-1833.

Thoma W.J. and Ugurbil K. (1989). Effect of adenine on liver nucleotide after loading by ^{31}P-NMR. Am. J. Physiol. Gastrointest. Liver. Physiol., 256: G949-G956.

Van den Berghe G. (1986). Fructose: metabolism and short-term effects on carbohydrate and purine metabolic pathways. Prog. Biochem. Pharmacal., 21: 1-32.

Van den Berghe G.M., Bronfman R., Vanneste R. and Hers H.G. (1977). The mechanism of adenosine triphosphate depletion in the liver after a load of fructose. A kinetic study of liver adenylate deaminase. Biochem. J., 162: 601-609.

Vincent M.F., Van den Berghe G and Hers H.G. (1986). Effect of fructose on the concentration of phosphoribosylpyrophosphate in isolated hepatocytes. Adv. Exp. Med. Biol., 195: 615-621.

Vincent M.F., Van den Berghe G. and Hers H.-G. (1989). Increase in phosphoribosyl pyrophosphate induced by ATP and P_i depletion in hepatocytes. FASEB J., 3: 1862-1867.

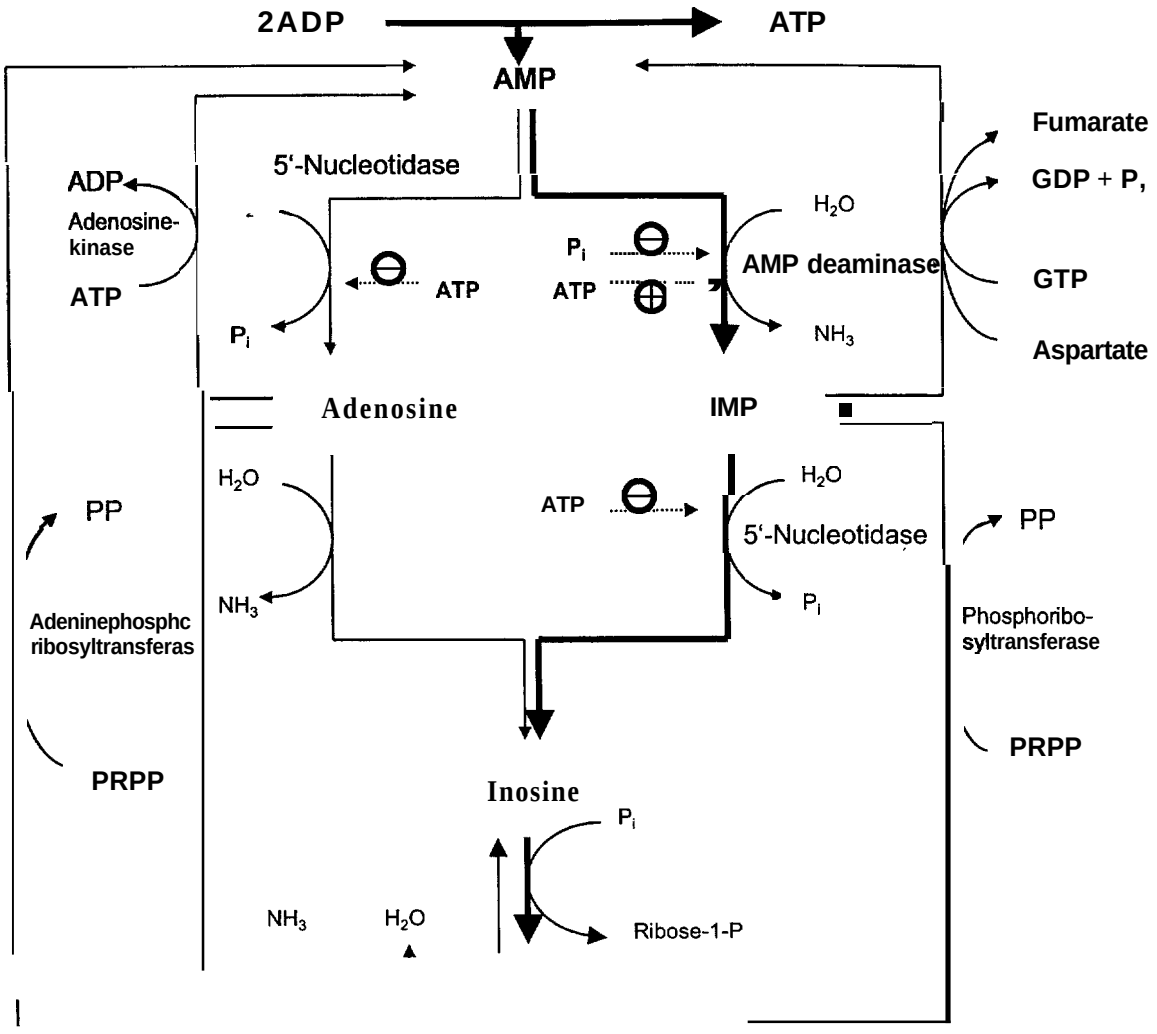
Zeid I.M., Bronk S.F., Fesmier P.J. and Gores G.J. (1997). Cytoprotection by fructose and other ketohexoses during bile salt-induced apoptosis of hepatocytes. Hepatology, 25: 81-86.

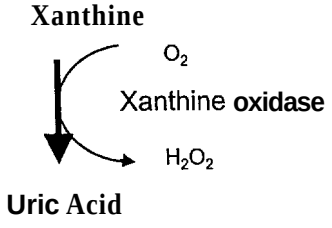
Pages 000200- 000201 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000202- 000202 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000203- 000203 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Figure 4 Catabolism in humans of adenine nucleotides to ox /purines (hypoxanthine, xanthine) and uric acid





Pages 000205- 000206 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000207- 000207 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000208- 000208 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000209- 000209 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000210- 000210 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000211- 000211 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000212- 000212 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000213- 000213 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000214- 000214 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

English Translations of Italian Studies

000215

STI

CERTIFICATION

Schreiber Tronslotions, Inc.

51 Monroe Street

Suite 101

Rockville, MD 20850

P: 301.424.7737

F: 301.424.2336

This is to certify that the attached English language document, identified as patent entitled Evaluations on Hyperuricemia From Fructose Loading Under Conditions of Altered Metabolism of Uric Acid, is a true and accurate translation of the original Italian language document to the best of our knowledge and belief.

Executed this 20th day of August, 2001

Schreiber Translations, Inc.
51 Monroe Street, Suite 101
Rockville, Maryland 20850
ATA Member 212207

Schreiber Translations, Inc. uses all available measures to ensure the accuracy of each translation, but shall not be held liable for damages due to error or negligence in translation or transcription.

translation@schreibernet.com

www.schreibernet.com

Pages 000217- 000221 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000222- 000227 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000228- 000231 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000232- 000237 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000238- 000240 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Pages 000241- 000243 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

Reference List for Industry Submission, GRN 000078

Pages	Author	Title	Publish Date	Source	BIB_Info
000200 - 000201	Boesch	Plasma uric acid before and after intake of a standard breakfast containing abut 99g starch and 15g D-tagatose or sucrose on day 1 of each treatment period (mean + SD)	2000	Regul. Toxicol. Pharmoacol.	Volume 33, pgs 257 - 267
000202 - 000202	Macdonald, I.	Effect of low single doses of fructose on serum wnc uric acid in male healthy subjects	1978	Am. J. Clin. Nutr.	Volume 31, pgs 1305 - 1311
000205 - 000205	Buemann, B.; Toubro, S.; Holst, J.J.; Rehfeld, J.F.; Bibby, B.M.; Astrup, A.	D-Tagatose, a stereoisomer of D-fructose, increased blood uric acid concentration	2000	Metabolism	Volume 49, pgs 969 - 976
000207 - 000207	Colling, M.; Wofram, G.	Zum Einfluss von DNS und RNS in Lebensmitteln auf die Harnsaurekonzentration im Serum des Menschen	1987	Z. Ernahrungswiss.	Volume 26, pgs 171 - 178
000209 - 000209	Caccetta, A. A.; Croft, K. D.; Beilin, L. J.; Puddey, I. B.	Ingestion of red wine significantly increases plasma phenolic acid concentrations but does not acutely affect ex vivo lipoprotein oxidizability	2000	Am. J. Clin. Nutr.	Volume 71, pgs 67 -74
000210- 000210	NA	NA	NA	NA	NA
000211- 000211	Menghini, S.; Della Corte, E.	Valutazioni Sull' ' Imperuricemia da carisco di fruttosio in condizioni di alterato metabolismo dell; acido urico	1987	Quad. Sclavo Diagn.	Volume 23, Number 4, pgs 441-446
000212 - 000212	Stirpe, F.; Della Corte, E.; Sonetti, E.; Abbondaza, A. Abbati, A. de Stefano, F.	Fructose-induced hyperuricemia	1970	Lancet	pgs 1310-1311
000213- 000213	Morozzi, G.; Berti, C.; Pellegrini, R.; Lulumera, M.; Marcolongo, R.	Inibizione dell'iperuricemia da fruttoso mediante pretrattamento con fosfati pretrattamento con fosfati	1982	Boll. SOC. It. Biol. Sper.	Volume 58, Number 8, pgs 491-496

NA- Not applicable

Pages	Author	Title	Publish Date	Source	BIB_Info
000214-000214	Park, Y.K.; Yetley, E.A.	Intakes and food sources of fructose in the United States	1993	Am. J. Clin. Nutr.	Volume 58, Suppl. S737-S747
000217-000221	Menghini, Sonia; Corte, Enrico Ella	Evaluations on Hyperuricemia from Fructose Loading under Conditions of Altered Metabolism of Uric Acid	1987	Quad. Sclavo Diagn	Volume 23, Number 4, pgs 441-446
000222 - 000227	Menghini, Sonia; Corte, Enrico Ella	Valutazioni Sull Iperuricemia da Carico di Fruttosio in Condizioni di Alterato Metabolismo dell Acido Urico	1987	Quad. Sclavo Diagn	Volume 23, Number 4, pgs 441-446
000228-000231	Morozzi, G.; Berti, C.; Pellegrini, R.; Lalumera, M.; Marcolongo, R.	Inhibition of Fructose-Induced Hyperuricemia by Means of Pretreatment with Phosphates	1982	Boll. Soc. It. Bio. Sper.	Volume, 58, Number 8, pgs 491-496
000232 - 000237	Morozzi, G.; Berti, C.; Pellegrini, R.; Lalumera, M.; Marcolongo, R	Inbizione Dell' Iferuricemia Da Fruttoso Mediante Pretrattam	1982	Boll. Soc. Hal. Biol. Spes.	Volume 58, Number 8
000238 - 000240	Roehrig, Karla L.	Carbohydrate Biochemistry and Metabolism	1984	NA	pg 98
000241 - 000243	Dorland, William Alexander Newman	Gout	2000	Dorlands Illustrated Medical Dictionary	29th Edition

NA- Not applicable