Bioresco
Food Scientific and Regulatory Services

D-Tagatose

Dossier prepared and submitted on behalf of
Arla Food Ingredients amba, Viby, Denmark
for evaluation pursuant to EU Novel Foods Regulation (EC) 258/97
by the UK Advisory Committee on Novel Foods and Processes

Author: Albert Bär PhD

Date: August 2, 2004

Bioresco Ltd. Bundesstrasse 29 CH-4054 Basel Switzerland
Telephone ++41 61 273 77 00 Telefax ++41 61 273 77 03 e-mail: contact@bioresco.ch Internet: http://www.bioresco.ch

CONTENTS

FIGURES 1-6

TABLES 1-6

Note about confidentiality

Under the "UK Novel Foods and Novel Food Ingredients (Amendment) Regulations 1999" and the corresponding "Guidelines on the disclosure of information and confidentiality in respect of applications made to the ACNFP", this dossier and the pertinent parts of the pivotal safety studies are to be made public. Information for which the applicant claims confidentiality because it pertains to production or marketing know-how and the specific properties of the applied immobilized lactase, which is purchased from a third party, is marked [redacted]. Annexes 1, 2, and 6 which contain details about the production process and the intended uses of D-tagatose, are marked "Confidential" in their entirety.

1. INTRODUCTION

D-Tagatose is a sugar (monosaccharide) and, more precisely, an enantiomer of D-fructose (inversion at C-4). It occurs naturally in small amounts in heat-treated dairy products, where it is formed from galactose by isomerisation.

Sugars, i.e., mono- and disaccharides, are generally considered to be foods, not food additives. This is also true for novel sugars as exemplified by trehalose which was authorized by a Commission Decision (2001/721/EC) under the Novel Food Regulation.

The non-cariogenicity, prebiotic activity, low glycemic impact and reduced energy value of D-tagatose are the main reasons for its use in sweet tasting foods. These nutritional purposes further underpin the food status of D-tagatose.

D-Tagatose has not been consumed so far in significant amounts in the European Union. Therefore, it must be regarded as a novel food. Since it is produced at a commercial scale from galactose, a component of lactose, by a one-step chemical isomerisation, it complies with the definition of a novel food according to article 1 (2)(c) of the Novel Food Regulation ("foods and food ingredients with a new or intentionally modified primary structure").

The present application for authorization of D-tagatose as a novel food was prepared according to the EU Commission's Guidelines on the scientific information necessary to support applications for the placing on the market of novel foods or novel food ingredients [Commission Recommendation (97/618/EC)]. D-Tagatose was identified as belonging to class 1.2 ("pure chemicals or simple mixtures from

non-GM sources with no history of food use in the Community"). Using the computerized decision tree developed by the UK Advisory Committee on Novel Foods and Processes, the data requirement for D-tagatose was identified. The outcome of this evaluation is shown in Annex 7.

For evaluation of a class 1.2 product, the following information is required according to the Commission Guideline, Table II:

I. Specification of the Novel Food (NF)

This information is presented in Section 3 and Section 5 of this Dossier.

II. Effect of the production process applied to the NF

This information is presented in Section 4 of this Dossier.

III. History of the organism used as the source of the NF

D-Tagatose is produced chemically from D-galactose, i.e., it is not obtained from an organism. Consequently, no information is required under this heading.

IV. Anticipated intake/extent of use of the NF

Information on the intended uses and resulting estimated daily intake is provided in Section 6.2 and Section 7 of this Dossier.

V. Nutritional information on the NF

Information on the nutritional benefits is provided in Section 6.1 of this Dossier.

VI. Microbiological information on the NF

There are no microorganisms involved in the production of D-tagatose. Data on the microbial purity of D-tagatose are presented in Annex 4.

VII. Toxicological information on the NF

This information is presented in Sections 8, 9 and 10 of this Dossier.

In addition to the information which is required according to the Guidelines, information is provided about an analytical method for the determination of D-tagatose in food (Section 6.3) and earlier safety assessments and the regulatory status in other countries of D-tagatose (Section 8).

The conceivable need for special labeling provisions of D-tagatose is discussed in Sections 10.7, 10.8 and 10.9.

An extensive summary may be found in Section 11.

2. ADMINISTRATIVE DATA

2.1 Purpose of the application

The purpose of this application is to obtain authorization for D-tagatose produced by a simple chemical process, as a novel food ingredient for use in solid, semi-liquid and liquid foods.

2.2 Petitioner

Arla Foods amba
Skanderborgvej 277
8260 Viby J
Denmark

2.3 Manufacturer

SweetGredients GmbH & Co KG
Küchenstrasse 9
38100 Braunschweig
Germany

2.4 Responsible persons

For Arla Food Ingredients amba:

Mr. Mads Vigh
Skanderborgvej 277
8260 Viby J
Denmark
Tel.: 0045.89.38.13.56
Mobile: 0045.20.75.35.58
e-mail: mads.vigh@arlafoods.com

For Bioresco Ltd.:

Dr. Albert Bär
Bundesstrasse 29
4054 Basel
Switzerland
Tel.: 0041.61.273.77.00
Fax.: 0041.61.273.77.03
e-mail: contact@bioresco.ch

2.5 Date of submission

February 9, 2005

3. NAME AND CHEMICAL STRUCTURE, PHYSICO-CHEMICAL AND CHEMICAL PROPERTIES, OCCURRENCE IN NATURE

3.1. Name and chemical structure

Common or usual name:	D-tagatose
Synonyms:	D-lyxo-hexulose, α-D-tagatose
CAS Registry Number:	87-81-0
EINECS No:	201-772-3
Empirical formula:	$C_6H_{12}O_6$
Molecular weight:	180.16 daltons
Structural formula:	D-Tagatose is an enantiomer of D-fructose (inversion at C-4) (see Fig. 1)

3.2. Physico-chemical properties

Solubility in water (g/100ml at 20°C): 160

Solubility in ethanol (g/100ml at 22°C):0.02

Specific rotation $[a]_D^{20}$ (1%, H_20): between -4° to -5.6°[1]

Melting point (°C): 133 - 137

Heat of solution (kJ/kg at 20°C): -42.3

[1] On dissolution in water, D-tagatose mutarotates (initial $[\alpha]_D$ is +2.7°) (Karabinos, 1952). At equilibrium, the following tautomers are present: 71% α-pyranose, 15-18% β-pyranose, 3-5% α-furanose, 8-9% β-furanose and about 0.3% keto-form (Wolff and Breitmaier, 1979; Que & Gray, 1974).

3.3. Other properties relevant to the use of D-tagatose as a food ingredient

3.3.1. Sweetness

D-Tagatose in a 10% (w/w) aqueous solution has a sweetness of 92% relative to that of sucrose (measured at 24°C) (Levin et al., 1995).

3.3.2. Hygroscopicity

Crystalline D-tagatose has a low hygroscopicity, similar to that of sucrose.

3.3.3. Chemical stability

Like other sugars, D-tagatose is stable under the pH conditions which are encountered typically in foods (pH >3). However, as a reducing sugar, D-tagatose undergoes Maillard reactions.

3.3.4. Thermal stability

Under the temperature conditions applied typically during the processing and storage of food, D-tagatose is stable. However, like other reducing sugars, D-tagatose caramelizes at elevated temperature.

3.4. Occurrence in Nature

D-Tagatose was identified as a component of a gum exudate of the cacao tree (Sterculia setigera) (Hirst et al., 1949). D-Tagatose was also detected as a component of an oligosaccharide in lichens of the *Rocella* species (Lindberg, 1955). In the bacterial metabolism of lactose, D-tagatose may be formed from galactose by enzymatic isomerization (catalyzed by L-arabinose isomerase) (Szumilo & Russa, 1982; Ibrahim & Spradlin, 1993). D-Tagatose-1-phosphate and D-tagatose-1,6-diphosphate are ordinary intermediate products of the lactose (galactose) metabolism of many bacteria (e.g., Staphylococci, Streptococci) (Schleifer et al., 1978).

Heat-treated dairy products contain traces of D-tagatose because small amounts of this sugar are formed from lactose at elevated temperature. Sterilized cow milk and milk powder were found to contain D-tagatose at concentrations between about 2 to 20 and 800 ppm, respectively (Troyano et al., 1991, 1996; Richards & Chandrasekhara, 1960). D-Tagatose was also detected in yoghurt (29 ppm), milk-based infant formulae (two brands with 4 and 23 ppm, respectively), and hot cocoa prepared with milk (two preparations with 140 and 173 ppm, respectively) (Saunders, 1998).

As a by-product, D-tagatose occurs in lactulose syrup which is used for regulating bowel function and for treating hepatic encephalopathy. Commercial lactulose syrups were found to contain about 0.7 – 1% D-tagatose (Parrish et al., 1980; Beadle cited in Zehner, 1994). It was estimated that in the US about 240,000 people ingest D-tagatose at a level of 0.2 – 0.3 g/person/day from the use of two common, lactulose-based OTC-preparations, Chronulac® and Cephulac®. These products are sold in the US by Marion Merrel Dow since 1983 and 1976, respectively (Zehner, 1994).

4. MANUFACTURING PROCESS

4.1. General aspects of D-tagatose production

D-Tagatose is produced from lactose in a two-step process. In the first step, lactose is enzymatically hydrolyzed to galactose and glucose. In the second step, the galactose is isomerized to D-tagatose under alkaline conditions using calcium hydroxide as a complexant. Calcium hydroxide shifts the isomerization equilibrium between galactose and D-tagatose in the direction of D-tagatose because it forms an insoluble complex with this sugar at elevated pH. In the precipitated calcium complex, D-tagatose is protected and does not participate in side-reactions that usually occur with sugars under the applied alkaline conditions (pH 12). Treatment of the suspension with carbon dioxide liberates D-tagatose by neutralizing the mixture and by precipitating calcium as calcium carbonate.

4.2. Detailed description of the manufacturing process

The flow-scheme of the D-tagatose production process is shown in Figure 2. The specifications of the raw material (lactose), all chemical reagents used in the process, the ion exchange resins, and the immobilized lactase are presented in Annex 1.

4.2.1. Dissolution of lactose and pH adjustment

Food-grade lactose is dissolved in hot water to the required concentration. A part of this solution is passed through a cation-exchanger [redacted]. Cations will be exchanged with H+ and the pH thereby lowered [redacted]. Untreated lactose solution is mixed with this acidified lactose solution [redacted].

For the regeneration of the resin, technical grade hydrochloric acid is used.

4.2.2. Enzymatic hydrolysis

The lactose solution is pasteurized (≥75°C, 15 sec), cooled with a heat exchanger to 10°C and passed through a column containing immobilized lactase [redacted]. The degree of lactose hydrolysis is regulated by controlling the flow through the column. The immobilized lactase must be replaced from time to time because the enzyme looses activity over time (for description and safety data of the applied immobilized enzyme, see Section 4.3, Annex 1, and Annex 2)

In order to avoid contamination of the immobilized lactase column with microorganisms, the column is treated periodically [redacted] with a solution of acetic acid, potassium sorbate and sodium benzoate. After exposure of the resin to this solution for 5-10 hours, the column is washed extensively with water.

4.2.3. Evaporation

The hydrolyzed lactose solution is concentrated by evaporation using a plate heat evaporator.

4.2.4. Chromatographic separation of glucose and galactose

The concentrated solution is fractionated [redacted] by simulated moving bed chromatography using a cation exchange resin in the Ca^{2+} form [redacted]. Two fractions are collected, one which is rich in galactose and one which contains the un-reacted lactose and most of the glucose [redacted]. The lactose/glucose fraction is concentrated by evaporation to a syrup for potential use in food and animal feed.

For regeneration and cleaning of the ion exchange resin, a concentrated solution of calcium hydroxide or calcium chloride is passed through the column.

4.2.5. Isomerization

The galactose fraction is cooled on a plate heat exchanger and pumped into a stirred stainless steel reaction tank. A suspension of technical-grade calcium hydroxide [$Ca(OH)_2$] is added [redacted]. This suspension may be produced by dissolving dry $Ca(OH)_2$ in water or by reacting technical grade calcium oxide (CaO) with water. The addition of $Ca(OH)_2$ increases the

pH of the galactose solution [redacted].

After completion of the isomerization step, [redacted], the solution is transferred to column in which carbon dioxide is bubbled through the solution in order to [redacted], precipitate calcium carbonate and thereby release tagatose from the calcium complex.

4.2.6. Press filtration

The precipitated calcium carbonate is separated from the neutralized isomerization solution using a press filter. Calcium carbonate is washed and dried on the filter.

4.2.7. Demineralization

The D-tagatose containing filtrate is demineralized [redacted]. In this step, which turns the dark-colored filtrate into an almost colorless solution, charged reaction by-products are removed as well.

For regeneration of the ion exchange resins, concentrated solutions of hydrochloric acid and sodium hydroxide are used.

4.2.8. Evaporation

The filtered and demineralized isomerization solution is concentrated on a plate heat evaporator.

4.2.9. Purification by chromatographic fractionation and decoloration

The concentrated D-tagatose solution is fractionated by simulated moving bed chromatography using a cation exchanger [redacted]. Two fractions are collected, one which is rich in D-tagatose and one which contains un-reacted galactose, residual glucose, as well as other products formed during the isomerization process. [redacted].

The D-tagatose containing fraction is decolorized by passing the solution through a column containing granular activated carbon. The decolorized solution is then passed through a sterile filter with a pore size of 5 μ.

4.2.10. Evaporation

The fraction containing D-tagatose is concentrated on a plate heat evaporator.

4.2.11. Crystallization

D-Tagatose is crystallized from the syrup in a continuous crystalliser.

4.2.12. Centrifugation

D-Tagatose crystals are removed from the mother liquor on a continuous screen centrifuge. The crystals are washed with water through nozzles. The mother liquor is recycled to the chromatographic purification step [redacted] to minimize loss of D-tagatose.

4.2.13. Continuous fluid bed drying

The centrifuged and washed crystals are dried on a standard continuous fluid bed dryer. The obtained crystals have a dry matter content of ≥99.8%.

4.3. Safety of raw materials and chemicals used in the process

Food-grade lactose with a minimum purity of 99% is used as the starting material (Specifications of lactose, all chemicals used in the process, and the ion exchange resins are presented in Annex 1). [redacted].

For the enzymatic hydrolysis of lactose, an immobilized lactase from Aspergillus oryzae is used [redacted], the resin which is used as a carrier for the lactase complies with US food additive regulations (21 CFR 173.357) and is acceptable for use in food processes in the EU. [redacted].

Food-grade acetic acid, potassium sorbate, and sodium benzoate are used to treat the IML-resin in regular intervals. After treatment, the IML column is rinsed extensively with water.

For separating galactose and glucose, the hydrolyzed lactose solution is passed through a of cationic ion-exchange resin [redacted]. The resin complies with the pertinent US regulations [21 CFR 173.25 (ion-exchange resins)] and the Council of Europe Resolution AP(97)1. The resin is regenerated from time to time by treatment with technical-grade calcium hydroxide [$Ca(OH)_2$] or technical-grade calcium chloride ($CaCl_2$) followed by exhaustive rinsing with water.

Technical-grade calcium oxide (CaO) or dry calcium hydroxide [$Ca(OH)_2$] is mixed with water to produce a concentrated solution of calcium hydroxide which is used for isomerizing galactose to D-tagatose. D-Tagatose is liberated from its calcium complex by bubbling CO_2 (liquid carbon dioxide for food use) through the solution.

Cation and anion exchange resins [redacted] are used for demineralization of the filtered isomerization solution. These resins comply with pertinent US regulations (21 CFR 173.25 (ion-exchange resins) and the Council of Europe Resolution AP (97)1. The resins are regenerated with technical-grade hydrochloric acid (HCl, 30%) and sodium hydroxide (NaOH, 27.65% solution), respectively. After regeneration, the resins are rinsed exhaustively with demineralized water.

[redacted]

5. SPECIFICATIONS

5.1. Potential impurities

5.1.1. Impurities from raw material and process chemicals

The lactose which is used as the raw material, is >99% pure and thus contains less than 1% of other components like peptides and minerals originating from milk. All applied chemicals all of which are used as processing aids, have a high purity and are low in heavy metals (for specifications, see Annex 1).

5.1.2. Impurities from side-reactions

Some side reactions occur during the isomerization of D-galactose. The reactions are well known because they occur commonly when monosaccharides (like D-fructose and D-glucose) are exposed to alkali. Base-catalyzed cleavage of D-galactose results in the formation of D-glyceraldehyde which can react with D-galactose or D-tagatose to form C-9 aldol condensation products. Furthermore, D-galactose may undergo base-catalyzed epimerization to D-talose. The aldol condensation of two D-galactose molecules, or a D-galactose molecule and a D-tagatose molecule, gives the respective C-12 aldol condensation products. Beta-elimination reactions and various disproportionation reactions that lead to the formation of more oxidized products (e.g., dicarbonyls, keto-acids, and carboxylic acids) are not expected to occur un-

der the conditions of the process because the temperature is too low and pH is not sufficiently high.

5.1.3. Purification steps

The cationic or anionic impurities are removed in the demineralization step that follows the isomerization of D-galactose [redacted]. Any remaining inorganic salts would be detected in the test for total ash. Lead would be detected by the specific test for lead.

The non-ionic impurities (e.g., galactose, talose, aldol condensation products, extractives from IML resin and ion exchange resins) are removed during the chromatographic purification and decoloration of the demineralized isomerization solution [redacted] and during the crystallization of D-tagatose. Quantitatively, the most important carbohydrate by-product is D-galactose. Its content is determined in the HPLC analyses which forms the basis for the Assay. HPLC analyses of six batches of D-tagatose produced at pilot-plant scale demonstrate that impurities other than galactose do not occur at detectable levels (see Annex 4).

Particulate matter that may originate from the ion-exchange resin used for the separation of galactose and glucose, or from the isomerization step would be removed during the calcium carbonate precipitation and the following press filtration [redacted].

Microorganisms that could be carried into the process with the raw material (lactose) are unlikely to survive the production process since it involves pasteurization of the lactose solution (≥75°C, 15 sec), heat treatment [redacted]

[redacted], exposure to alkali (pH 13) [redacted], and sterile filtration after treatment with granular charcoal [redacted].

5.2. Specifications

Complete specifications as adopted by JECFA at its 55th meeting and amended at its 61st meeting are presented in Annex 3.

For the purpose of the safety assessment, the following purity criteria are relevant.

Assay:	Not less than 98% of D-tagatose[1]
Total ash:	Not more than 0.1%
Lead:	Not more than 1 ppm

JECFA did not deem it necessary to include purity criteria for other heavy metals or microbiological parameters in the specifications of D-tagatose.

Analytical data of six batches of D-tagatose produced in the factory at Nordstemmen (Germany) are presented in Annex 4.

[1] On an anhydrous basis. Quantitatively the most important impurity of D-tagatose is D-galactose.

6. INTENDED USES IN FOODS

6.1. Nutritional benefits

Being more expensive to produce than sucrose, glucose or fructose, D-tagatose is likely to be used not primarily for its sweetness[2] but for its advantageous nutritional properties.

6.1.1. Noncariogenicity

It is generally accepted that a distinction between non-cariogenic and potentially cariogenic foods and food ingredients can be made on basis of plaque-pH tests in which the acid formation by human plaque is measured during and for 30 minutes after consumption (De Paola, 1986). If the food can potentially deposit on the plaque surface, the pH measurement must be made at the inner plaque surface, i.e., with an indwelling electrode (Schachtele et al., 1986).

If non-acidogenic (and thus non-cariogenic) food is to be distinguished from acidogenic (and thus potentially cariogenic) food on basis of a plaque-pH test, it is necessary to define a "critical plaque-pH". For the in-vivo method with measurement of the plaque-pH by an indwelling electrode, a pH of 5.7 is considered to represent the critical level. This value has been derived from studies on the dissolution of tooth enamel by acids and it contains a safety margin [Bössmann, 1977; Imfeld, 1983 (at p. 4)]. A comparison of plaque-pH

[2] D-Tagatose was determined to be 92% as sweet as sucrose in a 10% (w/w) aqueous solution (Levin et al., 1995). At a concentration of 1%, it has a relative sweetness of about 75% that of sucrose.

tests and rat caries experiments on several sugars and sugar substitutes supports the adequacy of this threshold value [Imfeld, 1983 (at p. 85/86)].

In the US, the Food and Drug Administration (FDA) issued a regulation for the "does not promote tooth decay" claim in 1996, citing the plaque-pH test with the indwelling electrode as the litmus test to distinguish between non-cariogenic and potentially cariogenic foods.

This test was applied to D-tagatose in two studies. In the first study, six subjects wearing a partial prosthesis with a plaque-covered, indwelling electrode rinsed for two minutes with 15 ml of a 10% aqueous solution of D-tagatose. No decrease of the plaque pH was observed that could be attributed to a fermentation of D-tagatose (Imfeld, 1996). In a subsequent study, it was examined whether the plaque bacteria might acquire the ability to ferment D-tagatose upon repeated exposure to this substrate. For this purpose, the same six subjects rinsed five times per day (2 times 2 minutes per occasion) during a 3-7 day period, during which plaque accumulated on the indwelling electrode. The thus conditioned plaque was then exposed again to a test rinse with 10% D-tagatose solution. Again, a lowering of the plaque pH due to a fermentation of D-tagatose was not observed (Imfeld, 1998).

On the basis of these results, the US-FDA accepted that D-tagatose is non-cariogenic [Fed. Reg., 68 (128): 39831-39833; 21 CFR§101.80].

The non-cariogenicity of D-tagatose forms the basis for its use in chewing gum, soft and hard candies, table top sweeteners, (lactose-free) chocolate and certain beverages which do not contain other, fermentable ingredients.

6.1.2. Reduced energy value

Owing due to its incomplete absorption, D-tagatose has a reduced physiological energy value.

Estimation of the energy value of D-tagatose by the factorial method gave a range of 1.1 – 1.4 kcal/g (Bär, 1998). In this method, the energy contributed by each metabolic step is evaluated separately taking into account data from all pertinent experiments (in vitro, in vivo, in humans, in experimental animals). The factorial approach also takes into account losses of energy which are caused indirectly by the fermentation of D-tagatose [e.g., increased fecal excretion of biomass and non-bacterial mass (mucous secretions)].

The energy value of D-tagatose was also evaluated in two studies in rats and one study in pigs. A net metabolizable energy value of –0.12 kcal/g was obtained for D-tagatose in the first rat study (Livesey & Brown, 1996). This unexpectedly low value was attributed to an interference of D-tagatose with the absorption of sucrose which was present in the basal diet, and/or to the relatively low amount of fermentable fiber in the basal diet. The second rat study suggested an energy value of about 1.2 kcal/g (Saunders, 1992). The pig study gave an energy value of 1.4 kcal/g for D-tagatose (Jørgensen & Laerke, 1998).

While the energy balance study in rats may offer the most precise caloric value (Livesey & Brown, 1996), its accuracy may suffer from the low fermentable fiber content and sucrose supplement of the basal diet. Moreover, while the rat is an accepted model for human metabolism, the pig may be better suited for studying the digestibility of nutrients. The results of the factorial calculations are fully con-

sistent with the energy value of 1.4 kcal/g observed in the pig study. On these grounds, it appears justified to apply a rounded caloric value of 1.5 kcal/g (6 kJ/g) for D-tagatose.

On basis of these data, the US FDA issued a no objection letter for the use of a physiological energy value of 1.5 kcal/g for D-tagatose (FDA, 1999).

It is clear that in the EU an energy value of 4 kcal/g applies to all sugars and that the allocation of a different, lower value for D-tagatose would require an amendment of the Nutritional Labeling Directive (90/496/EEC) which is based on an assessment of the scientific date by EFSA. It is not expected, therefore, that the ACNFP speaks out on the caloric value of D-tagatose in its assessment of this Dossier. Nonetheless, the applicant considered it appropriate to inform the ACNFP about the benefit of a lower energy value of D-tagatose, because this may form the basis for some food applications of D-tagatose in future, such as table-top sweeteners.

6.1.3. Low glycemic impact

D-Tagatose and fructose are metabolized via the same biochemical pathway. However, ingested fructose is absorbed quantitatively, while D-tagatose is absorbed more slowly and incompletely (see Section 9.1.1).

The metabolism of D-tagatose and fructose yields D-glyceraldehyde and dihydroxyacetone phosphate from which glucose may be synthesized. However, the gluconeogenic potential of D-fructose is low as shown by

its low glycemic index (GI) of about 20 (relative to that of glucose = 100). Considering the slow and incomplete absorption of D-tagatose, its glycemic impact is expected to not exceed 5 (relative to that of glucose = 100). This was confirmed in a study in which 12 healthy people consumed 50 gram portions of tagatose or glucose, dissolved in 200 mL water, on different mornings whereupon changes in their blood glucose and insulin levels were measured over a two-hour period. Compared to glucose, which had glycaemic and insulinaemic responses of 100%, D-tagatose produced very low glycemic and insulinemic responses of only 3%. Since the 50 gram dose of tagatose was not completely digested and absorbed, these are relative glycemic responses for equal-weight portions of the two sugars, rather than true GI and II values (Sugirs, 2004).

In another human study in which the ingestion of 75 g D-tagatose was tested, a corresponding result was obtained (Donner et al., 1996, 1999). In this study it was also found that D-tagatose had an attenuating effect on the glycemic response of co-ingested glucose. This effect was further examined in twelve subjects with elevated fasting glucose levels (110-140 mg/dl). The glycemic and insulinemic response was measured for a 3 hour period after intake of glucose (75 g), D-tagatose (7.5 g) and a mixture of both. As expected, D-tagatose alone did not evoke a significant glycemic or insulinemic response. If administered together with glucose, D-tagatose lowered the glycemic index of glucose by about 20% (Madenokoji et al., 2003).

The very low glycemic impact of D-tagatose and its slight glucose-blunting effect may be the basis for its use in certain cereal or fruit bars and other foods designed for use in a low-glycemic diet.

6.1.4. Prebiotic activity

While D-tagatose is not fermented by bacteria of the dental plaque, not even after repeated exposure, certain members of the more varied microflora of the intestinal tract are able to utilize D-tagatose as a substrate. D-Tagatose fermentation appears to be particularly widespread among Lactobacilli, Enterococci and other lactic acid bacteria (Bertelsen et al., 2001).

Studies on the prebiotic activity of D-tagatose confirmed that D-tagatose promotes the growth of lactobacilli and leads to an increased production of butyrate which is considered to have a beneficial trophic effect on the colonic mucosa (Jensen & Buemann, 1998, Venema et al., 2004).

The prebiotic effect of D-tagatose may be the basis for its use in breakfast cereals, cereal bars, meal replacements and yoghurt.

6.2. Applications

D-tagatose may be used like other sugars (sucrose, glucose, fructose) as a sweet-tasting, nutritive substance. It differs from some of these sugars by some of its physiological benefits (non-cariogenic, prebiotic, low-glycemic, energy-reduced) but exerts the same secondary, technological functions (depending upon application, for example, as a bulking agent, humectant, texturizer and stabilizer). At lower concentrations, D-tagatose enhances, like other sugars, the flavor of certain foods and the "mouthfeel" of low-calorie beverages.

Considering its nutritional and sensoric benefits and its technological properties, D-tagatose is expected to be used in ready-to-eat cereals, health bars and diet soft candies, diet soft drinks and diet teas, coffee-based beverages and coffee drinks, low/non-fat ice cream and frozen yogurt, other flavored yoghurt, hard and soft candy, smoothies, icings, formula diets, dietary supplements, table top sweeteners and chewing gum. The use levels of D-tagatose in these applications and the functions that it fulfills, are presented in Table 1. The intakes of D-tagatose that result from each of these application per eating occasion are presented in Annex 6 (Tables 2-10). A complete discussion of the estimated daily intake (EDI) is presented in Section 7.

6.2.1. Ready-to-eat cereals

D-Tagatose may be used for the production of pre-sweetened (i.e., ready-to-eat) breakfast cereals.

From a nutritional perspective, D-tagatose has value as carbohydrate with a prebiotic effect. It is considered that an intake of about 3 g D-tagatose in combination with the dietary fiber that is naturally present in many breakfast cereals (about 4 g/100 g) and the lactose that is present in the milk (about 4.5 g/100 ml) suffices to obtain the intended prebiotic effect. The use level of D-tagatose in ready-to-eat cereals is therefore limited to 3 g/serving. In terms of concentration this amount corresponds to a D-tagatose content of 20% in cereals weighing <20 g per cup, 10% in those weighing between 20 and <43 g per cup, 10% in high fiber cereals containing ≥28% fiber, and 5.45% in cereals weighing ≥43 g per cup and biscuit types. (Serving

sizes of ready-to-eat cereals are based on their density as defined in 21 CFR 101.12).

From a technological view point, D-tagatose offers some advantages over sucrose and high-fructose syrups in the frosting of breakfast cereals, such as a quick crystallization on the cereal flake and a low hygroscopicity on storage.

At an intake of about 4.6 g D-tagatose with ready-to-eat cereals per eating occasion for the average consumer (or about 7.6 g for the 90th percentile consumer, the so-called "heavy consumer") D-tagatose is known to be well-tolerated (see section 9.3.1). Even children would be expected to tolerate D-tagatose at the projected levels of intake per eating occasion [3.2 (5.6) and 4.6 (7.4) for the average (90th percentile) 2-5 and 6-12 year old child, respectively] without intestinal symptoms (except perhaps some increased flatulence) as indicated by a tolerance study of xylitol, another incompletely absorbed carbohydrate (polyol), in 7-16 year old children and teenagers (Akerblom et al., 1981).

6.2.2. Carbonated and non-carbonated diet soft drinks, diet teas and coffee drinks

In carbonated and non-carbonated diet soft drinks, diet teas and coffee drinks, which are sweetened with intense sweeteners, the addition of small amounts of D-tagatose offers some technological benefits. First, D-tagatose exhibits a pronounced sweetness synergy with aspartame. The addition of 0.2% D-tagatose permits beverage manufacturers to lower the aspartame concentration in cola-type beverages by 40 ppm. At the envisaged maximum level of use (1%), D-tagatose could re-

place 110 ppm aspartame. Second, D-tagatose accelerates the sweetness onset. Third, it improves the "mouthfeel" and flavor profile (by reducing bitter notes) of carbonated and non-carbonated diet soft drinks, diet teas and coffee drinks.

At the maximum use level of 1%, the intake of D-tagatose with carbonated diet soft drinks and diet teas would remain well within a range that is tolerated without gastrointestinal symptoms (about 4 and 7 g per eating occasion for the mean and 90th percentile user, respectively).

6.2.3. Low-fat/non-fat ice cream, frozen yogurt

D-Tagatose may be used in frozen dairy-type products (ice cream, yogurt) at a level of up to 3% for its prebiotic and flavor enhancing effect. The addition of higher levels of D-tagatose is not feasible as it would unfavorably affect the freezing process.

6.2.4. Diet and health bars, diet soft candy

D-Tagatose may be used as a bulk sweetener with prebiotic activity for the production of low-fat or reduced-fat diet and health bars, bars serving as a meal replacement, energy bars, nutrient fortified bars, breakfast bars, and diet soft candies. At the (maximum) use level of 10%, products belonging to this category provide an average intake of D-tagatose per eating occasion of about 4.1 - 6.5 g which in combination with other dietary fiber typically present in this type of products is considered sufficient for achieving the desired prebiotic effect.

D-Tagatose is also useful for formulating chocolate-type coatings of diet and health bars, and diet soft candy because it is almost equi-sweet with sucrose, has a low water content, and contributes positively to the chocolate flavor and taste.

6.2.5. Icings

D-Tagatose may be used at concentrations of up to 30% in icings (non-fat glaze) as used typically on products such as cookies, pastries, brownies, certain cakes, etc. It also will facilitate the formulation of "lite" products and/or products which are positioned for use in a diabetic diet. The low-hygroscopicity of D-tagatose will improve the stability of the icing.

6.2.6. Hard candy and chewing gum

D-Tagatose is not fermented by the dental plaque to a relevant extent (Imfeld, 1996, 1998). Therefore, it may be used as a sweetener in toothfriendly hard candy and chewing gum.

Since D-tagatose has a smaller "cooling effect" than the monosaccharide polyols (xylitol, sorbitol, mannitol) but has a higher sweetness than the disaccharide polyols (isomalt, lactitol, maltitol) it is particularly suited for use in fruit- or cinnamon-flavored chewing gum. The maximum use level of D-tagatose in chewing gum is 60%.

For the production of stable hard candies, D-tagatose must be mixed with compounds having a high glass-transition-temperature such as

certain polyols or polydextrose. The use level of D-tagatose in hard candies is limited to about 10-15% because at higher levels D-tagatose will produce an undesirable, slightly "burning" aftertaste like other monosaccharides (e.g., fructose).

The projected intake of D-tagatose from its application in hard candies and chewing gum is not expected to produce significant gastrointestinal side-effects based on experience from the current use of polyols which are used already as bulk sweeteners in confectionery at similar or higher levels. In this regard it is important to note that the use of D-tagatose will not increase the total intake of incompletely absorbed carbohydrates from chewing gum and hard candies but rather will substitute for some of it.

6.2.7. Formula diets for meal replacement

Formula diets for meal replacement ("diets for weight control or weight reduction") typically contain sucrose or fructose as a source of carbohydrate (about 15-20 g per serving). From a nutritional perspective, it would appear useful to partially replace these carbohydrates by D-tagatose because of its prebiotic and low-glycemic properties.

If fermentable dietary fiber is absent from the formulation, about 5 g D-tagatose is required to achieve a prebiotic effect. This would correspond to the replacement of about 25-30% of sucrose or fructose by D-tagatose. If fermentable fiber is present, slightly lower additions of D-tagatose may suffice for achieving the intended prebiotic effect.

In protein-based drinks, the addition of 1% D-tagatose will reduce the bitterness which is associated with certain sources of protein and protein hydrolysates.

6.2.8. Baked goods

D-Tagatose can be used at levels of up to 2% in certain types of baked goods to increase moisture, improve moisture retention and enhance the flavour of, for example, short breads and muffins.

6.2.9. Soft chewy candies

D-Tagatose contributes prebiotic properties to soft candies. Also, D-tagatose supports the flavor development and facilitates production by reducing the viscosity of the product mass.

6.2.10. Table top sweeteners

In combination with intense sweeteners D-tagatose enhances the sweetness (synergistic effect), improves the flavor profile by reducing bitterness, and shortens the sweetness onset-time. D-Tagatose is a preferred carbohydrate in mixtures with intense sweeteners because it provides only few calories and, unlike lactose or maltodextrin which are usually used as carriers for intense sweeteners, is tooth-friendly.

6.2.11. Yogurt

D-Tagatose (3-5 g per serving) may be added to yogurts for its prebiotic properties. D-Tagatose is not fermented during the fermentation process. Its ability to improve the flavour profile of high intensity sweeteners in diet fruit-type yogurts requires the addition of only about 0.2 – 1%.

6.2.12. Food supplements

D-Tagatose can be used in the formulation of food supplements as it has a reduced caloric content, is safe for teeth, has a prebiotic effect and has no glycemic impact.

6.3. Determination of D-tagatose in foods

D-Tagatose can be quantitated efficiently by HPLC with a RI (refractive index) detection unit.

For extraction of D-tagatose from the food matrix, and for separation of the bulk of other components (solids, fat-soluble components), standard techniques may be used.

A detailed description of the HPLC method along with validation data (analysis of D-tagatose in breakfast cereals, soft drink, chocolate and ice cream) are presented in Annex 5.

7. ESTIMATED DAILY INTAKE

The estimated daily intake (EDI) of D-tagatose from its different projected uses in food (as specified in Table 1), excluding chewing gum and food supplements, has been calculated for the US population by ENVIRON (Arlington, VA) using the dietary survey approach (Annex 6). This calculation model relies on food consumption data from the 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (94-96,98 CSFII). In 94-96 CSFII data were collected from a representative sample of individuals residing in households in the US. CSF98 was a supplemental survey of children (0-9 year old). Each individual was surveyed for two non-consecutive days using 24-hour recall interviews. The foods consumed were coded according to a system which contains about 6,000 different categories.

For the purpose of the present EDI calculation it was assumed that each food (or food component) which may contain D-tagatose, indeed contained D-tagatose at the highest, feasible concentration (as specified in Table 1). Where D-tagatose was used in a component of the food (e.g., in the icing), the intake of that component was calculated from data on food composition.

The EDI of D-tagatose averaged over the two observation days and expressed in g/kg bw/d was calculated for each food category in which D-tagatose may be used, and for all these food categories combined, except for chewing gum and food supplements for which intakes had to be estimated separately because the intake of these products was not recorded in CSFII (Table 2). Mean and 90^{th} percentile intakes were calculated for users of the following age groups: 2 years and older; preschoolers 2-5 years of age; elementary school

children 6-12 years of age; teenagers 13-19 years of age; and the adult U.S. population 20+ years of age. "Users" were defined as individuals who consumed food in the concerned category on at least one occasion. Since food intake was recorded by time of day and by eating occasion (breakfast, brunch, lunch, dinner, supper, snack, and extended eating occasion), D-tagatose intake could also be calculated per eating occasion of each observation day (Table 3).

The detailed results of these calculations are presented in Annex 6. For the entire population, estimated exposure to D-tagatose from all proposed uses, excluding chewing gum and food supplements, is 0.08 and 0.19 g/kg bw/day at the mean and 90th percentile, respectively. This corresponds to intakes of about 4.6 and 9.8 g/person/d for the 2-day average of the mean and 90th percentile consumer, respectively. The subpopulation with the highest estimated exposure to D-tagatose from all proposed uses, excluding chewing gum and food supplements, is preschoolers (0.19 and 0.37 g/kg bw/d for the 2-day average of the mean and 90th percentile user) (Table 2 and Annex 6).

A comparison between the intakes from the various food categories and the total intake from all sources demonstrates that many uses are mutually exclusive. In other words, a consumer of ready-to-eat cereals is unlikely to eat a diet and health bar or frozen yogurt at the same eating occasion. Consequently, the D-tagatose intake from all sources is much smaller than the arithmetic sum of intakes from the different food categories (Annex 6).

The data on D-tagatose intake per eating occasion demonstrate that the consumption of D-tagatose is evenly distributed over the day (Table 3). At no occasion levels are reached which would be expected to produce gastrointestinal symptoms (see Section 9.3.1).

The estimation of the D-tagatose intake from chewing gum of the sugarfree type is based on a separate survey in which 1044 households reported their one-day intake of regular and sugarfree gum by mail. The survey, which was conducted in 1995, reveals that the average number of pieces of (sugarless) gum consumed per day varies between 1.6 for preschoolers and 3.0 for teenagers (2.5 for the total population) [Annex 6 (Table 23)]. With the weight per piece of gum varying between 2-3 g and D-tagatose representing not more than 30% of the gum, this corresponds to a D-tagatose intake of about 1.8 – 2.1 g/d or 0.03 - 0.035 g/kg bw/d. [Slightly higher values are shown in Annex 6 (Table 23) because a standard serving size of 3 g was applied].

The intake of D-tagatose with food supplements, mainly in the form of tablets, is unlikely to exceed 3 g/person/d. Since D-tagatose cannot be directly compressed to tablets, it is likely to be used only in mixture with other ingredients such as lactose or sorbitol.

8. REGULATORY STATUS AND EARLIER SAFETY ASSESSMENTS

8.1. United States

In the United States, a GRAS Notice was submitted to the Food and Drug Administration (FDA) on May 11, 2001. The GRAS Notice was supported by the report of a panel of independent experts which concluded that D-tagatose under the intended conditions of use, resulting in an estimated intake of 6.6 g/person/day at the mean and 14.9 g/person/day at the 90th percentile, presents no risk to human health and is "generally recognized as safe" ("GRAS") based on scientific procedures. On October 25, 2001, the FDA informed Arla that the agency has no questions regarding Arla's conclusion that D-tagatose is GRAS under the intended conditions of use (FDA, 2001).

Already prior to the review of the safety data, the FDA examined a request to permit a physiological energy value of 1.5 kcal/g for D-tagatose. On October 25, 1999, the FDA issued a no objection letter for the use of 1.5 kcal/g in calculating the caloric value of D-tagatose (FDA, 1999).

On January 9, 2002, Arla petitioned the FDA to amend 21 CFR 101.80 to authorize a non-cariogenicity dental claim for D-tagatose. Based on the available evidence, the FDA concluded that there was enough scientific agreement to indicate that D-tagatose does not promote dental caries. The interim rule, published in Fed. Reg. Vol. 67, Number 231, p. 71461-71470, was adopted by the FDA in July 2003 without change.

8.2. Korea

In 2003, the Korean Food & Drug Administration (KFDA) evaluated Arla's petition to authorize the use of D-tagatose in foods. On July 23, 2003, the KDFA issued a letter according to which the use of D-tagatose is allowed in the processing and manufacturing of food products.

8.3. Australia / New Zealand

An application to permit the use of D-tagatose as a novel food ingedient was submitted to Food Standards Australia/New Zealand (FSANZ) in July 2002. FSANZ issued a favorable Final Assessment Report in February 2004.[3]

On this basis, D-tagatose was then added to Standard 1.5.1 (Novel Food) for unrestricted use in foods. In addition, D-tagatose was introduced in Standard 1.2.8 (Nutrition Information Requirements) with an energy value of 11 kJ/g (Gazette Food Standards, No. FSC 12, 29 April 2004).

8.4. JECFA

D-Tagatose was evaluated by JECFA at its 55th, 57th, 61st and 63rd meeting. An ADI "not specified" was allocated at the 63rd meeting (June 2004) (WHO, 2001, 2002, 2004a,b). The uses and the resulting EDI which were considered by the Committee, correspond to those presented in Sections 6 and 7 of this Dossier.

[3] http://www.foodstandards.gov.au/_srcfiles/A472_D_tagatose_FAR.pdf.

9. BIOLOGICAL DATA

9.1. Biochemical aspects

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 so-called facilitated diffusion of fructose through the intestinal mucosa (Crouzoulon, 1978; Sigrist-Nelson & Hopfer, 1974; Tatibouët et al., 2000).

9.1.1. Absorption, distribution and excretion

9.1.1.1 Rats

The absorption, distribution and excretion of D-tagatose was examined in rats using [U–^{14}C]-labeled D-tagatose (Saunders et al., 1999b). Upon intravenous administration of the radio-labeled D-tagatose at a dose of 620 mg/kg bw, about 36.6% of ^{14}C was recovered in respiratory CO_2, 42.6% in urine, and 4.8% in feces. Additional small amounts were detected in the intestinal contents (0.5%), selected tissues (1.2%) and the remaining carcass (6.2%) (rats were killed 48 hours after dosing). The disposition of orally administered ^{14}C-D-tagatose was studied in germfree and conventional rats that had not been treated with D-tagatose before, and in conventional rats who had been adapted to D-tagatose by receiving a diet with 10% of this sugar for 28 days before dosing. In the germfree rats (n=2), 21.8% of the ^{14}C was detected in the respiratory CO_2, 3.8% in the urine, and 6.2% in tissues

and the carcass (animals were killed 24 hours after dosing). Together these percentages account for 31.8 of the dose representing the absorbed fraction. 63.4% of the administered dose was found in the intestinal contents and feces, representing the unabsorbed fraction. The conventional, not adapted rats (n=3) excreted 49.4% of the administered ^{14}C with respiratory CO_2, 5.8% with the urine and 28.7% with feces. 8.8% remained in tissues and the carcass (animals were killed 72 hrs after dosing). The conventional, adapted rats (n=4) exhibited a somewhat higher ^{14}C expiration (67.9%) than the not adapted rats, a similar urinary excretion (5.2%), a lower fecal excretion (11.4%), and a similar retention in tissues and the carcass (6.2%).

While the number of animals in this study was small, the obtained values give a consistent picture of the disposition of D-tagatose, which closely resembles that of polyols. The findings in the germfree rats demonstrate that the intestinal absorption of ingested D-tagatose is incomplete. In the germfree rats about 30% of the administered D-tagatose was absorbed. In a study of D-psicose, a stereoisomer of D-tagatose which has identical diffusion properties but is not metabolized in the mammalian organism, an intestinal absorption rate of 25% was observed in conventional rats (Whistler et al., 1974). The seemingly slightly higher absorption of D-tagatose was explained by a possible absorption of D-tagatose also in the more distal gut segments of the germfree rats. The appearance of ^{14}C in the respiratory CO_2 of intravenously dosed rats and orally dosed germfree rats demonstrates that the absorbed D-tagatose is readily metabolized (via the glycolytic pathway as shown below). From the expiration of about 14% of the radiolabel within 6 hours after dosing in germfree rats, it may be estimated that about 20-23% were absorbed (typically about 60-70% of C atoms of absorbed and metabolized carbohydrates are expired as CO_2 within a few hours). The higher excretion of $^{14}CO_2$ in

adapted rats compared to not adapted, conventional rats is the result of a faster and more complete fermentation of unabsorbed D-tagatose by the intestinal microbiota. This notion is supported by an examination of the time course of respiratory $^{14}CO_2$ appearance. In adapted rats, $^{14}CO_2$ appears at a fast rate, reaching a maximum at about 3 hours after dosing, while in not adapted rats the $^{14}CO_2$ expiration follows a more protracted time course and does not reach as high levels as in the adapted rats.

9.1.1.2 Pigs

The absorption of D-tagatose from the small intestine was measured in pigs adapted and un-adapted to a diet containing D-tagatose. The formation of short-chain fatty acids (SCFAs) from D-tagatose by the intestinal microflora of the adapted pigs was examined as well (Jensen & Laue, 1998). Seventeen male castrated pigs were surgically fitted with cannulas in the portal vein, a mesenteric vein, and a mesenteric artery. Animals were fed a diet with 20% sucrose for 7 days, at which time blood samples were taken. On day 10, the pigs were switched to a diet with 20% D-tagatose and blood samples were taken again. After 7 days on the D-tagatose diet, blood samples were taken once more (day 17). The diets were administered twice daily. On the examination days, p-aminohippuric acid (PAH) was infused continuously in the mesenteric vein to determine portal vein blood and plasma flow. Blood samples were collected simultaneously from the mesenteric artery, the mesenteric vein, and the portal vein before the first feeding in the morning, every 30 minutes for 4 hours after feeding, and every 60 minutes thereafter to 12 hours. The samples were analyzed for D-tagatose, SCFAs (i.e., acetate, propionate, butyrate, etc.), PAH, and

hematocrit. Urine was collected during the 12 hour experimental period and analyzed for D-tagatose.

In adapted and not adapted pigs, the D-tagatose concentration increased in the portal vein blood shortly after feed intake reaching a maximum after 90-150 minutes. The D-tagatose concentration in the arterial blood reached a level of nearly 75% of that of the portal vein blood, indicating a low clearance of D-tagatose on first passage through the liver. D-Tagatose levels decreased after 150 minutes and returned to baseline levels after about 10 hours. Both adapted and not adapted animals had the highest absorption of D-tagatose 1-3 hours after feeding. Adapted animals absorbed 32 to 85 grams of D-tagatose per kilogram of feed, equal to 16% to 43% of ingested D-tagatose (mean: 27.6%). Not adapted animals absorbed 34 to 68 grams of D-tagatose per kilogram of feed, equal to 17% to 36% of ingested D-tagatose (mean 26.3%). The urinary excretion of D-tagatose ranged between 3% and 7% of ingested D-tagatose in adapted and not adapted animals. About 18% (range 11% to 36%) of the absorbed D-tagatose was excreted in urine, with no significant difference between not adapted and adapted pigs.

The analyses of the blood samples for SCFAs showed that there were no changes in portal or arterial concentrations after intake of the sucrose diet. However, intake of D-tagatose produced changes in blood SCFA concentrations. Particularly pronounced was the increase of butyric acid concentrations which reached five times higher levels after intake of the tagatose diet (for additional data on large intestinal fermentation, see section 9.1.2.2).

In another study with pigs, the disappearance of ingested D-tagatose from the small intestine was calculated from analyses of the intesti-

nal contents for D-tagatose and a not absorbed marker (chromium oxide). The data indicated that about 26% of ingested D-tagatose had disappeared from the digesta (by absorption and some minor bacterial fermentation) by the time they had reached the distal third of the small intestine (Laerke & Jensen, 1999).

9.1.1.3 Humans

In humans, the absorption of D-tagatose cannot be measured directly. However, human data on the absorption of L-rhamnose provide a good basis for an estimate. L-Rhamnose (6-deoxy-D-mannose) 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 by its molecular volume and lipophilicity (Hamilton et al., 1987), the intestinal absorption of L-rhamnose is expected to be somewhat 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). Consequently, 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%.

This value is not in line with the result of a study in which the absorption of D-tagatose was examined in ileostomic patients. In this study, only about 20% of ingested D-tagatose (15 g) was recovered from the 24-hour ileal effluent (Normén et al., 2001). This result was interpreted by the authors as indicative of an 80% intestinal absorption. However, similarly high absorption rates were suggested earlier also for sorbitol, maltitol and isomalt from studies in ileostomates although there is strong evidence that these polyols are poorly digested and absorbed (Langkilde, 1994; Bär, 1990). Inconsistent results also were reported from a study on the absorption of L-rhamnose in ileostomic patients. After intake of 1 g L-rhamnose, 17.6% of the ingested dose was recovered in the 24-hour urine, suggesting an absorption of about 25%. On the other hand, 51.1% of the ingested dose was detected in the total ileal effluent suggesting an absorption of nearly 50% (Jenkins et al., 1994). Different factors have been invoked for explaining such excessive absorption rates in ileostomic patients, such as fermentation of the test compounds by the small-intestinal microflora (e.g., by filaments which are firmly attached to the mucosa), incomplete analytical recovery of the test compound from the ileal effluent, and altered permeability of the intestinal mucosa. Although a definitive explanation is not yet available, it appears that the ileostomate model has a limited value for determining absorption rates of malabsorbed monosaccharides and polyols.

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 resulted in the same mild intestinal side-effects (e.g., flatulence) as a single dose of 20 g lactitol, a not absorbed disaccharide sugar alcohol (Lee &

Storey, 1999). An increased expiration of H_2, an indicator of colonic fermentation of malabsorbed carbohydrates, was observed after ingestion of 29 g D-tagatose (Buemann et al., 1998).

9.1.2. Biotransformation

9.1.2.1 Metabolism in the mammalian organism

The metabolism of absorbed D-tagatose proceeds along well established biochemical pathways.

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. Fructokinase has a higher affinity to 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. After ingestion of 30 g D-tagatose the liver concentration of D-tagatose-1-phosphate did not exceed 1 mM in healthy human volunteers (Buemann et al., 2000a).

While tagatose 1,6-diphosphate also serves as a substrate for aldolase (Lardy, 1951; Totten, 1949), no enzyme has been described that could phosphorylate D-tagatose-1-phosphate to D-tagatose-1,6-diphosphate in the animal organism. Since there is no evidence for an enzyme (hexokinase) that could phosphorylate D-tagatose to D-tagatose-6-phosphate (Michalcakova et al., 1986), the phosphorylation by phosphofructokinase of D-tagatose-6-phosphate to D-tagatose-1,6-diphosphate has no practical implications either (Fishbein et al., 1974; Koerner et al., 1976).

It follows from these observations that D-tagatose and D-tagatose-1-phosphate are the only new molecular species to which the animal body is exposed upon ingestion of this sugar. After the initial phosphorylation step, the metabolism of D-tagatose converges with that of fructose. 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 was found only in

these organs (Mayes, 1993; Malaisse et al., 1989; van den Berghe, 1986).

The formation of D-tagatose-1-phosphate was observed directly and non-invasively by magnetic resonance spectroscopy (MRS) of the human liver (Buemann et al., 2000a). 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 concentration of ATP decreased transiently by about 11%.

9.1.2.2 Fermentation in the large intestine

The unabsorbed portion of ingested D-tagatose is fermented nearly completely in the large intestine by intestinal microorganisms to SCFAs which then are absorbed almost completely. No D-tagatose was found in the feces of pigs after ingestion of this carbohydrate (Laerke & Jensen, 1999; Jorgensen & Laerke, 1998). In adapted rats, about 2% of an oral dose of ^{14}C-D-tagatose was recovered unchanged from the feces (Saunders et al., 1999b).

The in vitro fermentation of D-tagatose by intestinal microbiota of pigs and humans yielded acetate, propionate and butyrate as the main SCFA end-products. Formate, caproate, valerate and lactate were also detected. Isobutyrate, isovalerate and heptanoate were only produced in very small amounts, if at all. Carbon dioxide, methane and hydrogen were the gaseous products of D-tagatose fermentation (Laerke et al., 2000; Jensen & Buemann, 1998). A comparison of the SCFAs produced by the human fecal microflora from D-

tagatose and sucrose reveals no pertinent differences (i.e., the profile of SCFAs produced from these two substrates is qualitatively and qualitatively similar) (Jensen & Buemann, 1998). In this regard, it also made no biologically meaningful difference whether or not the subjects were adapted to tagatose (30 g/d) for a period of 14 days prior to feces collection (Jensen & Buemann, 1998). However, the adaptation to D-tagatose appeared to lead to a faster fermentation of D-tagatose and somewhat higher formation of butyrate in humans and pigs (Jensen & Buemann, 1998; Larke & Jensen, 1999b).

Since the formed SCFAs are absorbed quite rapidly and completely in vivo, only small concentrations can be detected in the digesta or in the feces of humans and pigs (Jensen & Buemann, 1998; Jørgensen & Laerke, 1998; Laerke & Jensen, 1999).

In an investigation of the capability of 34 different human intestinal bacteria to utilize D-tagatose as the sole carbon source under anaerobic conditions, it was found that only 5 strains could degrade D-tagatose. None of the 11 strains of pathogenic enteric bacteria had this ability. D-Tagatose fermenters included Clostridium innocuum, Enterococcus faecalis and Lactobacillus sp. A subsequent examination of bacteria which are used in the dairy industry confirmed that D-tagatose fermentation is widespread among Lactobacilli, Enterococci and other lactic acid bacteria (e.g., Lactococcus, Pediococcus, Leuconostoc) (Bertelsen et al., 2001). This observation is in line with the result of a human study in which the daily ingestion of D-tagatose (10 g three times daily for 13 days) was found to increase the density of lactobacilli and lactic acid bacteria in the feces (Jensen & Buemann, 1998). Since the density of Bifidobacteria, which are unable to fermentate D-tagatose, re-

mained unchanged, this increase is likely to be the result of the metabolic advantage that D-tagatose fermenting bacteria had under the conditions of the study, and not the unspecific consequence of a lower fecal pH.

9.2. Toxicological studies

9.2.1. Acute toxicity

A single dose of D-tagatose (10 g/kg bw) was administered by gavage to 10 rats (5 per sex) and 5 male mice. One rat died immediately post-dosing due to a dosing accident (confirmed at necropsy). Otherwise no mortalities occurred and there were no reactions or behavioral changes to the treatment. Body weights increased during the 3-14 day observation period after dosing (Trimmer, 1989).

9.2.2. Short-term studies of toxicity

9.2.2.1 Sprague-Dawley rats

Groups of 20 approximately 6-week old Sprague Dawley rats of each sex were fed diets containing D-tagatose at concentrations of 0, 5, 10, 15 or 20%. An additional control group received a diet with 10% fructose and 10% cellulose ("isocaloric control group"). The individually housed animals were examined daily for clinical signs of toxicity; body weights and food consumption were recorded in weekly intervals. An ophthalmoscopic examination was performed on all animals prior to start of the treatment and during the final week. Standard hematological and clinical chemical parameters were measured in blood sam-

ples collected at termination. Urinary parameters were not examined. Rectal temperature was measured prior to killing. Animals were killed after at least 90 days of treatment (animals were fasted over night prior to killing). All animals were subjected to gross necropsy. The absolute and relative weights of main organs were determined. Organs and tissues were preserved for histopathological examination. The microscopic examination was done on all tissues and organs of all animals of the 0 and 20% D-tagatose groups, the isocaloric control group, two animals of other groups that died spontaneously during the study, and those organs and tissues that exhibited changes at gross necropsy. In addition, the livers of six randomly selected animals/sex/group from all groups except the isocaloric control group were sectioned and stained for glycogen with periodic acid-Schiff reagent (PAS). However, only sections of the control and high dose animals were examined microscopically for glycogen because there were no differences of liver glycogen between these two groups.

With the exception of soft stools which most animals of the 15 and 20% dose group exhibited from day 1-3, there were no clinical signs or reactions that could be attributed to the treatment. Three animals died spontaneously for undetermined causes during the study (one rat of each the 10, 15 and 20% dose group). All groups gained weight during the study but mean body weights were slightly yet significantly below control levels in the 15 and 20% dose groups on some occasions. At the end of the treatment period, body weights of the 20% dose group were about 10% below those of the control group (0% dose group). The difference compared to the isocaloric control group was smaller although the animals of this group consumed more food to compensate for the lower caloric density of their diet.

The hematological examination revealed slight yet statistically significant reductions of hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin in males and females of the 15 and 20% dose groups. Minor changes of these parameters in other dose groups or of other hematological parameters occurred in one sex only. Differential white blood cell counts did not differ between treated and control animals. With the possible exception of serum alanine aminotransferase, which was slightly reduced, and serum cholesterol, which was increased in the 15 and 20% dose groups (as compared to the 0% control group), there were no dose-related changes that occurred in either sex. Total serum protein, albumin, and triglycerides were increased in males of the 15 and 20% dose groups but not even a trend for such changes was detected among females. Rectal temperatures were within the normal range and did not reveal biologically meaningful differences between dose groups. On ophthalmoscopic examination, all animals of the 20% dose group were found to be free of abnormalities.

The absolute and relative weight of livers were increased in males and females of the 10, 15 and 20% dose groups. At the 5% dietary level corresponding to a dose of 3.7 and 4.1 g/kg bw/d for male and female rats, respectively, absolute and relative liver weights did not differ significantly from those of the control groups. A number of other, statistically significant, yet in absolute terms smaller, changes of relative organ weights were detected. The increases of relative brain and testes weights were not confirmed by corresponding changes of absolute organ weights and are likely to be a consequence of the lower body weight in the 15 and 20% dose groups. The same applies most probably for the increased relative (but not absolute) heart, spleen and kidney weights.

The observation of increased relative weights of the brain, testes, kidneys, spleen, and heart (males) in rats fed a diet with 20% lactitol or 25% lactose for 13 weeks supports the notion that the observed organ weight changes (except for the liver) are of an unspecific nature (Sinkeldam et al., 1992). Since the feeding of high doses of D-tagatose (and other low digestible carbohydrates) is associated not only with a slight decrease of body weights, but also with an increase of the lean-to-fat body mass ratio (Lina & de Bie, 2000d; Saunders, 1992; Livesey & Brown, 1996; Grenby, 1985), it is conceivable that changes of relative organ weights would not be seen (or, in the case of the liver, would become somewhat smaller) if the lean body mass (rather than the total body weight) was applied as normalizing factor. The decrease of relative weights of the epididymal fat pads is another consequence of a smaller fat deposition in response to the treatment.

On gross necropsy, a "thickened" liver was noted in 4 and 5 males of the 15 and 20% dose group, respectively. All other observations occurred in a few animals only and did not exhibit any association with the treatment. Histopathological examination of organs and tissues did not reveal any changes that could be attributed to treatment except for the occurrence of hepatocellular hypertrophy in the 15% dose group (4/20 males and 4/20 females) and the 20% dose group (7/20 males and 11/20 females). This effect was characterized microscopically by a prominence of centrilobular areas of the liver lobules due to a minimal increased size (hypertrophy) of centrilobular hepatocytes. The affected hepatocytes had a more dense eosinophilic cytoplasm. Microscopic examination of the PAS-stained liver sections showed variability in the amount and distribution of hepatocellular glycogen that was, however, not influenced by the treatment. There was no discernible difference in the levels of glycogen that remained

in the livers after the overnight fasting period prior to killing (Trimmer et al., 1993; Kruger et al., 1999c).

9.2.2.2 Wistar rats

In order to gain insight into the time-course and longer term consequences of D-tagatose induced liver enlargement, groups of 60 male Wistar rats received diets containing 0, 5 and 10% D-tagatose, 20% fructose, and 10% D-tagatose + 10% fructose for a period of up to 6 months. At the start of the study, the rats were about 10 weeks old. Ten rats of each group were killed on day 3, 7, 14, 28, 94 and 182 of the study after an overnight fasting period.

The group-housed animals were examined daily for clinical signs of toxicity; body weights were recorded in weekly intervals and at necropsy. Food consumption was recorded weekly during the first 13 weeks and then for a one-week period every month. A number clinical-chemical parameters were analyzed in plasma which was collected from all rats at necropsy. These parameters included liver enzymes (ALP, ASAT, ALAT, GGT), lipids (cholesterol, triglycerides, phospholipids), bilirubin, albumin, globulin, total protein, and inorganic phosphate. After killing, the rats were subjected to gross necropsy. The livers were removed and weighed. A representative part of the liver was used for standard histopathological examination (H&E stained sections). An other part of the liver was used for measurement of nuclear density and determination of the BrdU labeling index (50 mg BrdU/rat was injected at 6 pm of the day prior to killing).

The remaining part of the liver was weighed, lyophilized and powdered. Aliquots of the liver powder were analyzed for total protein,

total lipids, glycogen, free glucose, and DNA. Moisture content was not determined. For determination of carcass composition, the content of the intestines were removed. The carcasses (without liver but with the empty intestines) were weighed, lyophilized, weighed again, and analyzed for ash, total protein, and total lipids.

There were no clinical signs or reactions that could be attributed to the treatments. One animal of the 10% D-tagatose group died spontaneously in week 20 for an undetermined cause. There were no noticeable differences of food intake between the groups except during the first week of the study during which animals of the 10% D-tagatose and 10% D-tagatose + 10% fructose group consumed a significantly smaller amount then the controls. The body weights were also slightly decreased in these two groups throughout the entire study. The analyzed plasma parameters did not reveal changes that could be attributed to the treatments. Absolute and relative liver weights did not differ between treated groups and controls at any time. The microscopic examination of livers did not reveal any treatment-related histopathological changes. The BrdU labeling index and nuclear density of liver tissue was not affected by the treatment.

The protein, lipid, glycogen, glucose and DNA contents of the (fasted!) livers (calculated in g/g wet liver) did not exhibit consistent changes that would be suggestive of a treatment-related effect. A significantly lower carcass weight was noted in the 10% D-tagatose and 20% fructose group. The fat content was significantly decreased in the 5 and 10% D-tagatose groups and in the 10% D-tagatose + 10% fructose group. It was concluded that the administration of D-tagatose at a dietary level of 10% (corresponding to an average dose of 5.8 g/kg bw/d for the first four weeks of the study and 4.8 g/kg bw/d for the entire 6-month period) was not associated with

any adverse effects on liver weight, liver function (as shown by clinical-chemical parameters), and liver morphology of Wistar rats (Lina & de Bie, 2000d).

9.2.2.3 Pigs

In the context of an energy balance study in pigs, four groups of two pigs each received for a period of 33 days either a regular pig diet (group 1), the diet with the addition of 20% sucrose (group 2), 20% D-tagatose (group 3), or a mixture of 10% sucrose and 10% D-tagatose. In group 3 (20% D-tagatose) the daily intake of D-tagatose was about 5 g/kg bw during the 33-day feeding period. Samples of liver tissue were collected at termination (about 6 h after the last meal) and were processed for electron microscopic examination. A few necrotic cells were seen in the control group. No ultrastructural changes were observed in the groups receiving D-tagatose (Mann, 1997).

9.2.3. Long-term toxicity/carcinogenicity tests

A 24-month chronic toxicity and carcinogenicity study was conducted in Wistar rats (HsdCpb:WU strain). Six groups of 50 rats/sex each received diets with 0, 2.5, 5 and 10% D-tagatose, 20% fructose, and 10% D-tagatose + 10% fructose. The additions of D-tagatose and fructose were made at the expense of pregelatinized potato starch. Body weights and food consumption were measured in regular intervals. Ophthalmoscopic examinations were conducted at start of the study and after 12 and 24 months of treatment. Hematological and clinical-chemical parameters were analyzed after 6 and 12 months and at termination of the study. All animals found dead or killed in extremis

during the study or killed at termination were subjected to detailed macroscopic examination. The determination of organ weights was limited to the liver which was the main target organ for this study, as well as the adrenals, kidneys, testes and cecum which are known to exhibit weight changes in relation to the feeding of low-digestible carbohydrates. The histopathological examination was, at first, limited to the liver. However, at the request of JECFA, it was subsequently extended to the kidneys, adrenals and testes.

The mortality rate was generally low and did not differ between the treatment groups. There were no treatment-related differences in behaviour and clinical signs. The body weights of the 10% D-tagatose group (with or without 10% fructose) were reduced in male and female rats. In the 5% tagatose group, a slightly lower body weight was observed in males on most occasions and in females during the first 10 months of the study. The food intake was slightly lower in the 5 and 10% tagatose groups and the 20% fructose group during the first four weeks of the study.

The ophthalmoscopic examinations and the clinical-chemical analyses did not reveal any treatment related effects. The hemoglobin concentration and hematocrit were decreased in females but not males of the 10% D-tagatose groups (with and without fructose). Other hematological changes or noteworthy changes of clinical-chemical parameters in response to the D-tagatose treatment were not observed.

The macroscopic examination at necropsy revealed enlarged adrenals in males of the 10% D-tagatose groups (with and without 10% fructose) and in females of the 2.5 and 10% D-tagatose group and 20% fructose group. This observation was paralleled by an increase of relative ad-

renal weights in the 5 and 10% D-tagatose groups, 10% D-tagatose + 10 % fructose group, and females of the 2.5% D-tagatose group.

The relative weight of the liver was increased in females of the 10% D-tagatose and 20% fructose groups and in both sexes of the 10% D-tagatose + 10% fructose group. The relative kidney weights were increased in females of all treated groups except the 2.5% D-tagatose group. The relative adrenal weights were increased in all tagatose groups except in males of the 2.5% dose group. The relative weight of the cecum (full and empty) was increased in males and females of the 10% D-tagatose groups (with and without fructose) and in males of the 5% D-tagatose group.

The microscopic examination of the liver did not reveal any histopathological changes that could be related to the administration of D-tagatose.

The incidence of nephrocalcinosis was significantly increased in males of all D-tagatose treatment groups and in females of the 10% D-tagatose groups (with and without 10% fructose). The incidence of transitional cell hyperplasia was increased in females of the 10% D-tagatose groups (with and without 10% fructose). This hyperplasia was ascribed to an irritating effect of the mineral depositions.

The incidence of adrenomedullary proliferative lesions (medullary hyperplasia and/or pheochromocytomas) was significantly increased in males and females of the 5 and 10% D-tagatose groups and the 10% D-tagatose + 10% fructose group, and in females of the 2.5% D-tagatose group.

It was concluded that the administration of D-tagatose has no adverse effect on the livers of Wistar rats even at high levels of exposure (4 g/kg bw/d) for a life-time (24 months) (Lina & Kuper, 2002). The histopathological examination of the kidneys and adrenals of rats revealed only changes that had been observed earlier in studies with other low-digestible carbohydrates. These changes are generally considered to not have relevance for human safety (Lina & Bär, 2003) (see Sections 10.2 and 10.3 for a more detailed discussion of the renal and adrenal effects).

9.2.4. Genotoxicity

The results of assays for genotoxicity are summarized in Table 4.

9.2.5. Developmental toxicity

In a range-finding embryotoxicity/teratogenicity study, groups of 5 presumed pregnant Sprague-Dawley rats received D-tagatose from day 6-15 of gestation by gavage at dose levels of 0, 4000, 8000, 12000, 16000 and 20000 mg/kg bw. The daily dose was given in two equal portions which were administered during the light period with an approximately 4-h interval in between. Animals were examined daily; body weights and food consumption were recorded in regular intervals. All animals were killed on day 20 of gestation. The dams were subjected to necropsy and parameters of reproductive performance were measured. The livers of all dams were weighed. The fetuses were examined for signs of toxicity and external malformations. No mortality occurred during the study. Mean body weights and weight gains were similar in all groups. Stool softening and diarrhoea due to the ad-

ministration of the test substance were observed in the dose groups receiving 12000 mg/kg bw/d or more. No adverse effects on reproductive performance, fetal weight, and external malformations were noted. The absolute and relative maternal liver weights did not differ between treated groups and controls. It was concluded that the highest dose level tested was suitable for inclusion in a full-scale embryotoxicity/teratogenicity study (Schroeder, 1994a).

In a study of embryotoxicity and teratogenicity, groups of 24 presumed pregnant Sprague-Dawley rats were given D-tagatose at doses of 0, 4000, 12000 and 20000 mg/kg bw by oral intubation of an aqueous solution on days 6-15 of gestation. The daily dose was given in two equal portions separated by approximately a 4-h interval. The animals were examined throughout the study, and body weights and food consumption were recorded. The rats were killed on day 20 and examined for parameters of reproductive performance. Complete macroscopic post-mortem examinations were performed on all dams. The livers of control and high-dose animals were weighed and examined microscopically. Fetuses were examined for signs of toxicity, external malformations, and soft-tissue defects, and were stained for detection of skeletal anomalies. Livers of one pup/sex/litter were weighed.

No deaths occurred during the study. During the treatment period, nearly all animals of the mid- and high-dose group had unformed or watery stools (osmotic diarrhoea). Maternal body-weight gain was similar in all groups except for a slight reduction in the high-dose group on days 6-9 of gestation. Necropsy of the dams showed no adverse effects that could be related to treatment. The mean relative liver weights were increased in the mid- and high-dose groups; the absolute liver weight was increased in the high-dose group. However, morphological changes were not seen on microscopic examination of the

livers. The number of viable litters, the number of corpora lutea, and the mean number of implantation sites were similar in all groups. Fetal length and body weight were also similar in all groups. Examination of the fetuses revealed no treatment-related increase in gross, skeletal, or visceral abnormalities. The weight of fetal livers did not differ between treated groups and controls. Under the conditions of this assay, D-tagatose was not embryotoxic or teratogenic and had no adverse effect on reproductive performance (Schroeder, 1994b; Kruger et al., 1999b).

9.2.6. Special studies

9.2.6.1. Mechanistic studies on liver enlargement

Six studies were conducted in rats in order to determine the characteristics and potentially underlying mechanism of D-tagatose induced liver enlargement.

In the first study, four groups of approximately 12-week old 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). The treatment lasted for 28 days. Ten rats of each of group A and B were killed after 14 days of treatment (interim kill). Ten rats of each of groups A-D were killed on day 28 (end of treatment period). The remaining animals of groups A and B continued the study on Purina diet until day 42 when they were killed (i.e., 2-week recovery period for group B; no recovery period was included for group D). Food remained available to all rats until they were killed (i.e., there was no fasting period prior to killing as in the 90-day study described in section 9.2.2.1). Body weights, and weights of wet and lyophilized

livers were determined. The lyophilized livers collected on day 28 from groups A and B were analyzed for protein, total lipid, glycogen, DNA, and residual moisture.

By day 14, relative wet liver weights had increased by 23% in group B. On day 28, the increase was 38% in group B (as compared to group A) and 44% in group D (as compared to group C). At the end of the recovery period, the increase had diminished to 14% in group B. Body weights were slightly lower in the D-tagatose treated groups. On day 28, liver glycogen content (expressed in % of liver weight) was significantly increased, and liver protein, lipid, and DNA contents were significantly decreased in group B (as compared to group A). Total amounts of protein, lipid, glycogen, and DNA (expressed in mg/liver) were significantly increased in group B (as compared to group A) by 26, 11, 94 and 23%, respectively.

This study demonstrated that the dietary administration of D-tagatose at a high level (20%) produced significant liver enlargement. This effect was reversible on cessation of the treatment. While in the 90-day toxicity study with Sprague-Dawley rats, referred to in section 9.2.2.1, no evidence of an increased glycogen storage was found because the animals were killed in fasted condition, in this study, in which the animals were killed in non-fasted condition, liver glycogen was increased under the influence of D-tagatose. However, the higher glycogen levels account for only part of the increase of liver weights. The increase of DNA and protein (per liver) demonstrates that there also was growth of liver tissue. Accordingly, liver weights were still somewhat higher in group B (as compared to group A) after the recovery period. For a complete regression of the additional liver tissue the 2-week recovery period may not have been long enough (Lina & de Bie, 1998a; Bär et al., 1999).

The second study was conducted in order to examine the dose-response relationship of D-tagatose induced liver enlargement in non-fasted rats, and in order to determine whether peroxisome proliferation was involved. For this purpose, four groups of 20 rats each received SDS diet with 0, 5, 10, and 20% D-tagatose for 29-31 days. The food was available until the time of sacrifice. At termination, plasma was obtained from 10 rats/group for clinical-chemical analyses. Five rats/group were subjected to whole-body perfusion, followed by processing of livers for qualitative and quantitative electron microscopic examination. Livers of 6 rats/group were analyzed for acyl-CoA oxidase and laurate 12-hydroxylase (cytochrome P450 4A1) activity, DNA synthesis (Ki-67 index), and number of nuclei per unit area of tissue.

Liver weights were significantly increased in linear relation to the D-tagatose intake. Under the conditions of the present study (non-fasted rats) liver weights were significantly increased at the 5% dose level. Plasma transaminases (but not gamma-glutamyl transferase and alkaline phosphatase) were increased in the 20% dose group. Except for increased glycogen accumulation in livers of the D-tagatose treated rats, no ultrastructural changes were seen on electron microscopic examination of livers of the control and 20% dose group. (In the absence of changes the livers of the lower dose groups were not examined). Morphometric analysis confirmed the increase of glycogen and the absence of alterations of endoplasmatic reticulum, mitochondria, and Golgi apparatus. The Ki-67 index did not differ between the groups. A dose-related decrease of the number of nuclei per unit area signified some hepatocellular hypertrophy. Acyl-CoA oxidase and CYP4A1 activity were significantly increased in the 10 and 20% dose groups, but these increases were small in comparison to the changes

that are typically seen with peroxisome proliferators. Moreover, electron-microscopic examination did not provide any evidence of peroxisome proliferation. The increased transaminase levels (ALAT, ASAT) were interpreted as a consequence of the increased glycogen storage (Chatila & West, 1996). It was concluded from this study that a dose-related increase of glycogen deposition is the main feature of the D-tagatose induced liver enlargement. There was no evidence for a treatment related stimulation of peroxisome proliferation. Hepatocellular hypertrophy and compensatory growth of liver tissue appear to be consequences of the increased glycogen storage. Degenerative ultrastructural changes were not seen (Lina et al., 1998; Bär et al., 1999).

The third study was conducted in order to determine whether the 5% dietary dose level was a no-effect level with regard to liver growth. For this purpose, groups of approximately 12-week old Sprague-Dawley rats received SDS diet (group A and C) or SDS diet with 5% D-tagatose (groups B and D) (groups A and B: 15 rats/group; groups C and D: 10 rats/group). All animals were killed on day 28. Groups A and B were fasted for 24 h before sacrifice; groups C and D had food available until sacrifice. Liver weights and liver composition were measured as in Study 1.

Relative wet and dry liver weights were increased in response to the treatment in rats killed under the fed condition, but not in rats killed under the fasted condition. The livers of the D-tagatose treated rats (group D) had an increased glycogen content in comparison to the respective controls (group C). In the livers of groups A and B there were no detectable levels of glycogen.

It was concluded that the 5% dose level is a no-effect level with regard to liver growth. This conclusion is in keeping with the result of the 90-day toxicity study in which there also was no increase of liver weights in rats of the 5% dose group (killed under fasted condition). In the 90-day study, the D-tagatose intake was 3.6 and 4.0 g/kg bw/d in males and females, respectively. In the present study, the D-tagatose intake was 2.6 – 2.8 g/kg bw/d (Lina & de Bie, 1998b; Bär et al., 1999).

Considering that only a fraction of ingested D-tagatose is absorbed and that the absorbed D-tagatose is channeled directly into the glycolytic pathway, it appeared unlikely that D-tagatose itself would act as a precursor of liver glycogen. Rather, it was hypothesized that D-tagatose-1-phosphate would promote the translocation of glucokinase from the nucleus in the cytoplasm and that the subsequently increased formation of glucose-6-phosphate would activate glycogen synthase which then would produce glycogen at an increased rate using glucose (from dietary sources) as a precursor (Figure 4). It was further hypothesized that an increase of glycogen deposition (above a certain threshold value) would stimulate (compensatory) liver growth (for details, see section 10.1).

This hypothesis was tested in the fourth study in which Wistar rats received D-tagatose together with or separate from a dietary source of glucose (Polycose™ was used as readily digestible glucose polymer). For the purpose of this study, four groups of 20 male Wistar rats each received semisynthetic powdered diets with either Polycose (6 g/d) (group A), D-tagatose (2.64 g/d) (group B), or Polycose + D-tagatose (6 + 2.64 g/d, resp.) (group C) during the dark period (feeding period). During the light period, these groups received by gavage (administered in two portions) a liquid diet consisting of ei-

ther peptone (2 g/d), peptone + polycose (2 + 6 g/d, resp.), or peptone (2 g/d). The amount of solid diet offered during the dark period was restricted to 18 g. The "placebo" compound in the solid diet of D-tagatose was Sunfiber (a fermentable dietary fiber), the "placebo" of polycose was cellulose. Otherwise, the diets contained equal amounts of fat, fish-meal, and essential nutrients. After 7 days of treatment, 10 rats/group were killed in the morning of the 8th day in the non-fasted condition, and 10 rats/group at the end of light period, i.e., after having received the liquid diet by gavage. Food intake was recorded daily (the animals were housed individually). Body weights were determined at start of the study (day 0), on the day of termination (day 7), and after killing. After gross necropsy, the liver was removed, weighed, frozen in liquid nitrogen, lyophilized, powdered, and analyzed for glycogen and free glucose.

There were no treatment-related clinical signs and all animals survived until the end of the study. However, all rats lost weight during the treatment period probably as a consequence of the reduced (restricted) food supply. At the end of the feeding period, the absolute and relative liver weights were decreased in group B (D-tagatose in solid diet) and increased in group C (D-tagatose + Polycose in solid diet) as compared to the control animals of group A (Polycose in solid diet). At the same time, liver glycogen was significantly below control levels in group B and significantly above control levels in group C. In the animals killed at the end of light period (i.e., after gavage), relative (but not absolute) liver weights were significantly higher in groups B and C. Liver glycogen was very low in groups A and C, and was increased (as a result of the Polycose administration by gavage) in group B.

These results demonstrate that D-tagatose leads to an increased liver glycogen deposition only if it is co-ingested with a source of glucose (Lina & de Bie, 2000a).

The fifth study pursued a similar purpose and thus had a similar design as the fourth study. However, Sprague-Dawley rather than Wistar rats were used (18 animals/group). Killing was performed after 5 days of treatment at three different times during the feeding cycle, namely near the end of the dark (feeding) period when liver glycogen was expected to have reached its zenith, during the light period before gavage, and during the light period after gavage (6 rats/group/killing occasion). In addition, the cellulose content of the diet was reduced. The same parameters were determined as in the fourth study.

No mortalities occurred during the study but body weights decreased slightly in all treatment groups. With regard to liver glycogen deposition, the results of this study confirm and extend the findings of the previous experiment. In addition, a comparison of animals of group A (controls) and group C (D-tagatose + Polycose) killed after gavage (of peptone), i.e., at a time when liver glycogen level was equally low in these two groups, revealed significantly higher absolute and relative liver weights in group C. In group B, liver weight was not increased at that time despite the presence of glycogen that was produced in response to the Polycose ingestion by gavage. These data support the hypothesis that an increased glycogen deposition, not D-tagatose per se, induces liver growth (Lina & de Bie, 2000b).

The sixth study was performed to further investigate the mode of tagatose ingestion (alone or in combination with Polycose) on liver weight. A similar protocol was applied as in the fifth study except

that the treatment period was extended to 26 days. Furthermore some changes were made in the dietary regime [casein was added to the solid diet; water (groups A and C) or a solution of Polycose (group B)] was given by gavage. The chemical analyses of the liver also included total protein, total lipid, DNA and residual moisture. In an attempt to kill the animals in an identical feeding conditions, all groups received control diet (diet of group A) ad libitum on day 26 and were deprived of food from 5 pm of day 27 until termination of the experiment in the morning of day 28.

There were no treatment-related differences in general condition or behavior between the groups. During the first 10 days of the study, the rats of all groups lost some weight. Therefore, the daily amount of Polycose was raised at that time from 6 to 6.4 g/d, and the amount of solid diet from 18 g to 19.2 g in groups A and C, and from 12 g to 12.8 g in group B. No further weight loss occurred after this change but body weights remained slightly lower in group B than in groups A and C (day 21). The D-tagatose intake of groups B and C was about 7 g/kg bw/d. In response to the ad libitum feeding of diet A on day 27, body weights raised in all groups by about 10% and returned again to previous levels after overnight fasting.

Relative liver weights were increased in groups B and C and absolute liver weights in group C (group A served as control). The total DNA content (mg DNA/liver) was significantly increased only in group C; it was similar in groups A and B. There were no significant differences in the liver concentrations of protein, glycogen, glucose, or moisture (g/g/liver). The liver lipid concentration was lower in group C as compared to groups A and B, and the liver DNA concentration was lower in group B (Lina & de Bie, 2000c).

The data on liver DNA (DNA/liver) appears to support the working hypothesis according to which D-tagatose induces liver growth if administered at sufficiently high level (in this study about 7 g/kg bw/d) together with a dietary source of glucose. On the other hand, the data on liver weights are not entirely consistent with this concept in that contrary to expectations relative liver weights were also increased in group B receiving D-tagatose and Polycose at separate occasions. A potentially confounding factor in this sixth study with unknown consequences for liver weight was the excessive feed intake on day 27 followed by overnight fasting.

The liver DNA content would be expected to be less sensitive to such short-term changes of food intake than the liver weight. Furthermore, it should be noted that in those studies in which the co-ingestion of D-tagatose ($\geq$6 g/kg bw/d) and dietary glucose precursors resulted in liver growth (Kruger et al., 1999c; Lina & de Bie, 1998a) the rats gained body weight, i.e., were in an anabolic condition, while under the dietary regimes of the 4th, 5th and 6th study the animals did not gain weight or in some cases lost weight.

9.2.6.2 Comparative study of D-tagatose induced liver enlargement in six strains of rats

As described in more detail in Section 9.2.2, D-tagatose administered at the 10% dietary level produced an increased liver weight in Sprague Dawley rats but not in Wistar rats (animals were fasted overnight prior to killing) (Kruger et al., 1999c; Lina & de Bie, 2000d). In order to test whether rat strains may differ with regard to their sensity for D-tagatose induced liver enlargement, a comparative feeding study was conducted with male rats of six different strains, viz.

Lewis, Fischer, Brown Norway, Lister Hooded, Sprague Dawley and Wistar rats. Groups of 15 males of each strain were fed either a control diet or the same diet to which 20% D-tagatose had been added at the expense of pregelatinized potato starch for a period of 28 days. At the end of the treatment, 10 rats were killed after overnight fasting and 5 rats without fasting. Body weights and liver weights were determined. Among the fasted rats, increased relative liver weights were observed in the tagatose group of all strains. Sprague Dawley rats exhibited the biggest and Wistar rats the smallest increase (20 and 6% increase, respectively). In the not fasted rats, the increase of relative liver weights due to the tagatose treatment was bigger than in fasted rats. Again, Wistar rats exhibited the smallest increase which did not attain statistical significance at the low number of animals tested (Appel, 2002).

9.2.6.3 Hemolysis

The susceptibility of dog erythrocytes to D-tagatose, glucose (negative control), and L-sorbose (positive control) was examined in vitro using concentrations of 0, 0.6, 6 and 60 mM of these sugars in the incubation medium. In line with earlier observations, L-sorbose induced significant hemolysis during the 24-hour incubation period at the mid and high-dose concentration. D-Tagatose did not have any adverse effect. It rather appeared to stabilize the cells (i.e. hemolysis was below control levels) (Bär & Leeman, 1999).

In this regard it is noteworthy that D-tagatose also had a protective effect on cultured murine hepatocytes (Zeid et al., 1997; Valeri et al., 1997; Paterna et al., 1998). The mechanism(s) responsible for this protective effect are not yet elucidated.

9.3. Observations in humans

9.3.1. Intestinal tolerance

Incompletely absorbed sugars (e.g., lactose) and polyols (e.g., sorbitol) can induce gastric discomfort and laxative effects if consumed in excessive amounts. These effects are not of a toxic nature. Furthermore, because of the short latency time, the affected individual would usually associate the side-effect with the type of food ingested. Yet, these gastrointestinal side-effects should be taken into account when considering appropriate levels of use of such substances.

In a screening test, 73 male volunteers received a single dose of 30g D-tagatose with a cake in the afternoon. Eleven subjects reported nausea. Mild, moderate, and strong diarrhea was reported by 11, 10 and 2 individuals, respectively (Buemann et al., 1999a).

In the context of a metabolic study, D-tagatose and sucrose were consumed daily for 15 days (1 dose of 30 g per day) (Buemann et al., 1998). Symptoms were recorded on the first and last day of each treatment period. Since the participants (3 males, 5 females) were preselected for good tolerance to D-tagatose (one 30-g dose had to be tolerated without nausea and diarrhoea), symptoms occurred only slightly more frequently or with higher scores after D-tagatose intake (Buemann et al., 1999a).

The intestinal tolerance to single doses of 29 g D-tagatose or 29 g sucrose administered with breakfast was examined in 23 women and 11 men in a double-blind cross-over study. Gastrointestinal symptoms

(scored on a scale from 0 - 4) and the number of stools were self-reported on structured questionnaires for the test days and the two subsequent days thereafter. 64% of the participants reported a higher score for one or several of the gastrointestinal symptoms. The difference of scores between the D-tagatose and sucrose (control) treatment was statistically significant for rumbling in the stomach and gut, distention, nausea, flatulence, and diarrhoea. The number of stools was higher after intake of D-tagatose. Six subjects reported diarrhoea, 3 of them describing it as "watery stool" and 3 as "loose stool". Except for distention, increased scores of the symptoms were reported only on the day of treatment but not on the two following observation days. Except for nausea which was scored as "mild" in most cases, the reported symptoms are those which typically occur after the consumption of low-digestible sugars and polyols. The nausea, which was reported by females only (mean body weight of females and males were 63.6 and 82.0 kg, respectively), was ascribed to the osmotic effect of unabsorbed D-tagatose in the small intestine (Buemann et al., 1999c).

In a subsequent study, the same investigators administered single doses of 29 g sucrose or 29 g D-tagatose with a continental breakfast to 20 male medical students who in a pre-test tolerated a single dose of 30 g D-tagatose without noticeable symptoms. One subject dropped out for an apparently treatment-unrelated reason (headache). No noteworthy gastrointestinal symptoms were reported except for two cases of moderate nausea and one case of strong flatulence after D-tagatose ingestion (Buemann et al., 2000b).

The tolerance of single 20-g doses of D-tagatose, sucrose and lactitol was studied in 50 healthy young male and female adults. The test substances were given with chocolate (40 g given in two equal por-

tions between 1 pm and 4 pm). The gastrointestinal side-effects were self-reported 24 hours after consumption of the chocolates. Consumption of D-tagatose in comparison to sucrose was associated with significantly more subjects experiencing nausea, bloating, borborygmi, colic, and flatulence. Thirst and loss of appetite were also reported more often. The number of stools and their consistency was not influenced by the treatment. Compared to the lactitol chocolate, the D-tagatose chocolate did not produce any of the symptoms more frequently, except for thirst (probably due to the higher osmotic effect in the small intestine) (Lee & Storey, 1999).

As part of a study on the effects of D-tagatose on glucose tolerance, 8 healthy and 8 type-2 diabetic subjects received increasing doses of D-tagatose with each meal. On day 1, subjects received 5 g with each meal. Dosage was then increased by 5 g/meal on each of the subsequent days. Gastrointestinal symptoms were recorded daily. The first symptoms appeared at an intake of 10-25 g/meal. No differences were noted between healthy and diabetic subjects (Donner et al., 1999).

Eight healthy and eight diabetic volunteers received 75 g D-tagatose daily for 8 weeks (daily 3 portions of 25 g each consumed together with main meals). Flatulence was experienced by 7 healthy and 7 diabetic subjects; diarrhoea of some degree was reported by 6 healthy and 6 diabetic subjects. No improvement was noticed for these intestinal side-effects over the 8-week treatment period (Saunders et al., 1999a).

Twelve healthy male volunteers received 45 g D-tagatose or sucrose daily for 28 days in a study in which effects of these treatments on liver volume and liver glycogen were examined. The test compound (D-tagatose) and placebo (sucrose) were given in three daily portions of

15 g each together with the three main meals. A double-blind two-way crossover design was applied. Mild to moderate laxative effects occurred occasionally during the D-tagatose period in 7 subjects. Flatulence and/or bloating occurred more often and to a higher degree during the tagatose treatment (Boesch et al., 2001).

9.3.2. Metabolic effects

9.3.2.1 Glycemia and insulinemia

Experiments with normal rats showed that D-tagatose administered p.o. at a dose of 1 g/kg bw does not elicit a glycemic or insulinemic response (Zehner et al., 1994). In healthy human volunteers, the ingestion of 50 g D-tagatose produced a very low glycemic and insulinemic response which was about 3% of that seen after ingestion of 50 g glucose (Sugirs, 2004).

In a feeding study with 5 lean and 5 diabetic, obese rats it was found that urinary glucose and polydipsia were reduced in the diabetic animals when they were fed a diet with 10% D-tagatose (Szepesi et al., 1996). These results suggested that D-tagatose might be useful for the formulation of foods with a low glycemic index (foods for people with diabetes, prediabetic conditions and increased risk of CHD). Subsequently, the suitability of D-tagatose for this purpose was evaluated in humans in a short-term and a long-term (12-month) clinical study (Donner et al., 1996, 1999; Makris, 1999).

Eight healthy and 8 type-2 diabetic subjects received single doses of 75 g glucose, 75 g D-tagatose, and 75 g D-tagatose followed (af-

ter 30 min) by 75 g glucose. The ingestion of D-tagatose did not result in an increase of blood glucose or insulin levels. When D-tagatose was administered 30 minutes prior to the glucose dose, the glycemic and insulinemic response was smaller than after administration of glucose alone. This effect of D-tagatose was seen in the healthy and diabetic subjects. When the experiment was repeated with lower doses of D-tagatose (10-30 g), it was found that the glycemia attenuating effect decreased with decreasing dose of D-tagatose. A 20-g dose still had a significant, though small effect (reduction of blood glucose AUC (0-3 h) by 20%) (Donner et al. 1996, 1999). In the absence of a comparison treatment with another incompletely absorbed carbohydrate (e.g., with sorbitol) it cannot be determined whether the "glucose blunting effect" on glycemia is specific for D-tagatose or whether it is an unspecific consequence of the osmotic effect (the influx of liquid in the intestine could accelerate small intestinal transit and reduce glucose absorption). It is also not known whether the "glucose bunting effect" would be seen with starch or when lower doses of glucose were administered.

In a 8-week cross-over tolerance study, 8 healthy and 8 type-2 diabetic volunteers received daily doses of 75 g D-tagatose or sucrose (25 g with each of the main meals). Plasma was collected at the start and end of each treatment period (subjects were fasted overnight). Liver enzymes bilirubin, uric acids, insulin, glucose, $Hb1A_c$, lipids and a number of other clinical-chemical parameters were determined. No significant changes were observed in the healthy and diabetic subjects that could be attributed to the D-tagatose treatment (Saunders et al., 1999a).

A 12-month clinical study was conducted in 4 male and 4 female type-2 diabetics. The subjects ingested 45 g D-tagatose per day (15

g with each of the 3 main meals). Standard clinical-chemical parameters as well blood pressure and body weights were determined in serum samples collected 2 months prior to start of the treatment, on day 0 of the study, and in 2-month intervals thereafter. During the first two weeks of treatment, increased flatulence was reported by 5 subjects. One female reported transient diarrhea during the first few days. However, none of the participants rated the side-effects as unacceptable and there were no drop-outs. Applying appropriate statistical analysis (ANOVA for repeated measures followed by Levene's test) it was found that body weights and glycosylated hemoglobin ($Hb1A_c$) decreased significantly over time. For the other analyzed parameters, including liver enzymes (AP, ALAT, ASAT), lipids (triglycerides, cholesterol), uric acid, bilirubin, fasting glucose and insulin, urea, minerals (Ca, Na, K, Mg, P_i, Cl), and blood pressure, there were no changes over time that would point to a treatment related effect (Makris, 1999). The decrease of body weights and $Hb1A_c$ started after 4 months of treatment only. In the absence of an untreated control group it cannot be decided whether these two changes are direct consequences of the D-tagatose treatment.

9.3.2.2. Serum uric acid levels and urinary uric acid excretion

The effect of single intakes of solutions with 30 g D-tagatose or 30 g fructose, or of pure water (control) on plasma uric acid levels was examined in 8 male fasted volunteers. A controlled lunch was given to the subjects 255 min after dosing. Samples of arterial blood were collected immediately prior to dosing and in regular intervals during a 7-hour period thereafter. The samples were analyzed for D-tagatose, uric acid, inorganic phosphate, glucose, lac-

tate, insulin, glucagon and other hormones [glucagon-like peptide (GLP-1), cholecystokinin (CCK), gastric inhibitory peptide (GIP), adrenaline, and noradrenaline].

Absorbed D-tagatose appeared in the arterial blood shortly after dosing, reaching a maximum concentration in serum of about 0.17 mMol/l after 50 minutes. Serum uric acid increased from about 360 to 430 µmol/l (6.1 to 7.2 mg/100 ml) during the same time (upper limit of normal range is given with <7.6 mg/100 ml for the method applied). The uric acid levels decreased thereafter but remained above the fasting level throughout the pre-lunch period. Inorganic phosphate levels were significantly decreased at 50 minutes after D-tagatose ingestion. The serum lactate levels were increased only slightly and an effect on energy expenditure was not seen. The excretion of urinary uric acid during the pre-lunch period was significantly higher after ingestion of D-tagatose than plain water.

With fructose, a smaller increase of plasma uric acid was seen than with D-tagatose. Serum phosphate levels remained unchanged. The marked increase of plasma lactate and energy expenditure after the ingestion of fructose (but not D-tagatose) reflects the fast degradation of this sugar. Serum glucose, insulin and glucagon levels were not significantly affected by the ingestion of fructose and D-tagatose. None of the other analyzed parameters exhibited noteworthy changes in response to the ingestion of D-tagatose or fructose (Buemann et al., 1999 b).

In order to examine whether D-tagatose would increase plasma uric acid levels also if it is ingested together with starch as a source of energy, six healthy, overnight fasted male volunteers were given a breakfast consisting of 200 g bread (proving 100 g starch), 30 g

D-tagatose and water. Plasma glucose, uric acid and phosphate concentrations were measured one hour before breakfast, at start of the breakfast, and in regular intervals for a 4-hour period thereafter. Plasma glucose and uric acid concentrations increased after intake of the test breakfast while phosphate concentrations decreased. At the zenith, plasma uric acid concentrations were on average 0.8 mg/dl above baseline values (range 0.7 – 1.2 mg/dl). However, uric acid concentrations remained within the normal range (<7 mg/dl) at all times. The maximum increase (about 16% above baseline) is similar to that observed in the studies by Buemann. This indicates that the co-ingestion of starch does not attenuate the uricemic effect of a high single dose of D-tagatose (Diamantis & Bär, 2001).

In another human study, plasma uric levels were measured in 8 healthy and 8 diabetic subjects after administration of single doses of 75 g D-tagatose or glucose. Plasma uric acid increased after ingestion of D-tagatose but not after ingestion of glucose. The highest levels were reached after about 60 minutes. Three hours after dosing, plasma uric acid concentrations had declined but without reaching pretreatment levels in any subject. For the applied uric acid test, a normal range of 3.5-8.5 mg/100 ml is given. The upper level of this range was transiently exceeded in three diabetic subjects, two of whom had plasma concentrations at or above this level already before dosing, and in all four healthy male volunteers of whom one had a value above the normal range before dosing. On average, plasma uric acid increased during the one-hour period after dosing by about 1.5 mg/100 ml (range –0.6 to 2.9 mg/100 ml) (Saunders et al., 1999a).

In a study on potential effects of D-tagatose on liver volume and glycogen deposition, 12 healthy male volunteers consumed a standard-

ized breakfast with 15 g D-tagatose (test substance) or 15 g sucrose (placebo). The breakfast provided 99 g starch. Plasma uric acid levels were determined before intake of the breakfast and in hourly intervals for a period of 7 hours thereafter. Plasma uric acid levels increased slightly after intake of the breakfast but there was no significant difference between the plasma uric acid profile after intake of D-tagatose and sucrose (Boesch et al., 2001). Since the co-ingestion of starch does not blunt the uricemic effect of a high single dose of D-tagatose (30 g), the result of this studies demonstrates that the 15-g dose of D-tagatose is a no-effect level with regard to a uricemic effect in healthy subjects.

In order to test whether a different (lower) no-effect level would apply under conditions of pre-existing hyperuricemia, 12 hyperuricemic male volunteers who had experienced at least one attack of gout, received a test breakfast of 200 g bread, 15 g D-tagatose and water after overnight fasting. Plasma glucose, uric acid, phosphate, creatinine and lactate were measured 1 hour before treatment, at start of the breakfast (0 h), and in regular intervals thereafter for a period of 4 hours. Urine was collected during this 4-hour period and a separate 24-hour period on the subsequent day. Urinary uric acid and creatinine were analyzed.

Plasma glucose and uric acid exhibited significant changes over the 5-hour observation period. Plasma uric acid increased steadily from -1 h to 0 h and from 0 h to 1 h by about 2.5% in each period. Thereafter, uric acid levels decreased again. At 3 to 4 h after intake of the breakfast, plasma uric acid levels were no longer different from baseline values. The observed transient increase of plasma uric acid levels is obviously very small and lacks clinical relevance. In addition, the time-profile suggests that the postprandial increase of

plasma uric acid represents probably the continuation of a pre-existing (diurnal?) trend rather than a treatment related effect. The urinary excretion of uric acid was not different between the 4-hour postprandial period and a full 24-hour cycle. The authors concluded that a single oral dose of 15 g D-tagatose represents a no-effect level in terms the uricemic potential of this substance also in hyperuricemic and gouty subjects (Diamantis & Bär, 2002).

9.3.3. Effect on liver size

A study was conducted in healthy male volunteers in order to determine whether the partial substitution of dietary sucrose by D-tagatose for 28 days would affect the volume of the human liver. In addition, it was examined whether the ingestion of D-tagatose together with a starch containing meal would increase postprandial liver glycogen concentration.

Twelve male volunteers (age: 21-30 years, body weight 70-93 kg, body mass index $\leq$25 kg/m^2) received three daily doses of 15 g D-tagatose (test substance) or 3 x 15 g sucrose (placebo) for a period of 28 days using a double-blind two-way crossover design. In the morning of day 1 and day 29 of each treatment period, liver volumes were determined in the overnight fasted subjects by Magnetic Resonance Imaging (MRI). Blood was collected prior to the MRI measurement for analysis of standard clinical-chemical and hematological parameters. On day 1 of each treatment period, the liver glycogen concentration was determined as well applying Magnetic Resonance Spectroscopy (MRS). After these baseline measurements in the fasted condition, the volunteers received, on day 1 of each treatment period, a standardized breakfast containing about 99 g starch (in the form of 190 g bread) and 15 g D-

tagatose or sucrose (with marmalade). Serum glucose, serum insulin, plasma glucagon and plasma uric acid were measured in hourly intervals for a period of 7 hours following ingestion of the breakfast. Liver volume and liver glycogen were measured about 5 hours after consumption of the breakfast.

The analyzed clinical-chemical and hematological parameters did not reveal any changes that could be attributed to the treatment with D-tagatose. Liver volumes tended to increase with duration of the study. However, there were no effects of treatment (and period or subject) on liver volume changes as shown by analysis of variance (ANOVA). Short-term effects on liver volume of the standardized breakfast with D-tagatose or sucrose could not be detected. Liver glycogen concentrations prior to - or five hours after consumption of the breakfast did not differ between the two treatments. The values of serum glucose and insulin demonstrate that the ingested starch was absorbed and metabolized almost completely within the first 1-2 hours after ingestion regardless of the type of added sugar (D-tagatose or sucrose). Since five hours lapsed between the consumption of the breakfast and the measurement of liver glycogen, and since the carbohydrate load was small (99 g starch), postprandial liver glycogen concentrations were not increased above baseline (fasting) values. Plasma uric acid levels were very slightly yet significantly elevated above baseline values at 1 hour after intake of the breakfast with D-tagatose. However, plasma uric acid levels did not differ at any time between consumption of D-tagatose and sucrose with the breakfast.

Gastrointestinal side-effects were reported more often during the D-tagatose than sucrose treatment period. However, their intensity was only mild to moderate and did not impair the compliance of the subjects with the dietary regime (Boesch et al., 2001).

10. DISCUSSION OF PARTICULAR ASPECTS OF THE SAFETY PROFILE OF D-TAGATOSE

10.1. Increased glycogen deposition in the liver and increased liver size

Liver enlargement was the only noteworthy effect of D-tagatose in a 90-day sub-chronic toxicity study in Sprague-Dawley rats. This effect was dose-dependent and occurred in the 10, 15 and 20% dose groups in males and females. The 5% dose level at which the rats consumed 3.6 (males) and 4.0 (females) g D-tagatose per kg bw per day was the NOEL with regard to effects on relative liver weight (Kruger et al., 1999c). A subsequent study of shorter duration (28 days) confirmed the effect of D-tagatose on liver weight (Lina & de Bie 1998a). The NOEL of 5% D-tagatose in the diet (2.6 - 2.8 g/kg bw/d) was confirmed as well in another 28-day study (Lina & de Bie, 1998b).

These observations triggered a number of additional studies with the aim of further characterizing the D-tagatose induced liver enlargment and eventually identifying the underlying mechanism. In a first series of three studies (described in more detail in section 9.2.6.1) it was demonstrated that liver glycogen was increased under the influence of D-tagatose in non-fasted rats in a dose-dependent manner (Lina & de Bie, 1998a; Lina et al., 1998; Lina & de Bie 1998b). At dietary levels of >10%, the increase of liver weight was attributable in part to an increased deposition of glycogen and in part to an increase of liver tissue mass. At the 5% dose level, the increase of liver weights in the non-fasted but not in the fasted rats was attributed to an increased glycogen deposition only (Lina & de Bie, 1998b). The increase of liver tissue mass was not associated with any

histopathological changes at light-or electron microscopic level. The results of these first three studies have been published (Bär et al., 1999).

The significance of D-tagatose induced liver enlargement for the safety assessment of D-tagatose was assessed on the basis of these results and additional data from the published literature in a review article (Bär, 1999). It was found that asymptomatic liver enlargement in rats is not a unique effect of D-tagatose but also occurs in response to the ingestion of high levels of fructose and sucrose (Figure 3). A comparison of the respective dose/response data of the different sugars demonstrates, however, that D-tagatose is about four times as efficient as fructose, and about eight times as efficient as sucrose.

Based on the results of a number of studies with fructose and D-tagatose, a plausible mechanism of fructose- and D-tagatose induced liver enlargement was then developed (Figure 4). Fructose and D-tagatose are phosphorylated in the liver to fructose-1-phosphate and D-tagatose-1-phosphate (Raushel & Cleland, 1977). These products abolish binding of glucokinase regulatory protein (GKRP) to glucokinase (GK) (Detheux et al., 1991). Thereby they promote the translocation of GK from the nucleus in the cytoplasm which leads to an increased phosphorylation of glucose to glucose-6-phosphate. Glucose-6-phosphate activates glycogen synthase (Gilboe & Nutall, 1982; Fernández-Novell et al., 1999; van Schaftingen et al., 1997). In vitro experiments with hepatocytes and short incubation times have shown that D-tagatose is as potent as fructose in stimulating the translocation of glucokinase and the subsequent phosphorylation of glucose (Agius, 1994; Van Schaftingen & Van der Cammen, 1989). In vivo, however, D-tagatose is more efficient than fructose in promot-

ing glycogen formation because D-tagatose-1-phosphate is eliminated more slowly than fructose-1-phosphate by aldolase B (see section 9.1.2.1 for references). The accumulation of glycogen in the liver above usual levels triggers compensatory (adaptive) growth of liver tissue. In rats and humans, a number of treatments and conditions which increase the deposition of liver glycogen, have been associated with asymptomatic liver enlargement (for references see Bär, 1999). For the fructose-induced glycogen accumulation and liver enlargement, a transient wave of proliferative activity has been observed in rat liver tissue within a few days after start of fructose ingestion (Kazdová et al., 1976).

According to the proposed mechanism D-tagatose does not act itself as a precursor of glycogen. Rather, its phosphorylation product (tagatose-1-phosphate) activates glycogen synthase which then produces glycogen from regular dietary precursors of glucose (i.e., starch). It follows that glycogen deposition and liver enlargement will occur only if D-tagatose and starch (or another glucose precursor) are ingested at about the same time.

This hypothesis was tested in a series of three feeding studies in which D-tagatose was administered together with - or separate from a dietary precursor of glucose (Polycose) (Lina & de Bie, 2000a,b,c). The results of these studies are entirely consistent with the working hypothesis in that the highest postprandial liver glycogen levels were seen in rats that had received D-tagatose and Polycose in combination. The data also suggest that an increased glycogen deposition triggers hepatocellular growth.

Considering the etiology of the D-tagatose induced liver enlargement, it is debatable whether the observed increase of liver mass may be

characterized as "hyperplasia" (Bender et al., 1972). To the extent that this term evokes the impression of an increase in the number of cells *above ("hyper")* a physiological level or even hints to a pre-neoplastic change, it is inappropriate. In the case of the D-tagatose induced liver enlargement the mass of liver tissue increases in order to cope with an increased work-load, i.e., just in order to maintain the physiological balance. A similar adaptive growth of liver tissue is seen, for example, in pregnant and lactating rats (Klinge & Blinzler, 1972; for additional references see Bär, 1999). By analogy, the adaptive growth of muscle tissue in response to strenuous exercise would probably be regarded as "growth" rather than as "hyperplasia".

Interestingly, liver enlargement was not observed at any time during a 6-month feeding study in which Wistar rats received a diet containing 0, 5 and 10% D-tagatose, 20% fructose or 10% D-tagatose + 10% fructose (Lina & de Bie, 2000d). In an earlier study with Wistar rats it was found that D-tagatose fed together with Polycose increased liver glycogen deposition also in Wistar rats (Lina & de Bie, 2000a). The absence of liver enlargement in this strain may, therefore, point to a difference in the responsiveness of the mechanism that triggers hepatocellular growth in response to an increased glycogen deposition.

Long-term feeding studies with fructose containing diets suggest that the fructose-induced increase of relative liver weight becomes smaller over time, i.e., the NOEL increases with duration of the treatment (Poulsom, 1986). Sucrose which is about eight times less efficient than D-tagatose for increasing liver weights, has been tested in a chronic rat toxicity test at the 20% dietary dose level. Increases of liver weight or adverse effects on the liver were not seen (Hunter et al., 1978 as cited in Bär, 1999). In earlier chronic

studies with administration of sucrose at higher levels there was some evidence of liver enlargement but adverse effects on this organ were not reported (Adams et al., 1959; Durand et al., 1968). Observations from a rat strain with glycogen storage disease also suggest that increased liver glycogen has no adverse consequences in the long term (Malthus et al., 1980; Clark & Haynes, 1988).

In humans, increased liver glycogen and liver enlargement were seen in response to poorly controlled diabetes. Although these changes had persisted for longer periods of time, they were not associated with degenerative changes (fibrosis) (Chatila & West, 1996). There also is no evidence for an association between insulin (which promotes glycogen deposition in the liver) and degenerative or proliferative disease of this organ.

Most relevant for the safety assessment of D-tagatose, however, is a clinical study in which the effect of D-tagatose on liver size was examined in 12 healthy male volunteers using Magnetic Resonance Imaging (MRI). The results of this study demonstrate the ingestion of 3 x 15 g D-tagatose per day (corresponding to an intake of about 0.6 g/kg bw/d) for a period of 28 days does not affect liver size (placebo treatment: 3 x 15 g sucrose/d) (Boesch et al., 2001).

It is concluded that the liver enlargement seen in rats in response to the consumption of D-tagatose is a physiological response to the treatment-induced increase of glycogen deposition. No hepatocellular growth was seen in Sprague-Dawley rats at the 5% and in Wistar rats at the 10% dietary level of D-tagatose suggesting that the increase of liver glycogen at this dose remained within a range which does not trigger compensatory growth of liver tissue. In humans, the ingestion of 0.6 g D-tagatose/kg bw/d for a period of 28 days was without con-

sequences on liver volume or clinical-chemical parameters related to liver function and integrity. Under the proposed conditions of use of D-tagatose, the EDI for the 90th percentile consumer ("heavy user") is 0.18 g/kg bw (2-day average of all age groups combined). This is considerably less than the dose that has been tested and found to not produce liver effects in human volunteers (0.6 g/kg bw). A comparison between the EDI of the average user (0.08 g/kg bw/d) and the NOEL in rats (3.7 and 4.1 g/kg bw/d in male and female Sprague-Dawley rats, respectively; 5.8 and 4.8 g/kg bw/d in male Wistar rats treated for 4 and 26 weeks, respectively) reveals an about 50-fold safety margin.

10.2. Nephrocalcinosis

In the 24-month chronic toxicity and carcinogenicity of D-tagatose in Wistar rats, the incidence of nephrocalcinosis (all types combined) was significantly increased among males of all D-tagatose treatment groups. A similar trend was observed in the males of the 20% fructose group, but the difference to controls was not statistically significant. The female rats exhibited a high spontaneous incidence of nephrocalcinosis in the control group (88%). A further, treatment-related increase reached statistical significance in the 10% D-tagatose group and 10% D-tagatose + 10% fructose group (100% incidence). A more refined analysis according to type (pelvic, cortical/corticomedullary, medullary) and severity of the observed nephrocalcinosis showed that the D-tagatose treatment promoted the formation of calculi mainly in the renal pelvis and medulla. The severity was generally rated as "very slight" or "slight".

Nephrocalcinosis, mainly of the pelvic and corticomedullary type, has been observed in several earlier feeding studies of polyols and other low-digestible carbohydrates in rats and mice (Bär, 1985a, b; Sinkeldam et al., 1992; Smits van Proije et al., 1990; Lina et al., 1996). The formation of renal calculi appears to be the direct consequence of the enhancing effect of these substances on intestinal calcium absorption (Lorinet, 1975; Brommage et al., 1993; Goda et al., 1993; Lacour et al., 1995; Yoshida et al., 1995; Fukahori et al., 1998; Younes et al., 1996). The mechanism by which polyols and other low digestible carbohydrates stimulate calcium absorption is not yet entirely elucidated (Sakuma, 2002, Scholz-Ahrens & Schrezenmeir, 2002). Yet, two consequences of the higher calcium absorption have been observed repeatedly in studies with polyols, namely an increased urinary excretion of calcium and an increased bone weight and/or bone density (Amman et al., 1988; Bär, 1987; Bär, 1985b; Knuuttila et al., 1989). Similar observations were made with polymeric, low-digestible carbohydrates such as fructo-oligosaccharides and certain chemically modified starches (Ohta et al., 1998; Lopez et al., 2000; WHO, 1982).

Fructose which is absorbed more slowly than glucose but faster than the polyols also increases the calcium absorption in rats if administered at high dietary levels (Kaul et al., 1996). Accordingly, fructose administered at a dietary concentration of 70% for 28 days had a weak nephrocalcinogenic activity in rats (Bergstra et al., 1993).

The incidence of transitional cell hyperplasia was increased in females of the 10% D-tagatose and 10% D-tagatose + 10% fructose group. This hyperplasia is most probably the consequence of an irritating effect by the mineral depositions. Focal hyperplasia of

the renal papillary and pelvic epithelium was observed for the same reason in other studies on low-digestible carbohydrates (de Groot et al., 1974; WHO, 1974, 1982; Lina et al., 1996).

In conclusion, an increased incidence of nephrocalcinosis in rats fed diets with 2.5, 5 and 10% D-tagatose for a life-time is not a unique finding but is a commonly observed consequence of the ingestion of low digestible carbohydrates in rats. The formation of renal calculi which is commonly observed in aged rats, is promoted by a treatment-induced hyperabsorption of calcium. There is agreement that this effect has no direct relevance for human safety because low-digestible carbohydrates, even at the highest tolerated dose, do by far not increase the intestinal calcium absorption in humans to the same extent as in rats, if at all; and because rats, especially the female rat, is uniquely prone to the development of nephrocalcinosis (Roe, 1989; WHO, 1982, 1983; SCF, 1985).

10.3. Adrenomedullary proliferative disease

In the 24-month chronic toxicity and carcinogenicity of D-tagatose in Wistar rats, the incidence of adrenomedullary proliferative lesions was significantly increased in males and females of the 5 and 10% D-tagatose group and the 10% D-tagatose + 10% fructose group, and in females of the 2.5% D-tagatose group. In males, which had a substantially higher spontaneous incidence of pheochromocytomas and medullary hyperplasia than females (62 vs. 16%, respectively), the administration of diets with 5 and 10% D-tagatose, and 10% D-tagatose + 10% fructose significantly increased the incidence of pheochromocytomas. In females fed D-tagatose containing diets, mainly the incidence of medullary hyperplasia was increased. Only

in females of the 10% D-tagatose and 10% D-tagatose + 10% fructose groups the incidence of pheochromocytomas was increased.

Treatment-related increases of adrenal weights and the incidence of adrenomedullary hyperplasia and/or neoplasia have been observed in several earlier studies on mannitol, xylitol, sorbitol, lactitol and lactose (Bär, 1988; Lina et al., 1996; Lynch et al., 1996; Sinkeldam et al., 1992; Yoshida et al., 1995). The results of the present study are in line with these earlier observations.

There is evidence that the increased calcium absorption due to the ingestion of low-digestible carbohydrates is a causal factor in the development of adrenomedullary proliferative disease in rats. This was for the first time demonstrated in a study in which it was shown that the xylitol-induced increase in the incidence of medium-size and large pheochromocytomas could be abolished partly or completely by reducing the dietary calcium level from 0.4% (control) to 0.2 and 0.05%, respectively. The reduction of the calcium level was associated with a lower severity (size) of the adrenomedullary lesions. However, since in the 20% xylitol/0.05% calcium group the urinary calcium excretion, and thus the intestinal calcium absorption, was still higher than in the controls (0% xylitol/0.4% calcium), the number of small and medium-size hyperplasias remained elevated (Bär, 1988). In other words, a reduction of the dietary calcium to even lower levels would have been required for reducing also the incidence of the initial stages of adrenomedullary proliferative disease (i.e., hyperplastic foci).

Further evidence for a role of calcium homeostasis in the etiology of adrenomedullary proliferative disease of rats is provided by studies in which a decreased dietary calcium supply was found to be

associated with decreased adrenal catecholamine levels (Baksi & Hughes, 1984; Hagihara et al., 1990). Furthermore, it has been demonstrated that the ingestion of elevated doses of vitamin D stimulates the proliferation of adromedullary cells in vivo and results in the formation of hyperplastic modules after treatment for 26-weeks (Tischler et al., 1996; Tischler et al., 1999). An association between hypercalcemia and increased incidence of pheochromocytomas has also been reported from a study on retinol acetate (RA) in rats. In that study, RA was administered to F344/DuCrj rats in drinking water for 2 years and was found to be associated with higher incidences of benign and malignant pheochromocytomas and hyperplasia of the adrenal medulla in the RA-treated groups. Hypercalcemia was also reported in the RA-treated rats, but without severe bone lesions. It was suggested that RA enhanced the absorption of calcium from the gut, which promoted the development of adrenal medullary proliferative disease (Kurokawa et al., 1985).

In conclusion, an increased incidence of adrenomedullary proliferative lesions in rats fed diets with 2.5, 5 and 10% D-tagatose for a life-time is not a unique finding but is an often observed consequence of the ingestion of low digestible carbohydrates (polyols, lactose) in rats. The formation of adrenomedullary hyperplastic or neoplastic foci which is commonly observed in aged rats, appears to be promoted by a treatment-induced hyperabsorption of calcium. There is agreement that this effect has no relevance for human safety because low-digestible carbohydrates, even at the highest tolerated dose, do by far not increase the intestinal calcium absorption in humans to the same extent as in rats, if at all; and because the adrenomedullary hyperplasia and neoplasia of rats differ from human pheochromocytomas not only with regard to their spontaneous occurrence but also their functionality (Bosland & Bär,

1984; Roe, 1989; Lynch et al., 1996; Capen, 2001; WHO, 1982, 1983; SCF, 1985; Pelgrom & van Raaij, 2001).

10.4. Effects on plasma uric acid concentrations

The ingestion of high doses of fructose or the infusion of fructose at high rates leads to an increase of plasma uric acid concentrations (Table 6). The underlying mechanism has been identified. Fructose entering the hepatocytes is rapidly phosphorylated by fructokinase in the presence of ATP. Since the next step (cleavage by aldolase B) proceeds at a lower rate, fructose-1-phosphate accumulates in the cells transiently. The consumption of ATP and the binding of phosphate to fructose produces a transient, partial depletion of ATP and a reduction of intracellular P_i concentrations. Together these changes lead to an increased degradation of purine nucleotides. Uric acid is the end-product of this well-known metabolic pathway (for references see Mayes, 1993). In view of the similarity between the metabolism of fructose and D-tagatose, it is not surprising that the ingestion of high single doses of D-tagatose ($\geq$30 g) also leads to increased serum uric acid levels (Buemann et al., 1999b; Saunders et al., 1999a; Diamantis & Bär, 2001).

For the safety assessment of D-tagatose it is relevant that the enhancing effect of this sugar (like that of fructose) on plasma uric acid levels is dose-dependent. With a single D-tagatose dose of 30 g, the increase of serum uric acid remained well within the normal (physiological) range (Buemann et al., 1999b; Diamantis & Bär, 2001). A single dose of 15 g D-tagatose did not produce a significantly different response than a single dose of 15 g sucrose (both ingested to-

gether with 99 g starch) in healthy volunteers (Boesch et al., 2001) (Figure 5). A similar result was obtained with a single 15-g dose of D-tagatose (ingested with about 100 g starch) in hyperuricemic subjects (Diamantis & Bär, 2002). At the lower intakes which are projected from the intended uses of D-tagatose in food (per eating occasion: 4.7 and 7.2 g for the mean and 90th percentile consumer, respectively), plasma uric acid concentrations are expected to not increase at all or only to a very small extent which is well within the range of the physiological, diurnal or diet-induced fluctuation of this parameter (Abu-Amsha et al., 2000; Brulé et al., 1992; Colling & Wolfram, 1987; Garrel et al., 1991; Reiser et al., 1989).

It also should be noted that effects of D-tagatose on uric acid levels, if there are any, would be transient and not cumulative over time. In studies in which D-tagatose was consumed for long periodes of time and/or at high levels, uric acid levels in plasma samples collected after overnight fasting were not elevated (Buemann et al., 1998; Saunders et al., 1999a; Makris, 1999; Boesch et al., 2001). Correspondingly, the 24-hour urinary excretion of uric acid was not increased in subjects consuming daily doses of 29 g D-tagatose for 15 days (Buemann et al., 1998).

It is concluded from these data that under the conditions of intended use D-tagatose will not adversely affect plasma uric acid levels, neither in healthy nor in hyperuricemic subjects.

10.5. Non-enzymatic glycation of proteins

Reducing sugars react with proteins in solution at a low rate. In a first step, so-called “Amadori products” are formed. Subsequent rearrangement of these products leads to the formation of “advanced glycation end products” (AGE). Different sugars have different reactivity vis-à-vis proteins. In a short-term (4-hour) in-vitro study, fructose caused a more rapid non-enzymatic glycation of hemoglobin than did glucose. D-Tagatose was less reactive than glucose (Bunn & Higgins, 1981). In another test system, the glycation of myofibrillar proteins was similar for glucose, fructose and D-tagatose after an incubation period of 48 hours (Syrový, 1994). In long-term in-vitro studies with incubation times of several weeks, fructose produced fluorescent AGE at a faster rate than glucose (Oimomi et al., 1989, Suárez et al., 1989).

It has been proposed that non-enzymatic glycation of proteins and subsequent formation of AGE in vivo accounts for some of the complications of diabetes, such as cataract formation (Monnier et al., 1979; Monnier, 1990), stiffening of collagen (Vishwanath et al., 1986), and vascular narrowing (Cerami et al., 1986). The late complications of diabetes (retinopathy, nephropathy, neuropathy, microangiopathy) are phenomenologically in fact linked to hyperglycemia (Nathan, 1996). However, it is not yet known whether the glycation of important structural or functional proteins directly causes this pathology, or whether the increased levels of glycation products in tissues and the circulating blood are merely a marker of a metabolic condition that accelerates the formation of degenerative disease.

The 24-month chronic toxicity and carcinogeneicity study of D-tagatose in rats indicates that the ingestion of diets with high levels of D-tagatose and/or fructose is not associated with an increased incidence of retinopathy (cataracts) or degenerative renal changes as are typically observed in diabetic rats (Lina & Kuper, 2002; Lina & Bär, 2003).

There are no in-vivo studies in which the protein glycating potential of D-tagatose was measured directly. Considering, however, that the glycating potential of D-tagatose is not higher than that of fructose, it may suffice to recapitulate the results of studies on fructose.

Two feeding studies in rats which were conducted to compare the protein glycating potential of fructose, glucose and sucrose gave conflicting results. In one study, metabolically healthy rats were fed a commercial diet for 1 year. The animals had free access to water or 25% solutions of fructose, glucose, or sucrose. Blood fructose, fructosamine, and glycated Hb levels were increased in the fructose treated rats. Collagen-bound fluorescence (representing AGE) and insoluble collagen were increased in this group as well. The type-III to type-I collagen ratio was decreased, suggesting faster aging in response to fructose ingestion (Levi & Werman, 1998; Werman & Levi, 1997).

In the other study, rats received diets with 66.5% starch, glucose, sucrose, fructose, or glucose + fructose for up to 26 months. Serum glucose, fructosamine and glycated Hb were analyzed. Collagen-associated fluorescence and pentosidine concentrations were measured in different organs. After 9 months of treatment, glycated Hb was about 10% higher in the fructose and the starch group. However, a

similar difference was not observed after 18 and 26 months. All the other markers of glycemic stress and accumulation of AGE did not differ between treatments (Lingelbach et al., 2000).

The reasons for the discrepant results of these two studies are not clear. However, it is well established that the administration of high doses of fructose leads to copper-deficiency in rats particularly if dietary copper levels are only marginally sufficient. Interestingly, copper deficiency was associated with increased glycated Hb, serum fructosamine, and serum pentosidine (Saari & Dahlen, 1999). In the absence of data on the copper status it cannot be excluded that the seemingly fructose-induced glycation and aging of collagen observed by Levy and Werman were in fact consequences of a fructose-induced copper deficiency. A fructose-induced decrease of insulin sensitivity and thus increased blood glucose levels might have contributed to the effect as well (Ruhe et al., 1996; Thorburn et al., 1989).

For the assessment of the safety of D-tagatose it is relevant that there exists no report in the open literature demonstrating that there is an association in humans between fructose intake and increased protein glycation. However, there are at least two observations which suggest that such an association does not exist.

(1) Glycation-related pathology is not a clinical feature of fructosuric subjects. People with benign hereditary fructokinase deficiency ("essential fructosuria") exhibit increased blood fructose levels after ingestion of fructose ($\leq$ 250 mg/l), yet are otherwise asymptomatic (Sachs et al., 1942; Marble, 1947; Silver & Reiner, 1934; Szombathy et al., 1969) [for a summary see also the On-line Mendelian Inheritance in Man database

(http://www3.ncbi.nlm.nih.gov/omim/)]. Fructosuric subjects do not exhibit increased levels of glycated hemoglobin (HbA_1 and HbA_{1c}) and do not appear to be more prone to cataract formation (Steinitz et al., 1963; Petersen et al., 1992; Gitzelmann et al., 1995).

(2) The ingestion of 30 g/d fructose for 18 months did not influence the incidence and progression of retinopathy in 72 diabetic subjects of which 32 subjects entered the study with preexisting retinopathy. The control group was matched with regard to age and glycemic status. Thirty-four control subjects had preexisting retinopathy, 179 subject were asymptomatic (Schartow, 1965).

In conclusion, dietary fructose at customary levels of intake appears to lack importance as a causative factor of non-enzymatic glycation reactions in the human body. Since fructose and D-tagatose have the same chemical reactivity, this conclusion may be extended to D-tagatose. The lower absorption of D-tagatose provides for an additional safety margin in this respect.

10.6. Metabolic interactions between fructose and D-tagatose

Fructose is consumed in considerable amounts with an ordinary human diet [added fructose: 10 g/d; fructose from all sources including sucrose: 37 g/d (Glinsmann et al., 1986; Park & Yetley, 1993)]. Since fructose and D-tagatose have a similar chemical structure and share a common metabolic pathway using the same enzymes (fructokinase and aldolase B), it needs to be considered whether there is a possibility of interaction if fructose and D-tagatose are consumed at the same

time. Interactions are conceivable at the following steps: intestinal absorption, (hepato)cellular uptake, phosphorylation by fructokinase, and cleavage by aldolase B.

As regards intestinal absorption, it has been shown that D-tagatose does neither promote nor inhibit the carrier-mediated diffusion of fructose through the intestinal mucosa (the GLUT5 and GLUT2 carriers are involved) (Crouzoulon, 1978; Sigrist-Nelson & Hopfer, 1974). The interaction of D-tagatose with the GLUT5 transporter is weak (Km=59 mM) (Tatibouët et al., 2000).

The rate of phosphorylation of fructose and D-tagatose on first hepatic passage is determined by the rate of cellular uptake (Sestoft & Fleron, 1974; Baur & Heldt, 1977; Okuno & Glieman, 1986). The GLUT2 carrier appears to be responsible for the carrier mediated uptake of fructose by hepatocytes. On the basis of data on intestinal absorption, it is expected that D-tagatose will not interfere with the hepatocellular uptake of fructose.

Fructokinase has a higher affinity to fructose than to D-tagatose (Sánchez et al., 1971; Raushel & Cleland, 1973, 1977). Accordingly, it is more likely that fructose slows down the phosphorylation of D-tagatose than vice versa. It is consistent with this notion that D-tagatose at a concentration of 0.35 mM reduces the phosphorylation of fructose (0.05 mM) in vitro by only 10% (Malaisse et al., 1989).

Under physiological conditions, D-tagatose will not reach intracellular concentrations that could be inhibitory to fructokinase.

ADP is known to inhibit fructokinase. However, the consumption of 30 g D-tagatose decreases ATP levels in the human liver by only about

10% (Buemann et al., 2000a). At the expected levels of intake of D-tagatose, this decrease will be much smaller. The corresponding increase of the ADP concentration would not suffice to produce any significant inhibition of fructokinase.

Fructose-1-phosphate inhibits fructokinase only at relatively high concentrations (K_i = 12 mM) (Raushel & Cleland, 1977). The K_i of D-tagatose-1-phosphate has not been determined. However, D-tagatose-1-phosphate concentrations will remain below 1 mM [maximum concentration found in the human liver after intake of 30 g D-tagatose (Buemann et al., 2000a)]. It is unlikely that D-tagatose-1-phosphate will significantly inhibit fructokinase at the low concentrations which are encountered after ingestion of D-tagatose.

In the perfused rat liver, fructose (0.5 mM) was phosphorylated rapidly even in the presence of D-tagatose (0.5 mM) (Quistorff & Therkelsen, 1999).

Since both fructose and D-tagatose induce increased glycogen deposition in the liver and, at high doses, transient compensatory hepatocellular growth, a concomitant administration of the two sugars could be thought to produce an additive (or even synergistic effect). The liver weight data of rats (retired breeders) that received diets with 15% sucrose, 15% D-tagatose, 5% D-tagatose plus 10% fructose, and 15% fructose for 90 days demonstrate that this is not the case. While absolute and relative liver weights were significantly increased in the 15% D-tagatose group, there was no difference of liver weights between the 5% D-tagatose + 10% fructose group and either of the two controls (15% sucrose and 15% fructose) (Saunders, 1992). The absence of an additive or synergistic effect on liver enlargement is confirmed by the results of a 6-month and a 24-month study in which one

group of rats received a diet with 10% tagatose + 10% fructose (Lina & de Bie, 2000d; Lina & Kuper, 2002).

In conclusion, there is no reason to expect an interference of D-tagatose on fructose absorption and fructose metabolism. The available enzyme kinetic data indicate that D-tagatose, at the levels which may occur transiently in the circulating blood or within cells after intake of D-tagatose containing foods, will not affect the metabolism of co-ingested fructose.

10.7. Intake of D-tagatose by individuals with hereditary fructose intolerance

10.7.1. Inadvertent consumption does not represent a risk for health

Subjects with hereditary fructose intolerance (HFI) are a subgroup of the population which should avoid the intake of fructose. HFI is a rare autosomal recessive metabolic disorder with an incidence of between 1 in 12,000 to 1 in 130,000 (Steinmann et al., 1975; James et al., 1996). HFI is caused by a defect in the gene encoding for aldolase B. This enzyme splits fructose-1-P (and tagatose-1-P) as well as fructose-1,6-diphosphate to produce two 3-carbon fragments. The symptoms of HFI result from the intracellular accumulation of Fru-1-P (which is osmotically active) and the corresponding sequestration of phosphate which leads to a depletion of ATP, hypophosphatemia and finally hyperuricemia due to AMP degradation.

Considering these mechanisms and the fact that fructose and D-tagatose are metabolised via the same biochemical pathways using

the same enzymes, it must be assumed that D-tagatose produces the same effects in HFI subjects as fructose. However, several-fold higher intakes of D-tagatose may be required to produce these effects because of its incomplete absorption (about 20% of the ingested dose).

In a strict, prescribed diet for older children, fructose intake is limited for HFI subjects to 20-40 mg/kg bw/d; in a self-imposed diet according to individual tolerance, fructose intakes of up to 100-200 mg/kg bw/d may be acceptable for older children and adults (van den Berghe, 1997). Considering the lower absorption of D-tagatose, about five-fold higher levels of this sugar may be tolerated. The inadvertent consumption of D-tagatose containing foods will, therefore, not expose HFI subjects to a significant health risk.

It should be noted that another substance which is on the market since a long time, namely sorbitol, should also be avoided in a HFI diet. Sorbitol is widely used as a non-cariogenic bulk sweetener and humectant. Like D-tagatose, it is absorbed only partly (about 20%) and is converted after absorption almost completely to fructose by sorbitol dehydrogenase.

10.7.2. Measures ensuring that HFI patients know to avoid D-tagatose containing foods

HFI is typically detected very early in life. Infants with HFI are perfectly healthy and free of symptoms as long as they do not ingest any food containing fructose. However, first symptoms arise when infants receive their first supplementary foods beside breast

or bottle feeding (adapted milks). Most of these food supplements contain sucrose and/or fructose (e.g., from orange juice, mashed fruits etc.) which then trigger the occurrence of symptoms.

In small children, the leading symptoms of HFI are failure to thrive, protracted vomiting, frequent attacks of hypoglycemia with occasional unconsciousness, jaundice, albuminuria and aminoaciduria (Froesch, 1978). Introduction of a fructose-free diet results in rapid alleviation of these symptoms and a subsequent recovery. Later, these children develop a strong aversion against all sweet foods including fruits, thereby protecting themselves against the sequelae of HFI. This distaste of sugar is most likely the result of the unpleasant effects like sweating, trembling, dizziness, nausea and vomiting which immediately follow the ingestion of even small amounts of fructose. This explains why a chronic picture of HFI does not exist in adults and why the teeth of HFI patients are, as a rule, in extraordinarily good condition.

Because of the symptoms of HFI, all subjects suffering from this disorder are very well aware of their condition. Despite the low incidence of HFI, health professionals (pediatricians, dietitians, etc.) are also well informed about this intolerance and about the importance of dietary counselling for HFI subjects.

In most countries, metabolic disorders are managed through network of health care professionals. In addition, HFI patients may be assisted directly by respective self-help groups. Therefore, the most appropriate and effective strategy for advising HFI individuals about the fructose-like properties of D-tagatose would be via notification of the relevant health professionals and HFI-individuals through the established organisations and the medical press. Re-

spective articles and letters should be released when D-tagatose is put on the market.

In view of the low incidence of HFI, the fact that this is not a life-threatening condition in adults, and the fact that HFI subjects are unlikely to consume products with D-tagatose because they have a general aversion against sweet foods, it does not appear justified to introduce a special information statement on the food label. Probably for these same reasons, such a statement is also not required for sorbitol containing foods which are on the market already. It apparently suffices to refer to the presence of D-tagatose (or sorbitol) in the list of ingredients and to ensure that the health profession is adequately informed about the fructose-like properties of D-tagatose.

10.8. Intake of D-tagatose by individuals with milk allergy

Milk contains different proteins that may act as allergens and can cause allergic reactions in subjects with milk allergy. Alpha-lactalbumin and beta-lactoglobulin, both present in the whey fraction of milk, are most often involved in IgE-mediated allergic reactions. In recognition of the risks of allergic reactions, many countries have introduced legislation which requires that the presence of milk and milk-derived products is clearly indicated on the label of processed foods. This requirement also applies for lactose which is produced from whey and may contain small amounts of milk protein.[4]

[4] Directive 2000/13/EC as amended by Directive 2003/89/EC requires that products derived from milk (including lactose) must be labeled in such a way that their origin from milk is clearly apparent to consumers who may suffer from an allergy to milk protein. Lactose may, for example, be referred to as "milk sugar" [FSA Guidance Notes on the Food Labeling (Amendment) (No. 2) Regulations 2004].

Since D-tagatose is produced from lactose, the question arises of whether traces of milk protein could be carried over from the raw material (lactose) into the final product. Considering the production process of D-tagatose which includes treatment with heat, alkali, ion exchange resins and activated carbon, it appears highly unlikely that protein could occur in the final product. Yet, in order to have experimental proof, D-tagatose was tested for the presence of whey protein using an ELISA test. For comparison, lactose (three different grades) and purified whey protein concentrate (Lacprodan 80, Arla Foods) were included in the test. There was no indication for the presence of whey protein in D-tagatose (Annex 4) (Taylor et al., 2005). Whey protein was found, however, in two of the three tested lactose products.

In view of the absence of whey protein in D-tagatose, no benefit is apparent for consumers, including those with established milk allergy, from a particular labeling that would identify D-tagatose as an indirectly milk-derived product.

10.9. Intestinal side-effects

Intestinal side-effects such as borborygmi, nausea, flatulence and stool softening, may occur in susceptible individuals after consumption of more than 10-15 g D-tagatose (ingested as a single dose) (Donner et al., 1999; Boesch et al., 2001). The tolerable total daily dose is a multiple of the tolerable single dose since the intestinal effects are not cumulative over time. Thus, total daily doses of 45 g D-tagatose did not produce unacceptable side-effects (Boesch et al., 2001).

The intake of D-tagatose from all intended uses combined does not reach 10 g per eating occasion for the 90th percentile consumer of any age group (Table 3). Therefore, there appears to be no need for a warning statement on the label of D-tagatose containing foods.

However, the list of intended uses is only exemplary (i.e., reflecting the current knowledge of D-tagatose uses) and thus may be subject to changes as new applications of D-tagatose emerge. The application of an information statement ("excessive consumption may produce laxative effects") may, therefore, be feasible on the label of foods containing more than 15 g D-tagatose per serving.[5]

[5] Council Directive 96/21/EC amending Directive 94/54/EC requires a corresponding statement on foods containing more than 10% added polyols. However, the occurrence of intestinal effects is related to the amount but not the concentration of unabsorbed polyols or, for the same purpose, unabsorbed sugars. Therefore, it appears more justified to use the expected intake rather than the applied concentration as parameter which triggers information labeling. This view also underlies the US regulation on sorbitol which requires that food whose reasonably foreseeable consumption may result in a daily ingestion of 50 grams of sorbitol shall bear the statement: "Excessive consumption may have a laxative effect" (21 CFR § 184.1835).

However, since the occurrence of intestinal effects is related more to the intake per eating occasion than the total daily intake, it would appear more feasible to make the information labeling dependent upon the foreseeable intake per serving (i.e., eating occasion).

11. SUMMARY AND CONCLUSIONS

D-Tagatose is an epimer of D-fructose inverted at C-4. It is obtained from D-galactose by base-catalyzed isomerisation in the presence of calcium. D-Galactose is produced from lactose by enzymatic hydrolysis using an immobilized lactase from Aspergillus oryzae. Aspergillus oryzae is an accepted non-pathogenic and non-toxigenic source organism for enzymes used in the manufacturing of foods (21 CFR 137.105; 21 CFR 173.150; GRAS Notices Nos. 8, 34). The safety of the immobilized lactase has been examined in a 90-day toxicity study in rats. A liquid extraction test according to FDA guidelines has been performed as well. Based on a comparison between the maximum potential exposure to total extractives and the NOAEL of the immobilized lactase established in the 90-day study, it is concluded that the immobilized lactase from A. oryzae is fit for use in the production of D-tagatose.

Purification of the synthesized D-tagatose is achieved by chromatographic separation, treatment with activated carbon and crystallization. The final product has a high purity (>98%). The only by-product that can be detected by HPLC chromatography (with RI detection) is D-galactose. Specifications have been adopted by JECFA at its 55th meeting (Annex 3).

D-Tagatose has a sweetness which is only slightly less than that of sucrose (about 75-92% depending upon concentration). Being absorbed only incompletely and serving as a substrate for lactobacilli and other lactic acid bacteria of the intestinal microbiota, D-tagatose has prebiotic activity (Jensen & Buemann, 1998; Bertelsen et al., 1999). In comparison to sucrose or fructose, D-tagatose offers the additional advantages of a reduced energy value, a low glycemic in-

dex, and toothfriendly (i.e., non-cariogenic) properties. Therefore, D-tagatose may be used as a sugar substitute with prebiotic activity in ready-to-eat cereals, low-fat/non-fat ice cream and frozen yogurt, diet and health bars and diet soft candy, and formula diets intended for use as a meal replacement in a weight control diet. Added at low concentrations ($\leq$1%) to carbonated diet soft drinks, smoothies, baked goods, milk chocolate, yoghurt, diet teas and coffee drinks, D-tagatose can improve the taste and mouth-feel of these products. In chewing gum, hard and soft candy, D-tagatose serves as a non-cariogenic, energy-reduced sweetener that has a low hygroscopicity and a lower cooling effect than some polyols. In icings, D-tagatose may be used because of its low hygroscopicity and reduced energy value. In table top sweeteners (spoon-for-spoon products), D-tagatose provides bulk and synergistically enhances the sweetness.

Using US food consumption data (94-96 CSFII and supplement 98) and assuming that D-tagatose would be used in all mentioned applications (except chewing gum and formula diets) at the highest feasible concentrations, the two-day average intake has been estimated at about 4.6 and 9.8 g per day for the mean and 90th percentile consumer ("user"), respectively. Expressed in terms of g D-tagatose/kg body weight/day, the highest intake is projected for preschool children (age 2-5 years) with values of 0.19 and 0.37 g/kg bw/d for the mean and 90th percentile consumer ("user"), respectively. For the users of all age groups combined the estimated intake is 0.08 and 0.19 g/kg bw/d for the mean and 90th percentile consumer. The additional intake from chewing gum is estimated at 0.030 - 0.035 g/kg bw/d for the average user (assuming a use level of 30%). The intake of D-tagatose is evenly distributed over the different daily meals and snacks with an average intake of 3.1 and 6.2 g per eating occasion for the mean and 90th percentile consumer, respectively.

The absorption, distribution and excretion of D-tagatose has been examined in rats. Absorption data are also available from two studies in pigs. About 20-25% of an oral dose is absorbed during small intestinal passage in rats and pigs. From numerous data on the absorption in humans of non-metabolizable hexoses of nearly identical molecular size and hydrophilicity as D-tagatose, in particular L-rhamnose, it is estimated that about 20% of an oral D-tagatose dose would be absorbed by man.

Absorbed D-tagatose is metabolized mainly in the liver. After phosphorylation by fructokinase to tagatose-1-phosphate, and cleavage of this intermediate product by aldolase B, the metabolism of D-tagatose converges with that of D-fructose. These first two steps proceed at a lower rate for D-tagatose than fructose because of a somewhat lower affinity of the two involved enzymes for D-tagatose and D-tagatose-1-phosphate than for fructose and fructose-1-phosphate, respectively. Since the K_m of aldolase B is higher than that of fructokinase, tagatose-1-phosphate accumulates in the liver transiently but its concentration remains low (<1mM after ingestion of 30 g D-tagatose) (Buemann et al., 2000a). A corresponding transient accumulation of fructose-1-phosphate occurs after intake (or infusion) of fructose.

Not absorbed D-tagatose is fermented by the intestinal microbiota like other low-digestible carbohydrates (dietary fiber) yielding short-chain fatty acids as the main, absorbable end-products. The ingestion of D-tagatose leads to an increased density of Lactobacilli and other lactic acid bacteria in the feces (prebiotic effect).

The toxicity of D-tagatose was examined in standard in vitro and in vivo toxicity tests. Tests for bacterial gene mutation, chromosomal

aberration, micronucleus formation (in vivo), and TK locus mutation gave uniformly negative results demonstrating that D-tagatose is not genotoxic (Kruger et al., 1999a). There was no mortality and no evidence of toxicity upon acute oral administration of D-tagatose (10 g/kg bw) in rats and mice (Trimmer, 1989).

The subchronic and chronic toxicity of D-tagatose was tested in a 13-week rat study according to standard guidelines. A subsequent 6-month study was focused on potential effects of D-tagatose on the liver because significant liver enlargement was observed in the 13-week study. A 24-month carcinogenicity study was conducted in Wistar rats but the histopathological examination was limited to the liver and those organs (adrenals, kidneys, testes) which exhibited changes in response to the chronic dietary administration of other low-digestible carbohydrates in earlier rat studies.

In the 13-week subchronic toxicity test, rats received D-tagatose with the diet at dietary levels of 0, 5, 10, 15 and 20%. The occurrence of soft stool in most of the rats of the 15 and 20% dose groups during the first few days of the study were attributed to the incomplete absorption of the test substance (osmotic diarrhea). Otherwise, no reactions to the treatment were observed. Body weights were about 10% below controls in the 20% dose group at the end of the study. No toxicological relevance was attributed to a slight, yet statistically significant reduction of hemoglobin and hematocrit in males and females of the 15 and 20% dose groups.[6] Serum cholesterol was increased in the 15 and 20% dose group. Serum enzymes levels (γ-GT, ALAT, ASAT) were not increased in response to the treatment. The terminal examination revealed increased absolute and relative liver weights in

[6] A separate in vitro study demonstrated that D-tagatose (unlike L-sorbose) does not cause hemolysis of dog erythrocytes but rather has a stabilizing effect on these cells as it has on cultured hepatocytes (Bär & Leeman, 1999).

males and females of the 10, 15 and 20% dose groups. A number of other, numerically small, yet statistically significant changes of relative but not absolute organ weights were attributed to changes of body weight and body composition[7]. Histopathological examination of organs and tissues revealed no changes in response to the D-tagatose treatment, except for a mild hepatocellular hypertrophy in some rats of the 15 and 20% dose groups.[8] With regard to liver enlargement, the 5% dietary level was the NOEL. At this level D-tagatose intake was 3.7 and 4.1 g/kg bw/d for male and female rats, respectively (Kruger et al., 1999c).

The effect of D-tagatose on the liver of rats was further examined in a 6-month study in which groups of 60 male Wistar rats received diets containing 0 (controls), 5 and 10% D-tagatose, 20% fructose, and 10% D-tagatose + 10% fructose. Ten rats of each group were killed on day 3, 7, 14, 28, 94 and 182 of the study after overnight fasting. Absolute and relative liver weights did not differ between controls and treated groups at any time. At the high dose level D-tagatose intake was 5.8 g/kg bw/d over the first four weeks and 4.8 g/kg bw/d over the entire 6-month period (Lina & de Bie, 2000d). In view of the significant, D-tagatose-induced liver enlargement in Sprague-Dawley rats, the different outcome in Wistar rats was unexpected. In order to investigate the possibility of a strain-related difference, the sensitivity of six different rat strains was, therefore, tested in a study of shorter duration (4 weeks) but applying a higher dietary level of D-tagatose in the test diet (20%). The relative liver weights of the overnight fasted rats were significantly increased in all strains (only males were tested). However, the magnitude of the

[7] D-tagatose fed rats have smaller fat deposits than control rats (Lina & de Bie, 2000d).

[8] No ultrastructural abnormalities were seen by electron-microscopic examination of the liver of a small number of pigs who had ingested D-tagatose at doses of about 2.5 and 5 g/kg bw/d for 33 days (Mann, 1997).

effect was biggest in Sprague Dawley rats and smallest in Wistar rats (Appel, 2002).

The biochemical and morphological characteristics of D-tagatose induced liver enlargement were examined in a 4-week study with male 10 to 11-week-old Sprague-Dawley rats. Groups of 20 rats received a diet containing 0, 5, 10 and 20% D-tagatose. The animals were killed in the non-fasted condition. The livers of 5 rats/group were processed for electron-microscopic examination. In the livers of 6 rats/group marker enzymes of peroxisome proliferation were measured. DNA synthesis (Ki-67 index) and nuclear density were determined in the livers of another 6 rats/group. Liver weights were significantly increased in linear relation to the D-tagatose intake. Except for an increased glycogen accumulation, no ultrastructural changes were seen on EM examination of livers of the high-dose group. Acyl-CoA oxidase and CYP4A1 activity were increased but the magnitude of this increase was considered too small for being indicative of peroxisome proliferation. EM examination confirmed the absence of peroxisome proliferation. The Ki-67 index did not differ between the groups but a dose-related decrease of the number of nuclei per unit area signified some hepatocellular hypertrophy or swelling probably due to the increased glycogen deposition (Lina et al., 1998; Bär et al., 1999).

A chronic (24-month) toxicity/carcinogenicity study was conducted in Wistar rats. For reasons of animal availability, the specific strain used in this study (Hsd Cpb:WU) was not the same as was used in the 6-month study (Cpb:WI(WU)BR) and in earlier chronic studies on other low-digestible carbohydrates at the same contract institute (TNO, Zeist, The Netherlands). The study was conducted according to standard OECD Guidelines except that the evaluation of organ weights was limited to the liver, kidneys, adrenals, testes and cecum and the

histopathological evaluation to these same organs except the cecum. The test substance was administered with the diet at levels of 0, 2.5, 5 and 10%. Two additional comparison groups received diets with 10% D-tagatose plus 10% fructose or 20% fructose.

The treatment had no adverse effect on mortality, ophthalmoscopic changes, hematological and clinical chemical parameters. Relative liver weights were increased in the 10% D-tagatose group (females only) and in the 10% D-tagatose + 10% fructose group (both sexes), demonstrating that D-tagatose at the high dose level produced the expected liver enlargement in the applied rat strain. The histopathological examination demonstrated, however, that this liver enlargement had no adverse consequence for the morphological integrity of this organ. Since plasma liver enzymes and other liver related clinical parameters were also not affected by the treatment, it must be concluded that the observed liver enlargement represents a physiological adaptive response to the D-tagatose treatment. Mechanistic studies suggest that a D-tagatose induced increase of the hepatic glycogen deposition triggers the (compensatory) growth of this organ (Lina & Kuper, 2002).

The relative weights of kidneys, adrenals and testes also appeared to be increased in response to the D-tagatose treatment. The histopathological examination revealed nephrocalcinosis and adrenomedullary proliferative changes. No alterations were observed in the testes. Nephrocalcinosis (mainly of the pelvic and corticomedullary type and predominantly in females) is a common observation in rats (and mice) fed low digestible carbohydrates. The main causative factor is a treatment-induced increase of the intestinal calcium absorption and thus urinary calcium excretion. Other factors, such as the mineral composition of the diet or a treatment related increase of urinary

uric acid, may have a modulating effect on the incidence and severity of the mineral deposition. Another more indirect consequence of the increased calcium absorption is the promotion of adrenomedullary proliferative disease. The spontaneous incidence and severity of this condition varies substantially between different strains of rats with males usually exhibiting a higher spontaneous incidence and a correspondingly higher sensitivity to treatment-induced effects. The Wistar strain applied in this study had a rather high spontaneous incidence of adrenomedullary hyperplasia and neoplasia. It is, therefore, not surprising that the incidence of adrenomedullary proliferations (all degrees combined) was increased even at the lowest D-tagatose level tested (Lina & Bär, 2003).

However, it must be noted that nephrocalcinosis and adrenomedullary proliferative changes are well-known consequences of the dietary administration of low-digestible carbohydrates (polyols, lactose) in rats which do not have any significance for human safety. This is reflected, for example, by the allocation of an ADI "not specified" by JECFA for sorbitol, xylitol, mannitol and lactitol, all of which produced nephrocalcinosis and adrenomedullary proliferative disease in rats.

An embryotoxicity/teratogenicity study was conducted in Sprague-Dawley rats with administration of D-tagatose by gavage at levels of up to 20 g/kg bw/d from day 6-15 of gestation. There were no signs of maternal toxicity, embryotoxicity or teratogenicity. Reproductive performance was not affected by the treatment. Relative liver weights of dams were increased in the mid and high dose groups (12 and 20 g/kg bw/day, respectively). No morphological changes were seen on microscopic examination of the livers (Kruger et al., 1999b).

The intestinal tolerance of D-tagatose was examined in several human studies. At doses of up to 15-20 g per eating occasion, flatulence was usually the only side-effect noted (Boesch et al., 2001; Lee & Storey, 1999; Donner et al., 1999). At higher levels, additional symptoms, such as nausea, borborygmi, colic and laxation, may occur reflecting the presence in the gut of an osmotically active substance (Buemann et al., 1999c; Saunders et al., 1999a). The spectrum of the intestinal symptoms and their approximate dose/response relationship are similar for D-tagatose and other incompletely absorbed carbohydrate or polyols (Lee & Storey, 1999).

The tolerance of D-tagatose with regard to a number of metabolic and clinical parameters was examined in healthy and type–2 diabetic volunteers. Under the applied conditions D-tagatose was well tolerated [75 g/d for 8 weeks in healthy and diabetic volunteers (Saunders et al., 1999a); 45 g/d for 12 months in healthy and type-2 diabetic volunteers (Makris, 1999); 45 g/d for 28 days in healthy volunteers (Boesch et al., 2001)]. No adverse effects were seen on fasting plasma uric acid levels, plasma enzymes, lipids and other clinical parameters.

The potential effects of D-tagatose on liver volume and glycogen deposition were examined in a study with 12 healthy male volunteers. D-Tagatose (test substance) or sucrose (placebo) were administered at a dose of 45 g/d (divided in three equal daily doses consumed with the main meals). Each treatment period lasted for 28 days (double-blind, two-way crossover design). Liver volume was measured in the morning of day 1 and 29 after overnight fasting. While there was a general increase of liver volumes during the study, there was no significant difference between the treatments. Plasma enzyme levels and other clinical-chemical parameters were not affected by the treat-

ment. The ingestion of a standard breakfast with 15 g D-tagatose or sucrose did not produce differences of liver volume and liver glycogen concentration 5 hours after intake as measured by Nuclear Resonance Imaging and Nuclear Resonance Spectroscopy, respectively (Boesch et al., 2001).

From the available metabolic and toxicological data of D-tagatose three issues were identified that required particular consideration in the safety assessment of this substance. These issues are, (1) the D-tagatose induced liver enlargement in rats, (2) a small increase of plasma uric acid observed in humans after ingestion of 30 and 75 g D-tagatose, and (3) the possibility of metabolic interactions between D-tagatose and fructose which is consumed in considerable amounts with a regular human diet.

(1) With regard to the D-tagatose induced liver enlargement in rats it is relevant that it was reversible on cessation of the treatment and that it was not accompanied by any morphological or ultrastructural changes, even in 24-month carcinogenicity study.[9] It appears, therefore, that the increased liver mass is the result of normal hepatocellular growth.

D-Tagatose administered at dietary concentrations of $\geq$5% leads to an increased accumulation of glycogen in the liver of rats. It is hypothesized that this increased work-load triggers a compensatory growth of the liver. Observations from a rat strain with chronically increased liver glycogen (due to a genetic defect) confirm that this condition has no adverse conse-

[9] A similar observation was made in a long-term feeding study with fructose. The increase of relative liver weight above control levels decreased rather than increased with duration of the treatment (Poulsom, 1986).

quences even in the long term (Malthus et al., 1980; Clark & Haynes, 1988).

Fructose and sucrose administered at dietary levels of >20% and >40%, respectively, also increase liver glycogen deposition and may produce liver enlargement in sensitive strains of rats (Bär, 1999). Nonetheless, the administration of sucrose (20% in the diet) for 2 years did not produce any adverse effects in the liver of rats (Hunter et al., 1978 cited in Bär, 1999).

In humans, increased liver glycogen and liver enlargement may be seen in subjects with poorly controlled diabetes. Even if persistent, this condition is not associated with degenerative changes (fibrosis) of the liver tissue (Chatila & West, 1996).

A study in adult human volunteers demonstrates that the daily ingestion of 45 g D-tagatose (0.6 g)/kg bw/d) for 28 days has no effect on liver volume (Boesch et al., 2001).

It follows from all these observations that D-tagatose under the conditions of intended use with an estimated intake of 0.08 and 0.19 g/kg bw/d for the mean and 90th percentile user, respectively will not adversely affect the human liver.

(2) With regard to effects of D-tagatose on plasma uric acid levels, it is well known that the intake of fructose also produces a slight raise of plasma uric acid levels. In fact, there appears to be a dose/response relationship of this effect for both tagatose and fructose. A comparison of the data suggests that D-tagatose is about 2-3 times more efficient than fructose in raising plasma uric acid levels (Table 4, Figure 6). Yet,

the data also demonstrate that, at the projected intake of D-tagatose from the intended use in food, the increase of plasma uric acid will be negligible in absolute terms and will remain well within the range of changes that result from the consumption of ordinary foods. This is also true for subjects with pre-existing hyperuricemia (Diamantis & Bär, 2002).

(3) With regard to the possibility of metabolic interactions between D-tagatose and fructose, enzyme kinetic considerations and data from a liver perfusion study indicate that D-tagatose at the levels which may result from its intended use, will not interfere with the metabolism of co-ingested fructose.

In conclusion, there is a substantial body of evidence to support the safety of D-tagatose as a food ingredient.

On the basis of the available toxicological data on D-tagatose and in consideration of the close similarity between the metabolic effects of D-tagatose and fructose, it is concluded that D-tagatose, at the intake which would result from its intended uses in food, does not present a significant risk for human health.

12. REFERENCES

Abu-Amsha Caccetta R., 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.

Adams M., Fisher M. and Koval G.J. (1959). The influence of dietary carbohydrate on kidney and liver damage and serum cholesterol in the rat. Fed. Proc. 18: 178 (abstr. 701).

Agius L. (1994). Control of glucokinase translocation in rat hepatocytes by sorbitol and the cytosolic redox state. Biochem. J. 298: 237-243.

Agius L. (1998). The physiological role of glucokinase binding and translocation in hepatocytes. Advan. Enzyme Regul. 38: 303-331.

Agius L., Peak M., Newgard C.B., Gomez-Foix A.M. and Guinovart J.J. (1996). Evidence for a role of glucose-induced translocation of glucokinase in the control of hepatic glycogen synthesis. J. Biol. Chem. 271 (48): 30479-30486.

Åkerblom H.K., Koivukangas T., Puukka R. and Mononen M. (1981). The tolerance of increasing amounts of dietary xylitol in children. In: Xylitol – Clinical Investigations in Humans (eds. Raunhardt O. and Ritzel G.). Bern, Stuttgart, Wien.

Ammann P., Rizzoli R. and Fleisch H. (1988). Influence of the disaccharide lactitol on intestinal absorption and body retention of calcium in rats. J. Nutr. 118: 793-795.

Appel M.J. (2002). A 28-day comparative study with D-tagatose in male rats of 6 different strains. Unpublished report No. V4252 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Bär A. (1985a). Safety assessment of polyol sweeteners – some aspects of toxicity. Food Chem. 16: 231-241.

Bär A. (1985b). Urolithiasis and nephrocalcinosis in xylitol- and sorbitol-fed male mice of two different strains. In: Xylitol and oxalate, Int. J. Vitam. Nutr. Res., Suppl. 28, Bär A. and Ritzel G. (eds.), p. 69-89.

Bär A. (1987). Toxicological aspects of sugar alcohols – studies with xylitol. In: Low digestibility carbohydrates, Leegwater D.C. , Feron V.J. and Hermus R.J.J. (eds.), Pudoc, Wageningen, p. 42-50.

Bär A. (1988). Sugars and adrenomedullary proliferative lesions: the effects of lactose and various polyalcohols. J. Am. Coll. Toxicol. 7 (1): 71-81.

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, p. 209-257.

Bär A. (1998). An evaluation of the caloric value of D-tagatose. Manuscript for MD Foods Ingredients amba.

Bär 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.

Baksi S.N. and Hughes M.J. (1984). Alteration of adrenal catecholamine levels in the rat after dietary calcium and vitamin D deficiencies. J. Autonomic Nervous System 11: 393-396.

Bär A. and Leeman W.R. (1999). L-Sorbose but not D-tagatose induces hemolysis of dog erythrocytes in vitro. Regul. Toxicol. Pharmacol., 29 (2,2/2): S43-S45.

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.

Bender A.E., Damji K.B., Khan M.A., Khan I.H., McGregor L. and Yudkin J. (1972). Sucrose induction of hepatic hyperplasia in the rat. Nature 238: 461-462.

Bergstra A.E., Lemmens A.G. and Beynen A.C. (1993). Dietary fructose vs. glucose stimulates nephrocalcinogenesis in female rats. J. Nutr. 123: 1320-1327.

Bertelsen H., Andersen H. and Tvede M. (2001). Fermentation of D-tagatose by human intestinal bacteria and dairy lactic acid bacteria. Microbial Ecology in Health and Disease 13: 87-95.

Bertelsen H., Jensen B.B. and Buemann B. (1999). D-Tagatose – a novel low-calorie bulk sweetener with prebiotic properties. World Rev. Nutr. Diet. 85: 98-109.

BIBRA (1990a). A 14-day range finding study with an immobilised enzyme system in rats. Unpublished report of the British Industrial Biological Research Association, Carshalton, Surrey (U.K.).

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

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.

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.

Bössmann K. (1977). Entwicklung und Anwendung einer Methode zur experimentellen Karieserzeugung in vitro. Die Quintessenz, Berlin.

Bosland M.C. and Bär A. (1984). Some functional characteristics of adrenal medullary tumors in aged male Wistar rats. Vet. Pathol. 21: 129-140.

Brommage R., Binacua C., Antille S. and Carrié A.-L. (1993). Intestinal calcium absorption in rats is stimulated by dietary lactulose and other resistant sugars. J. Nutr. 123: 2186-2194.

Brulé D., Sarwar G. and Savoie L. (1992). Changes in serum and urinary uric acid levels in normal human subjects fed purine-rich foods containing different amounts of adenine and hypoxanthine. J. Am. Coll. Nutr. 11 (3): 353-358.

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}P magnetic resonance spectroscopy. Metabolism 49: 1335-1339.

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.

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., 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.

Capen C.C. (2001). Overview of structural and functional lesions in endocrine organs of animals. Toxicol. Pathol. 29 (1): 8-33.

Cerami A., Vlassara H. and Brownlee M. (1986). Role of nonenzymatic glycosylation in atherogenesis. J. Cell. Biochem. 30: 111-120.

Chatila R. and West A.B. (1996). Hepatomegaly and abnormal liver tests due to glycogenosis in adults with diabetes. Medicine 75 (6): 327-333.

Cifone M.A. (1994). Mutagenicity test on D-tagatose in the L5178Y TK+/- mouse lymphoma forward mutation assay. Report of Hazleton Washington, Inc., Vienna, VA for Biospherics Inc., Beltsville, MD.

Clark D. and Haynes D. (1988). The glycogen storage disease (gsd/gsd) rat. Curr. Top. Cell. Reg. 29: 217-263.

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

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.

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.

De Groot A.P., Til H.P., Feron V.J., Dreef-van der Meulen H.C. and Willems M.I. (1974). Two-year feeding and multigeneration studies in rats on five chemically modified starches. Fd. Cosmet. Toxicol. 12: 651-663.

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.

De Paola D.P. (1986). Executive summary of scientific consensus conference on methods for assessment of the cariogenic potential of foods. J. Dent. Res. 65: 1540-1543.

Detheux M., Van der Cammen A. and Van Schaftingen E. (1991). Effectors of the regulatory protein acting on liver glucokinase: A kinetic investigation. Eur. J. Biochem. 200: 553-561.

Diamantis I. and Bär A. (2001). Effect of an oral 30-g dose of D-tagatose on the plasma uric acid levels of healthy male volunteers. Unpublished study report.

Diamantis I. and Bär A. (2002). Effect of an oral 15-g dose of D-tagatose on the plasma uric acid levels of hyperuricemic male volunteers. Unpublished study report.

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.

Durand A.M.A., Fisher M. and Adams M. (1968). The influence of type of dietary carbohydrate: Effect on histological findings in two strains of rats. Arch. Pathol. 85: 318-324.

FDA (1999). US Food and Drug Administration, Letter dated October 25, 1999 from J. Hoadley, Division of Technical Evaluation, Of-

fice of Food Labeling, Center for Food Safety and Applied Nutrition (CFSAN), to M. Wertzberger.

FDA (2001). US Food and Drug Administration, Letter dated October 25, 2001 from A. Rulis, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition (CFSAN), to D. McColl.

Fernández-Novell J.M., Castel S., Bellido D., Ferrer J.C., Vilaró S. and Guinovart J.J. (1999). Intracellular distribution of hepatic glucokinase and glucokinase regulatory protein during the fasted to refed transition in rats. FEBS Letters 459: 211-214.

Fishbein R., Benkovic A., Schray K.J., Siewers I.J., Steffens J.J. and Benkovic S.J. (1974). Anomeric specificity of phosphofructokinase from rabbit muscle. J. Biol. Chem. 249 (19): 6047-6051.

Förster H., Boecker S. and Ziege M. (1972). Anstieg der Konzentration der Serumharnsäure 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 Serumharnsäurekonzentration nach oraler Zufuhr von Fructose, Sorbit und Xylit. Z. Ernährungswiss. 10: 394-396.

Froesch E.R. (1978). Essential fructosuria, hereditary fructose intolerance, and fructose-1,6-diphosphatase deficiency. In: "The metabolic basis of inherited diseases" (Stanbury, J.B., Wyngaarden, J.B., Fredrickson, D.S., eds.), 4th Ed., New York: McGraw-Hill, pp. 121-136.

Fukahori M., Sakurai H., Akatsu S., Negishi M., Sato H., Goda T. and Takase S. (1998). Enhanced absorption of calcium after oral administration of maltitol in the rat intestine. J. Pharm. Pharmacol. 50: 1227-1232.

Garrel D.R., Verdy M., PetitClerc C., Martin C., 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.

Gilboe D.P. and Nutall F.Q. (1982). Stimulation of liver glycogen particle synthase D phosphatase activity by caffeine, AMP, and glucose 6-phosphate. Arch. Biochem. Biophys. 219(1): 179-185.

Gitzelmann R., Steinmann B. and Van den Berghe G. (1995). Disorders of fructose metabolism. In: The metabolic and molecular bases of inherited disease (C.R. Scriver, Beaudet A.L., Sly W.S., Valle D. eds.). 7th ed. 1: p. 905-934, McGraw-Hill, New York.

Glinsmann W.H., Irausquin H. and Park Y.K. (1986). Evaluation of health aspects of sugars contained in carbohydrate sweeteners. J. Nutr. 116 (Suppl. 11): S1-S216.

Goda T., Takase S. and Hosoya N. (1993). Maltitol-induced increase of transepithelial transport of calcium in rat small intestine. J. Nutr. Sci. Vitaminol. 39: 589-595.

Grenby T.H. (1985). Dental and lipogenic effects of polyols and Lycasins replacing sucrose in the diet of laboratory rats. Adv. Diet. Nutr. (Int. Congr.), p. 86-89.

Hagihara M., Togari A., Matsumoto S. and Nagatsu T. (1990). Dietary calcium deprivation increased the levels of plasma catecholamines and catecholamine-synthesizing enzymes of adrenal glands in rats. Biochem. Pharmacol. 39 (7): 1229-1231.

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.

Hirst E.L., Hough L. and Jones J.K.N. (1949). The structure of Sterculia setigera gum. Part I. An investigation by the method of paper partition chromatography of the products of hydrolysis of the gum. J. Chem. Soc., 1949: 3145-3151.

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.

Ibrahim O.O. and Spradlin J.E. (1993). D-Tagatose production by enzymatic isomerization of D-galactose. EP 0 552 894 A2.

Imfeld T. (1983). Identification of low caries risk dietary components. In: Monographs in Oral Science, 11: 1-198. Myers H.M. (ed.), Karger, Basel.

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 product's qualification as being "safe for teeth". Study performed after different plaque-adaptation periods. Dental Institute, University of Zurich, Switzerland.

James C.L., Rellos P., Ali M., Heeley A.F. and Cox T.M. (1996). Neonatal screening for hereditary fructose intolerance: frequency of the most common mutant aldolase B allele (A149P) in the British population. J. Med. Genet., 33: 837-841.

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.

Jørgensen H. and Laerke H.N. (1998). The influence of D-tagatose on digestibility and energy metabolism in pigs (unpublished report).

Karabinos J.V. (1952). Psicose, sorbose and tagatose. Adv. Carbohydr. Chem. Biochem. 7: 99-136.

Kaul P., Sidhu H., Sharma S.K. and Nath R. (1996). Calculogenic potential of galactose and fructose in relation to urinary excretion of lithogenic substances in vitamin B_6 deficient and control rats. J. Am. Coll. Nutr. 15 (3): 295-302.

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

Kelsay J.L., Behall K.M., Moser P.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.

Klinge O. and Blinzler M. (1972). Schwangerschaftsbedingte Epithelreaktionen der Rattenleber. Virchows Arch. Abt. B Zellpath. 11: 257-262.

Knuuttila M., Svanberg M. and Hämäläinen M. (1989). Alterations in rat bone composition related to polyol supplementation of the diet. Bone and Mineral 6: 25-31.

Koerner T.A.W., Voll R.J., Ashour A-L.E. and Younathan E.S. (1976). The fructose 6-phosphate site of phosphofructokinase. Epimeric specificity. J. Biol. Chem. 251 (10): 2983-2986.

Kruger C.L., Whittaker M.H. and Frankos V.H. (1999a). Genotoxicity tests on D-tagatose. Regul. Toxicol. Pharmacol., 29 (2,2/2): S39-S42.

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

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

Kurokawa Y., Hayashi Y., Maekawa A., Takahashi M., Kukubo T. (1985). High incidences of pheochromocytomas after long-term administration of retinol acetate to F344/DuCrj rats. J. Natl. Cancer Inst. 74: 715-723.

Lacour B., Ohan J., Aznag A. and Drüeke T.B. (1995). Stimulating effects of sorbitol and L-xylose on rat ileal Ca transport in vitro. Miner. Electrolyte Metab. 21: 391-397.

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 mi-

crobiota from the gastrointestinal tract of pigs. J. Nutr. 130: 1772-1779.

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

Lardy H.A. (1951). Phosphofructokinase and aldolase. In: Phosphorus metabolism (McElroy W.D. and Glass B., eds.), p. 116. John Hopkins Press, Baltimore.

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.

Levi B. and Werman M.J. (1998). Long-term fructose consumption accelerates glycation and several age-related variables in male rats. J. Nutr. 128 (9): 1442-1449.

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 Bär A. (2003). Chronic toxicity and carcinogenicity study with D-tagatose and fructose in Wistar rats. Addendum 1 to Report V45333 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lina B.A.R., Bos-Kuijpers M.H.M., Til H.P. and Bär A. (1996). Chronic toxicity and carcinogenicity study of erythritol in rats. Reg. Toxicol. Pharmacol. 24: 264-279.

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 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 si-

multaneously 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.

Lina B.A.R. and Kuper C.F. (2002). Chronic toxicity and carcinogenicity study with D-tagatose and fructose in Wistar rats. Unpublished report No. V4533 of TNO Nutrition and Food Research Institute, Zeist, The Netherlands for MD Foods amba, Denmark.

Lindberg B. (1955). Studies on the chemistry of lichens. VIII. Investigation of a Dermatocarpon and some Roccella species. Acta Chem. Scand. 9 (6): 917-919.

Lingelbach L.B., Mitchell A.E., Rucker R.B. and McDonald R.B. (2000). Accumulation of advanced glycation endproducts in aging male Fischer 344 rats during long-term feeding of various dietary carbohydrates. J. Nutr. 130 (5): 1247-1255.

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

Lopez H.W., Coudray C., Levrat-Verny M.-A., Feillet-Coudray C., Demigné C. and Rémésy C. (2000). Fructooligosaccharides enhance mineral apparent absorption and counteract the deleterious effects of phytic acid on mineral homeostasis in rats. J. Nutr. Biochem. 11: 500-508.

Lorinet A. (1975). Étude comparée de l'effet du glucose et du fructose libres et combinés sur l'absorption et la rétention du calcium. Ann. Nutr. Alim. 29: 313-320.

Lynch B.S., Tischler A.S., Capen C., Munro I.C., McGirr L.M. and McClain R.M. (1996). Low digestible carbohydrates (polyols and lactose): significance of adrenal medullary proliferative lesions in the rat. Reg. Toxicol. Pharmacol. 23: 256-297.

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.

Madenokoji N., Iino H., Shimizu T., Hayakawa J. and Sakashita M. (2003). Blunting effect of D-tagatose on blood glucose when administered orally with glucose in volunteer donors of boundary glycemic level. J. Jap. Soc. Clin. Nutr. 25(1): 21-28.

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.

Malthus R., Clark D.G., Watts C. and Sneyd J.G.T. (1980). Glycogen-storage disease in rats, a genetically determined deficiency of liver phosphorylase kinase. Biochem. J. 188: 99-106.

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.

Marble A. (1947). The diagnosis of the less common meliturias including pentosuria and fructosuria. Med. Clin. N. Am. 31: 313-325.

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

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

Maxton, D.G., Bjarnason, I., Reynolds, A.P., Catt, S.D., Peters, T.J. and Menzies, I.S. (1986). Lactulose ^{51}Cr-labelled ethyl-

enediaminetetra-acetate, L-rhamnose and polyethyleneglycol 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.

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.

Michalcakova S., Zalibera L., Sturdik E. and Liptaj T. (1986). NMR study of monosaccharide phosphorylation by yeast hexokinase. Biologia (Bratisl.) 41 (12): 1157-1166.

Monnier V.M. (1990). Nonenzymatic glycosylation, the Maillard Reaction and the aging processes. J. Gerontol. 45: B105-B111.

Monnier V.M., Stevens V.J. and Cerami A. (1979). Nonenzymatic glycosylation, sulfhydryl oxidation , and aggregation of lens proteins in experimental sugar cataracts. J. Exp. Med. 150: 1098-1107.

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.

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.

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

Nathan D.M. (1996). The pathophysiology of diabetic complications: how much does the glucose hypothesis explain? Ann. Intern. Med. 124 (1 pt 2): 86-89.

Niculescu L., Veiga-Da-Cunha M. and Van Schaftingen E. (1997). Investigation on the mechanism by which fructose, hexitols and other compounds regulate the translocation of glucokinase in rat hepatocytes. Biochem. J. 321: 239-246.

Normén L., Laerke H.N., Langkilde A.-M. and Andersson 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.

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.

Ohta A., Ohtsuki M., Baba S., Hirayama M. and Adachi T. (1998). Comparison of the nutritional effects of fructo-

oligosaccharides of different sugar chain length in rats. Nutr. Res. 18 (1): 109-120.

Oimomi M., Sakai M., Ohara T., Igaki N., Nakamichi T., Hata F. and Baba S. (1989). Acceleration of fructose mediated collagen glycation. J. Int. Med. Res. 17: 249-253.

Okuno Y. and Gliemann J. (1986). Transport of glucose and fructose in rat hepatocytes at 37°C. 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.

Parrish F.W., Hicks K. and Doner L. (1980). Analysis of lactulose preparations by spectrophotometric and high performance liquid chromatographic methods. J. Dairy Sci. 63: 1809-1814.

Paterna J.C., Boess F., Stäubli A. and Boelsterli U.A. (1998). Antioxidant and cytoprotective properties of D-tagatose in cultured murine hepatocytes. Toxicol. Appl. Pharmacol. 148:117-125.

Pelgrom S.M.G.J. and van Raaij M.T.M. (2001). Pheochromocytomas. In: Factsheets for the (Eco)toxicological Risk Assessment Strategy of the National Institute of Public Health and the Environment (RIVM), Luttik R. and van Raaij M.T.M. (eds.), Bilthoven, p. 39-47.

Petersen A., Steinmann B. and Gitzelmann R. (1992). Essential fructosuria: increased levels of fructose 3-phophate in erythrocytes. Enzyme 46: 319-323.

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

Que L. and Gray G.R. (1974). ^{13}C nuclear magnetic resonance spectra and the tautomeric equilibria of ketohexoses in solution. Biochemistry 13 (1): 146-153.

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

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. Biochemistry 16 (10): 2169-2175.

Reiser S., Powell A.S., Scholfield D.J., Panda P., Ellwood K.C. and Canary J.J. (1989). Blood lipids, lipoproteins, apoproteins, and uric acid in men fed diets containing fructose or high-amylose cornstarch. Am. J. Clin. Nutr. 49: 832-839.

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.

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

Roe F.J.C. (1989). Relevance for man of the effects of lactose, polyols and other carbohydrates on calcium metabolism seen in rats: a review. Human Toxicol. 8: 87-98.

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

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

Ruhe R.C., Coordt M.C. and McDonald R.B. (1996). Effects of caloric restriction and source of dietary carbohydrate on glycemic status of the Fischer 344 rat. Aging Clin. Exp. Res. 8: 287-291.

Saari J.T. and Dahlen G.M. (1999). Early and advanced glycation end-products are increased in dietary copper deficiency. J. Nutr. Biochem. 10: 210-214.

Sachs B., Sternfeld L. and Kraus G. (1942). Essential fructosuria: its pathophysiology. Am. J. Dis. Child. 63: 252-269.

Sakuma K. (2002). Molecular mechanism of the effect of fructooligo-saccharides on calcium absorption. Biosci. Microflora 21 (1): 13-20.

Sánchez 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. (1992). Multiple fat rat study. Unpublished report of Biosperics, Inc., Beltsville, MD.

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

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.

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

SCF (1985). Reports of the Scientific Committee for Food, 16th Series.

Schachtele C.F. et al. (1986). Human plaque acidity models. J. Dent. Res., 65 (Spec. Iss.): 1530-1531.

Schartow D. (1965). Langzeitergebnisse nach Fructoseverabreichung an Diabetiker. Inaugural-Diss. Med. Fakultät der Ludwig-Maximilians-Uni, München. (Direktor: Prof. Dr. W. Seitz).

Schleifer K.H., Hartinger A. and Götz F. (1978). Occurrence of D-tagatose-6-phosphate pathway of D-galactose metabolism among staphylococci. FEMS Microbiology Letters 3 (1): 9-11.

Scholz-Ahrens K.E. and Schrezenmeir J. (2002). Inulin, oligofructose and mineral metabolism – experimental data and mechanism. Br. J. Nutr. 87 (Suppl. 2): 179-186.

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.

Seegmiller J.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. USA 87: 8326-8330.

Seoane J., Gómez-Foix A.M., O'Doherty R.M., Gómez-Ara C., Newgard C.B. and Guinovart J.J. (1996). Glucose 6-phosphate produced by glucokinase, but not hexokinase I, promotes the activation of hepatic glycogen synthase. J. Biol. Chem. 271 (39): 23756-23760.

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.

Silver S. and Reiner M. (1934). Essential fructosuria: report of three cases with metabolic studies. Arch. Intern. Med. 54: 412-426.

Sinkeldam E.J., Woutersen R.A., Hollanders V.M.H., Til H.P., Van Garderen-Hoetmer A. and Bär A. (1992). Subchronic and chronic toxicity/carcinogenicity feeding studies with lactitol in rats. J. Am. Coll. Toxicol. 11 (2): 165-188.

Smits van Proije A.E., de Groot A.P., Dreef-Van der Meulen H.C. and Sinkeldam E.J. (1990). Chronic toxicity and carcinogenicity study of isomalt in rats of mice. Food Chem. Toxicol. 28: 243-251.

Steinitz H., Steinitz K. and Mizrachi O. (1963). Essentielle Fructosurie. Untersuchungen des intermediären Stoffwechsels bei intravenöser Fructosebelastung. Schweiz. Med. Wschr. 93: 756-760.

Steinmann B., Baerlocher K. and Gitzelmann R. (1975). Hereditäre Störungen des Fruktosestoffwechsels. Belastungsproben mit Fruktose, Sorbitol und Dihydroxyaceton. Nutr. Metabol. 18 (Suppl. 1): 115-132.

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

Suárez G., Rajaram R., Oronsky A.L. and Gawinowicz M.A. (1989). Nonenzymatic glycation of bovine serum albumin by fructose (fructation). J. Biol. Chem. 264 (7): 3674-3679.

Sugirs (2004). Sydney University's Glycaemic Index Research Service, Unpublished Report for Arla Foods, March 2004).

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

Szepesi B., Levin G., Zehner L. and Saunders J. (1996). Antidiabetic effect of D-tagatose in SHR/N-cp rats. FASEB J. 10 (3): A 461.

Szombathy G., Teichmann F. and Jezerniczky J. (1969). Essential benign fructosuria. Acta Paediatr. Acad. Scientiar. Hungar. 10 (3-4): 283-290.

Szumilo T. and Russa R. (1982). Accumulation of D-tagatose by galactose-grown Mycobacteria. Ann. Univ. Mariae Curie Sklodowska, Sect. D. Med., 37 (2): 11-18.

Tatibouët 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.

Taylor S.L., Lambrecht D.M. and Hefle S.L. (2005). Tagatose and milk allergy. Allergy 60 (3): 412-413.

Thorburn A.W., Storlien L.H., Jenkins A.B., Khouri S. and Kraegen E.W. (1989). Fructose-induced in vivo insulin resistance and elevated plasma triglyceride levels in rats. Am. J. Clin. Nutr. 49: 1155-1163.

Tischler A.S., Powers J.F., Downing J.C., Riseberg J.C., Shahsavari M., Ziar J. and McClain R.M. (1996). Vitamin D_3, lactose, and xylitol stimulate chromaffin cell proliferation in the rat adrenal medulla. Toxicol. Appl. Pharmacol. 140: 115-123.

Tischler A.S., Powers J.F., Pignatello M., Tsokas P., Downing J.C. and McClain R.M. (1999). Vitamin D_3-induced proliferative lesions in the rat adrenal medulla. Toxicol. Sci. 51 (1): 9-18.

Totten E.L. (1949). I. The synthesis of D-tagatose and D-tagatose-6-phosphate and the metabolism of this phosphorylated sugar by animal tissues. Thesis, University of Wisconsin.

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.

Trimmer G.W., Phillips R.D. and Lonardo E.C. (1993). 90-Day dietary oral toxicity study in rats with D-tagatose (MRD-92-456). Study report by Exxon Biomedical Science, Inc., East Millstone, N.J., Biospherics Inc., Beltsville, MD.

Troyano, E., Olano, A., Fernández-Díaz, M., Sanz, J. and Martínez-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.

Valeri F., Boess F., Wolf A., Göldlin C. and Boelsterli U.A. (1997). Fructose and tagatose protect against oxidative cell injury by iron chelation. Free Radical Biology and Medicine 22:257-268.

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

Van den Berghe (1997). Hereditary fructose intolerance (HFI). Presentation for European Metabolic Group, 30th Conference, Knokke (B), June, 1997.

Van Schaftingen E. and Vandercammen A. (1989). Stimulation of glucose phosphorylation by fructose in isolated rat hepatocytes. Eur. J. Biochem. 179: 173-177.

Van Schaftingen E., Veiga-da-Cunha M. and Niculescu L. (1997). The regulatory protein of glucokinase. Biochem. Soc. Trans. 25 (1): 136-140.

Venema K., Vermunt S.H.F. and Brink E.J. (2004). D-Tagatose increases butyrate production by the colonic microbiota in healthy men and women. Manuscript to be submitted for publication.

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.

Vishwanath V., Frank K.E., Elmets C.A., Dauchot P.J. and Monnier V.M. (1986). Glycation of skin collagen in type I diabetes mellitus. Correlation with long-term complications. Diabetes 35: 916-921.

VTT (1998). Research Report No. BEL 399/98 of VTT Biotechnology and Food Research, Espoo, Finland.

Werman M.J. and Levy B. (1997). The chronic effect of dietary fructose intake on glycation and collagen cross-linking in rats. Am. J. Clin. Nutr. 66 (7/1): A 94.

Whistler R.L., Singh P.P. and Lake W.C. (1974). D-Psicose metabolism in the rat. Carbohydr. Res. 34: 200-202.

WHO (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. 17th Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, p. 368-371 (phosphated distarch phosphate).

WHO (1982). Toxicological evaluation of certain food additives and food contaminants. 26th Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, p. 95-150 (modified starches).

WHO (1983). Toxicological evaluation of certain food additives and food contaminants. 27th Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, p. 82-94 (lactitol), p. 161-202 (xylitol).

WHO (1987). Toxicological evaluation of certain food additives. 31st meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additive Series No. 22, p. 5-9 (enzymes derived from A. Oryzae), WHO, Geneva.

WHO (2001). Safety evaluation of certain food additives and contaminants. 55th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series No. 46, p. 21-47, WHO, Geneva.

WHO (2002). Safety evaluation of certain food additives and contaminants. 57th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series No. 48, p. 79-87, WHO, Geneva.

WHO (2004a). Safety evaluation of certain food additives and contaminants. 61st Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additives Series No. 52, WHO, Geneva.

WHO (2004b). Summary and Conclusions 63rd Meeting of the Joint FAO/WHO Expert Committee on Food Additives, p. 1-18

Wolff G.-J. and Breitmaier E. (1979). ^{13}C-NMR-spektroskopische Bestimmung der Keto-Form in wässrigen Lösungen der D-Fructose, L-Sorbose und D-Tagatose. Chem. Ztg. 103 (6): 232-233.

Yoshida M., Ishibashi S., Nakazawa M., Tamura H., Uchimoto H., Kawaguchi K., Yoshikawa K., Hamasu Y. and Sumi N. (1995). The mechanism of lactitol (NS-4) in inducing adrenomedullary proliferative lesion in rats. J. Toxicol. Sci. 20 (Suppl. 1): 37-45.

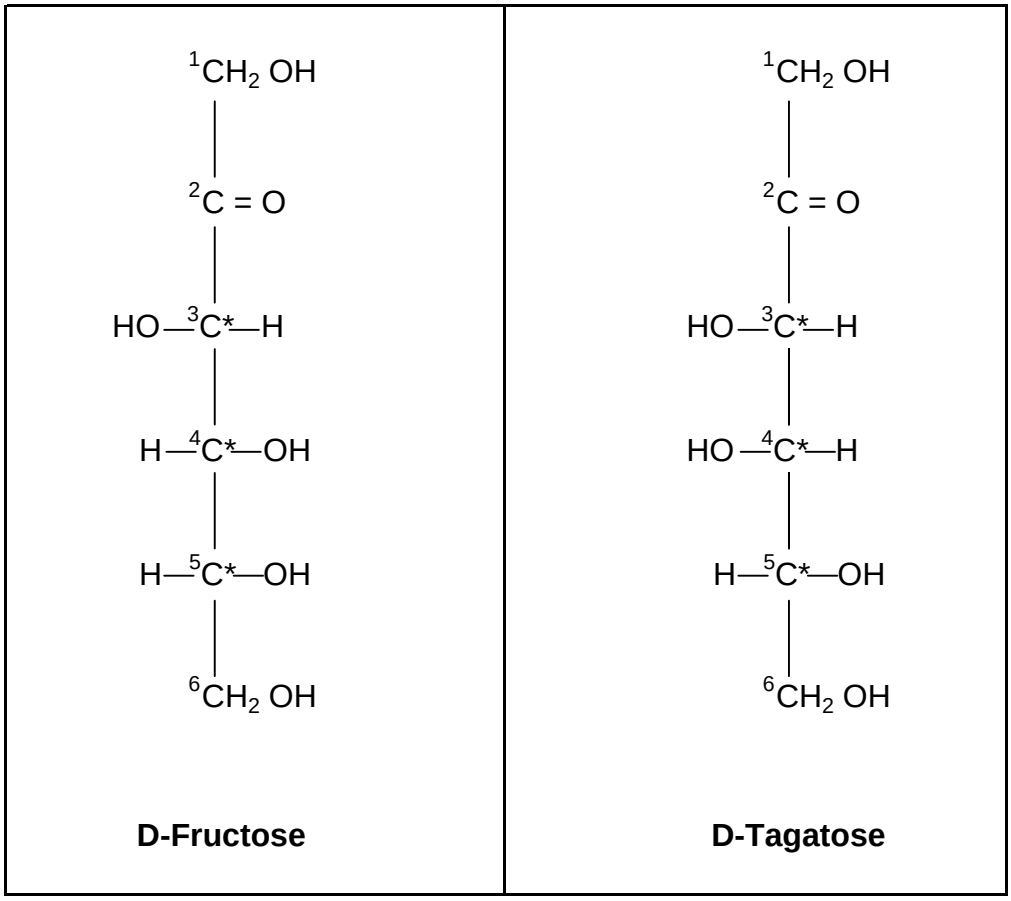
Younes H., Demigné C. and Rémésy C. (1996). Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. Br. J. Nutr. 75: 301-314.

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

Zehner L.R., Levin G.V., Saunders J.P. and Beadle J.R. (1994). D-Tagatose as anti-hyperglycemic agent. United States Patent No. 5,356,879 of Biospherics Inc., Beltsville, Md.

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.

Figure 1 Molecular Structures



* chiral carbon

Figure 2 Production of D-tagatose

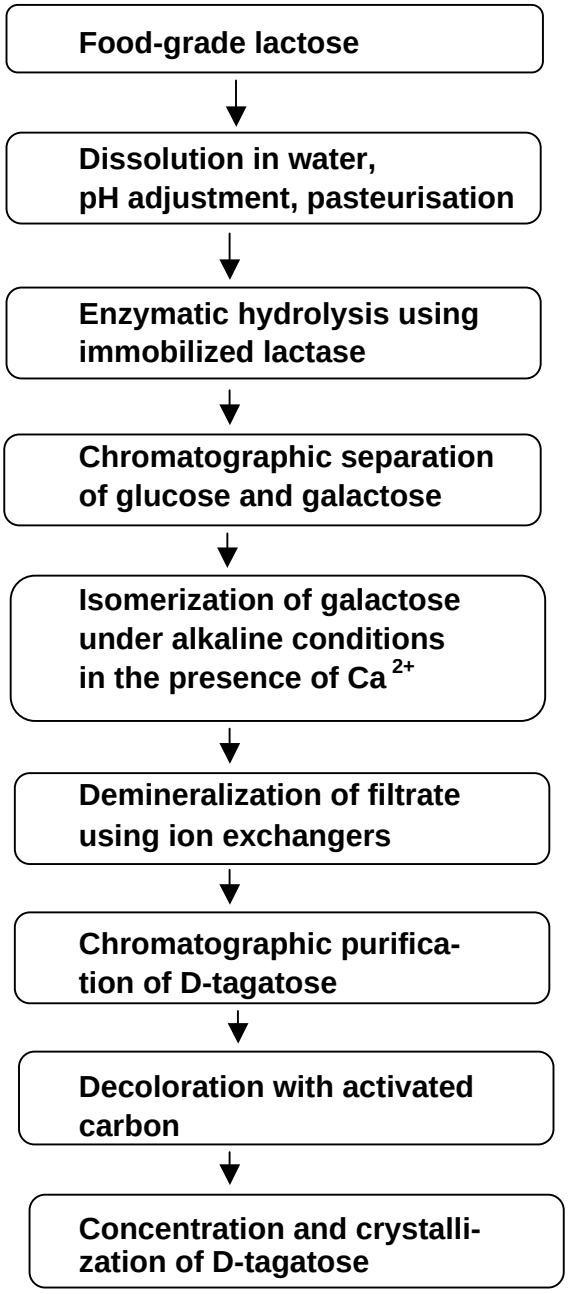
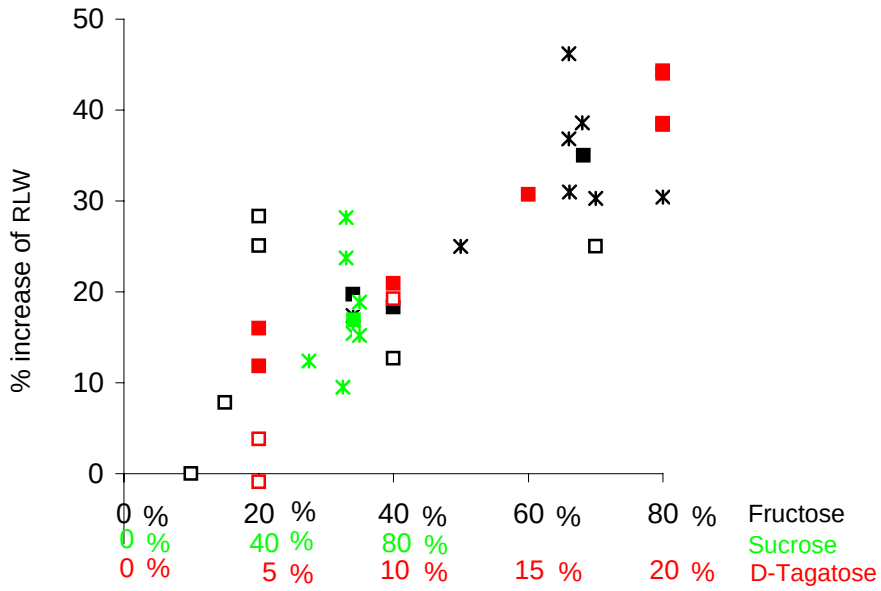


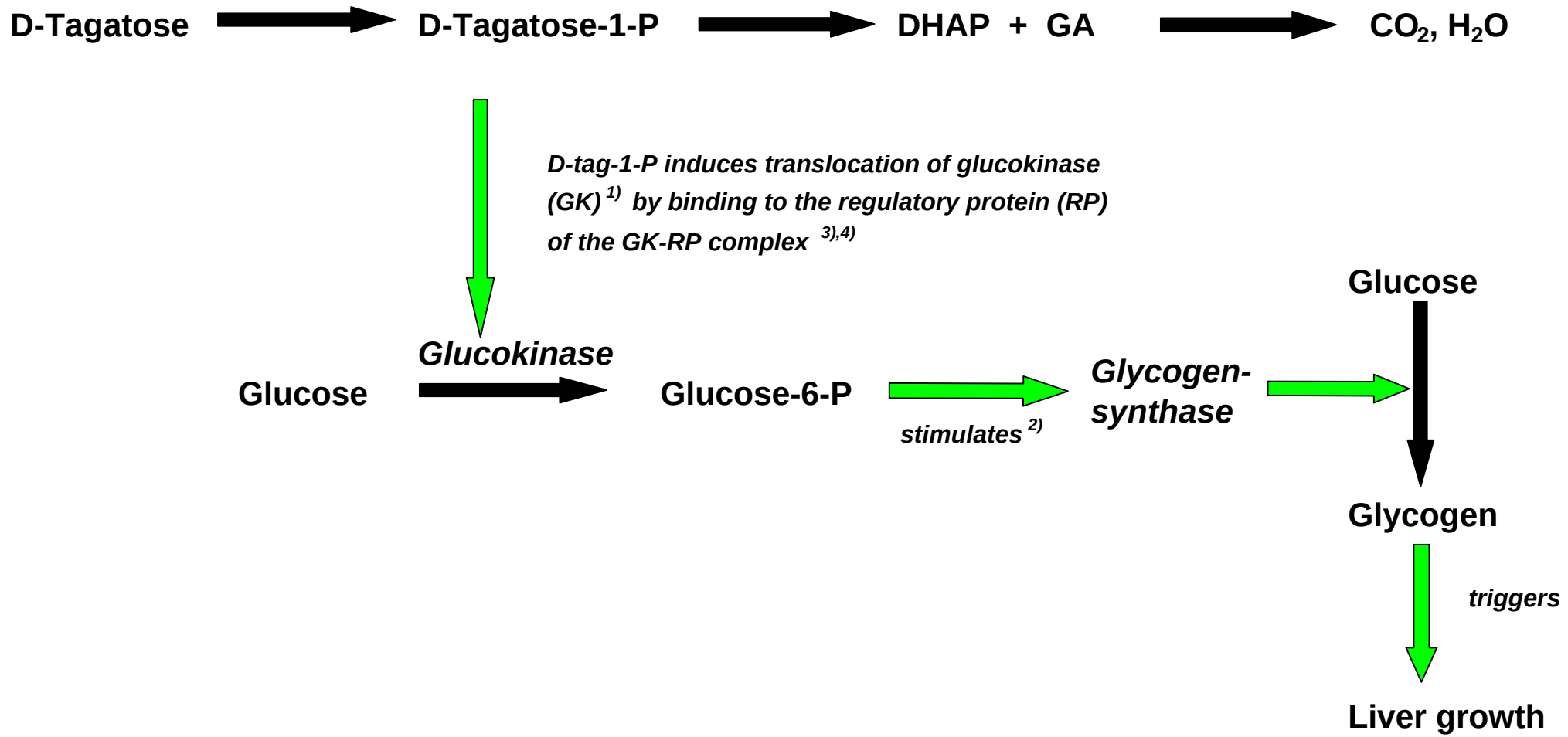
Figure 3 **Increase of relative liver weights in rats ingesting diets with different levels of fructose, sucrose and D-tagatose for ≥ 3 weeks (data from 20 different studies[a])**



Legend: Black, green and red symbols represent liver weights of rats fed diets with fructose, sucrose a D-tagatose, respectively. Controls received corresponding diets with glucose or starch. Filled and empty symbols signify killing of rats under non-fasted or fasted conditions, respectively. A crossed symbol was used when the feeding condition was not specified.

(For references see Bär, 1999)

Figure 4: Mechanism of D-tagatose induced liver enlargement in rats



References

1) Agius L. (1998). The physiological role of glucokinase binding and translocation in hepatocytes. Adv. Enzyme Regul. 38: 303-331.

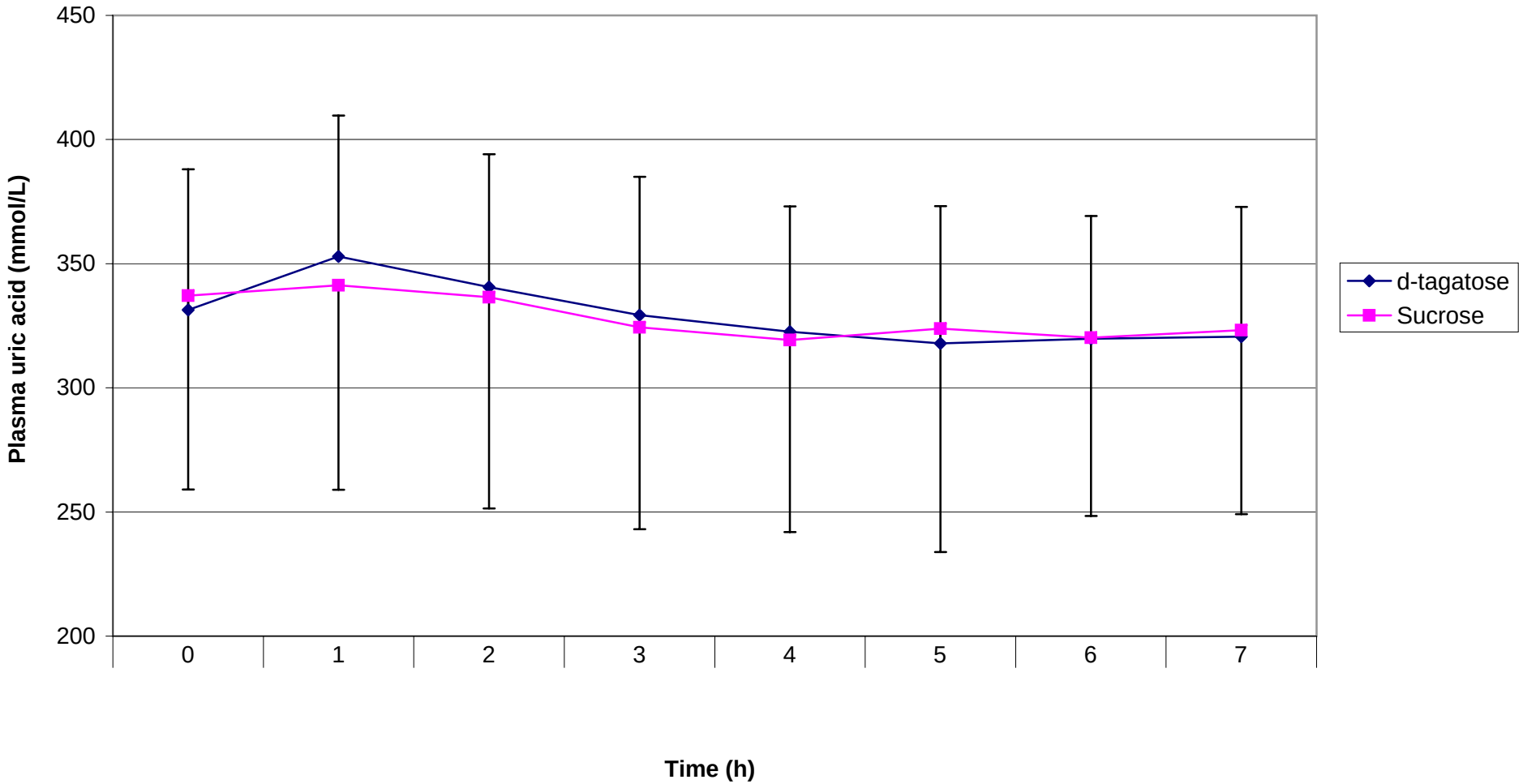
2) Seoane J., Gómez-Foix A.M., O'Doherty R.M., Gómez-Ara C., Newgard C.B. and Guinovart J.J. (1996). Glucose 6-phosphate produced by glucokinase, but not hexokinase I, promotes the activation of hepatic glycogen synthase. J. Biol. Chem. 271 (39): 23756-23760.

3) Niculescu L., Veiga-Da-Cunha M. and Van Schaftingen E. (1997). Investigation on the mechanism by which fructose, hexitols and other compounds regulate the translocation of glucokinase in rat hepatocytes. Biochem. J. 321: 239-246.

4) Agius L., Peak M., Newgard C.B., Gomez-Foix A.M. and Guinovart J.J. (1996). Evidence for a role of glucose-induced translocation of glucokinase in the control of hepatic glycogen synthesis. J. Biol. Chem. 271 (48): 30479-30486.

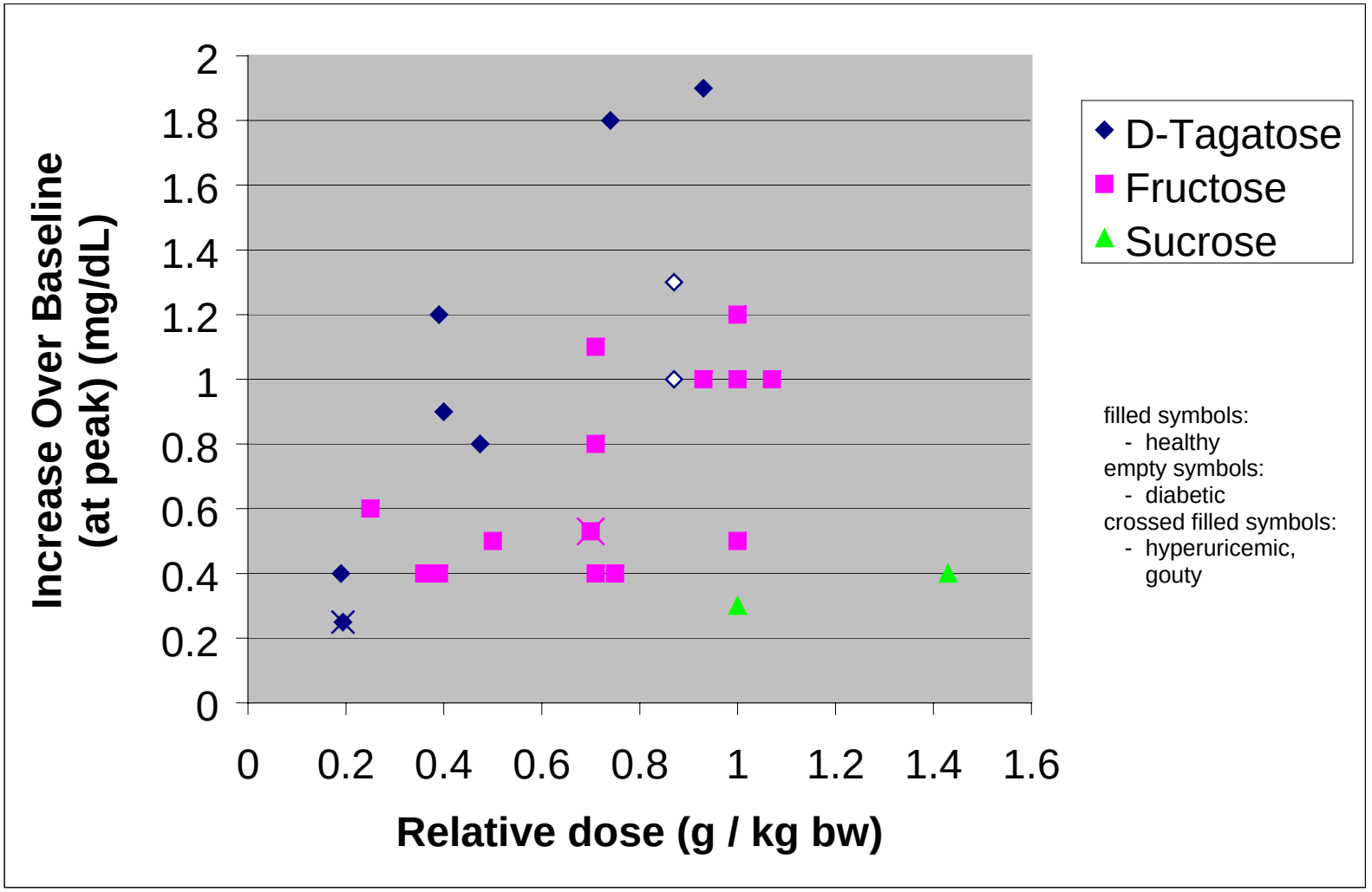
Figure 5 **Plasma uric acid before and after intake of a standard breakfast containing about 99g starch and 15 g D-tagatose or sucrose on day 1 of each treatment period (Boesch et al., 2000)**

Note. Values are means ± SD (n = 12 male volunteers). The value at 1 hour after intake of the breakfast with D-tagatose was significantly elevated above the baseline value ($p \leq 0.05$) but it was not different from the value at 1 hour after intake of the breakfast with sucrose.



GRAS_MANU1_FIG 5_200104.xls

Figure 6 **Effect of single oral doses of D-tagatose, fructose or sucrose on serum uric acid (maximum postprandial increase above baseline)**



Note: Results of 14 publications are shown; n = 5-12 subjects/treatment.

References: Boesch et al., 2001; Buemann et al., 2000a, b; Davies et al., 1998; Förster & Ziege, 1971, Diamantis & Bär, 2001, 2002; Förster et al., 1972; Kelsay et al., 1977; Macdonald et al., 1978; Menghini & Della Corte, 1987; Reiser et al., 1984;Saunders et al., 1999a; Seegmiller et al., 1990, Stirpe et al., 1970.

Table 1 Intended applications of D-tagatose in foods and maximum levels of use

Food Category	Description of Foods	Functionality	Proposed Use of D-Tagatose (g per 100 g food as consumed)
Baked goods	Cookies, quick breads, muffins, and quick bread type coffee cakes	Flavor enhancer	2
Beverages: diet/reduced calorie carbonated and non-carbonated beverages	"Diet" and "sugar-free" carbonated beverages; non-carbonated beverages sweetened with low-calorie sweeteners – includes milk-based beverages, juices, juice drinks, teas, and coffee-based beverages (ready-to-drink, prepared from mix, and dry mix forms)	Flavor enhancer	1
Coffee drinks	Coffee drinks such as cappuccino and latte	Flavor enhancer	1
Frozen milk-based desserts, reduced/low fat	Light ice cream (ice milk), frozen milk desserts, low fat and nonfat frozen yogurts, and related frozen novelties	Nutritive substance	3
Hard candies	Hard candies, including regular and dietetic candies	Nutritive substance	15
Health bars and diet soft candies	Low fat, reduced fat, diet meal, energy, or nutrient fortified bars; dietetic soft candies	Nutritive substance	10
Icings	Icings (or glazes), such as those used on cookies, pastries, brownies, and angel food, chiffon, and pound cakes [c]	Nutritive substance	30
Meal replacement/supplement beverages	Meal replacement beverages, diet meal beverages, nutrient supplement beverages (ready-to-drink, prepared from mix, and dry mix forms)	Nutritive substance	5 g per serving (240 mL)
	Protein drinks, including supplements and diet beverages (ready-to-drink, prepared from mix, and dry mix forms)		1
Milk chocolate	Milk chocolate candies and coatings/coverings	Flavor enhancer	3
Ready-To-Eat cereals	All Ready-To-Eat cereals	Nutritive substance	3 g per serving
Smoothies	Fruit and dairy "smoothie" type beverages	Flavor enhancer	1
Soft/chewy candies	Soft/chewy candies such as caramels, toffees, taffies, nougats, creams, fudges, fondant, and fruit-based confectionery (excluding marshmallows, soft jellies, gummis, panned candies, and licorice)	Flavor enhancer and nutritive substance	3
Chewing gum	Toothfriendly (non-cariogenic) chewing gum	Nutritive substance	30
Table top sweeteners, low calorie	Sugar substitutes/replacements	Nutritive substance	1 g per serving
Yogurt	Yogurt	Flavor enhancer	2

Table 2 Estimated two-day average intake of D-tagatose from its proposed uses in food[1)]

Age group	Intake[2)]			
	g/kg bw/d		g/person/d	
	mean	90th perc.	mean	90th perc.
Total population (age ≥ 2 years)	0.08	0.19	4.6	9.8
Preschoolers (age 2-5 years)	0.19	0.37	3.2	6.2
Elementary school children (age 6-12 years)	0.14	0.28	4.3	8.5
Teenagers (age 13-19 years)	0.08	0.16	4.7	9.5
Adults (age ≥ 20 years)	0.06	0.14	4.8	10.5

1) Chewing gum and food supplements are not included (see section 5 for a discussion of EDI from these uses).

2) Data source: 1994-96, 1998 Continuing Survey of Food Intakes by Individuals; includes individuals with 2-day diet recalls; excludes breastfeeding children and pregnant and/or lactating females.

Table 3 **Estimated intake of D-tagatose per eating occasion from its proposed uses in food combined[1)]**

Age Group	Eating occasion	Intake per user (g/person/d)	
		mean	90th perc.
Total population (age ≥ 2 years)			
	Per eating occasion	3.1	6.2
	Breakfast	3.9	7.4
	Lunch[2]	2.9	5.8
	Dinner	2.8	5.8
	Supper	2.9	6.0
	Snack	2.4	4.9
Preschoolers (age 2-5 years)			
	Per eating occasion	2.2	4.5
	Breakfast	3.2	5.8
	Lunch	1.6	3.6
	Dinner	1.7	3.6
	Supper	1.6	3.6
	Snack	1.4	3.4
Elementary school children (age 6-12 years)			
	Per eating occasion	2.8	6.0
	Breakfast	4.3	7.4
	Lunch	1.6	3.8
	Dinner	2.2	5.0
	Supper	2.2	4.7
	Snack	2.1	4.5
Teenagers (age 13-19 years)			
	Per eating occasion	3.6	7.4
	Breakfast	4.9	8.5
	Lunch	3.0	6.2
	Dinner	3.1	5.9
	Supper	3.4	6.6
	Snack	2.7	5.9
Adults (age ≥ 20 years)			
	Per eating occasion	3.2	6.2
	Breakfast	3.8	7.4
	Lunch	3.2	6.0
	Dinner	3.0	5.9
	Supper	3.0	6.0
	Snack	2.5	5.0

[1] Based on USDA CSFII 1994-96 data, intake from chewing gum and formula diets not included

[2] Lunch combines reported consumption at both lunch and brunch

Table 4 Results of genotoxicity studies with D-tagatose

Test	Test system	Concentration	Result	Reference
Bacterial gene mutation [a]	S. typhimurium (TA 1535, TA 1537, TA 1538, TA 98, TA 100); E.coli (WP2uvrA$^-$)	100 - 5000 mg/plate	Negative	Lawlor, 1993; Kruger et al., 1999a
Chromosomal aberration [a,b]	Chinese hamster ovary cells	1250 - 5000 mg/ml	Negative	Murli, 1994a; Kruger et al., 1999a
Micronucleus formation [d]	CD-1 mouse bone marrow	1250-5000 mg/kg bw (p.o.)	Negative	Murli, 1994b; Kruger et al; 1999a
TK-locus mutation [a,c]	Mouse lymphoma cells (L5178Y)	500 - 5000 mg/ml	Negative	Cifone, 1994; Kruger et al., 1999a

[a)] with and without exogenic metabolic activation (rat liver S9 fraction).
[b)] treatment time, 7.4 h (without activation), 2 h (with activation); harvest time, 10 h.
[c)] treatment time, 4 h.
[d)] termination 24, 28 and 72 h after dosing.

Table 5 Results of oral toxicity studies with D-tagatose

Type of study	Species (n)	Dose level (% of diet)	Results	NOAEL (% of diet and/or g/kg bw/d)	References
Acute toxicity test	Rats (5/sex) Mice (5♂)	10 g/kg bw (single dose)	No mortality or reaction to treatment	10 g/kg bw	Trimmer, 1989
Subchronic (90-d) toxicity study	S-D rats (20/sex/group)	0, 5, 10, 20% 10%fruc+10%cellulose	Soft stools (day 1-3); reduced weight gain in 20% group; increased abs. and rel. liver weights in 10, 15, 20% tag groups, some hypertrophy of hepatocytes in 15, 20% group[a)]	5%[c)] [3.7(♂) and 4.1(♀) g/kg bw/d]	Trimmer et al., 1993 Kruger et al., 1999c
Subchronic (29-31 d) study on liver parameters[e)]	S-D rats (20♂/group)	0, 5, 10, 20% tag	Dose-dependent increase of liver glycogen and liver weight[b)]. No ultrastructural (EM) changes of liver tissue except increased glycogen deposition. Slight hypertrophy of hepatocytes. Slightly increased ALAT, ASAT in 20% tag group probably in response	n.d.[d)]	Lina et al., 1998; Bär et al., 1999
Subchronic (6-month) toxicity study	Wistar rats (60♂/group)	0,5,10% tag, 20% fru, 10% tag + 10% fru. Interim kills on days 3, 7, 14, 28, 94, 128 (10♂/group)	Only liver and plasma parameters were examined. No increase of liver weight and no histopathological changes[a)]; no changes of plasma parameters.	10% of diet [5.8 g/kg bw/d (day 1-28); 4.8 g/kg bw/d (day 1-128)]	Lina & de Bie, 2000d

Table 5 continued

Type of study	Species (n)	Dose level (% of diet)	Results	NOAEL	References
Chronic (24-month) toxicity/carcinoge-nicity study	Wistar rats	0, 2.5, 5, 10% tag, 20% fru, 10% tag + 10% fru	Examination of organ weights and his topathology limited to liver, kidneys, adrenals and testes (cecum: weight only). Liver enlargement in 10% tag (♀), 20% fru (♀), 10% tag + fru (♂,♀) but no morphological changes. Increased nephrocalcinosis in♂♂ of all tag dose groups and in 10% tag (♀) and 10% + 10% fru (♀). Increased incidence of adrenomedullary proliferative disease in 2.5% tag (♀), 5% tag (♂,♀), 10% tag (♂,♀) and 10% + 10% fru (♂,♀).	2.5% of diet [< 1 g/kg bw/d]	Lina & Kuper, 2002 Lina & Bär, 2003
Energy balance study (33-d)	Pigs (2/group)	0, 20% tag, 20% suc, 10% tag + 10% suc	No ultrastructural (EM) changes of liver tissue	5 g/kg bw/d	Mann, 1997
Embryotoxicity / teratogeni-city study (range finding)	S-D rats (5♀/group)	0, 4, 8, 12, 16, 20 g tag/kg bw/d (day 6-15 of gestation)	Soft stool and diarrhoea at >12 g/kg bw. (No adverse effects otherwise).	20 g/kg bw/d (11 g/kg bw/d)	Schroeder, 1994a
Embryotoxicity / teratogeni-city study	S-D rats (24♀/group)	0, 4, 12, 20 g tag/kg bw/d (day 6-15 of gestation)	Maternal liver weight increased in 12 and 20 g/kg bw group. No morphological changes in liver. No adverse effects otherwise.	20 g/kg bw/d	Schroeder, 1994b; Kruger et al., 1999b

Abbreviations: tag, D-tagatose; fru, fructose; suc, sucrose; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; S-D, Sprague-Dawley; n.d., not determined; bw, body weight.

[a)] animals killed after overnight fasting

[b)] animals killed in the fed condition

[c)] based on effects on liver weight

[d)] liver weight cannot be used as a basis for determination of the NOAEL since rats were killed in the fed condition (increased weight is partly due to liver glycogen accumulation). D-Tagatose intake was about 11.4 g/kg bw/d at the high-dose level.

[e)] A series of additional studies on the effects of D-tagatose on liver weight and glycogen accumulation was performed but their results are not shown in this table because toxicological end-points (e.g., histopathology) were not examined.

Table 6 Effects of acute oral doses of D-tagatose vs. fructose on human blood uric acid levels

Reference	Type of Subjects	n	Sugar	Dose (g)	Relative Dose (g/kg bw)	Time of Peak Response (minutes after dose)	Normal Range (mg/dL)[a]	Baseline (Time 0) Level (mg/dL)	Peak Level (mg/dL)[a]	Increase Over Baseline Level (measured at time of peak response) (mg/dL)
Boesch et al., 2001	Healthy males	12	D-Tagatose	15[d]	0.19	60	--	5.6	5.9	0.4
Diamantis & Bär, 2002	Hyperuricemic males	12	D-Tagatose	15[d]	0.19	90	<7.0	10.2	10.4	0.2
Macdonald et al. 1978	Healthy males	9	Fructose	~18[b]	0.25	30	--	7.0	7.6	0.6
Förster et al. 1972	Healthy males	6	Fructose	25	~0.36[b]	60	--	5.4	5.8	0.4
Buemann et al. 1999b	Healthy males	8	Fructose	30	0.39	50	<7.6	6.1	6.5	0.4
	Healthy males	8	D-Tagatose	30	0.39	50	<7.6	6.0	7.2	1.2
Diamantis & Bär, 2001	Healthy males	6	D-Tagatose	30[d]	0.47	90	<7.0	4.9	5.7	0.8
Macdonald et al. 1978	Healthy males	9	Fructose	~35[b]	0.50	15	--	7.0	7.5	0.5
Förster and Ziege 1971	Healthy males	10	Fructose	50	~0.71[b]	30	--	6.5	7.6	1.1
Förster et al. 1972	Healthy males	9	Fructose	50	~0.71[b]	30	--	6.4	7.2	0.8
Oberhaensli et al. 1987	Healthy subjects	8	Fructose	50	~0.71[b]	60	3.4-7.6	[c]	[c]	0.4
Macdonald et al. 1978	Healthy males	9	Fructose	~52[b]	0.75	30	--	7.6	8.0	0.4
Saunders et al. 1999a	Healthy 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
	Healthy females	4	D-Tagatose	75	0.93	60	2.5-7.0	3.8	5.7	1.9
Reiser et al. 1984	Healthy males and females	15 M, 8 F	Fructose	69	0.93	30	--	5.1	6.1	1.0
Macdonald et al. 1978	Healthy males	9	Fructose	~70[b]	1.00	30	--	7.8	9.0	1.2
Stirpe et al. 1970	Healthy males	6	Fructose	~70[b]	1.00	60	--	4.8	5.3	0.5
Davies et al. 1998	Healthy males	19	Fructose	75	~1.07[b]	120	--	5.2	6.2	1.0

[a] 10 mg/dL is generally regarded as the level above which plasma uric acid becomes clinically relevant (Fessel 1979).
[b] Assumed body weight is approximately 70 kg.
[c] No data were avaible.
[d] Ingested together with 100 g starch