D-TAGATOSE

A Human Health Risk Assessment

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CONTENTS

SUMMARY

D-Tagatose is a naturally occurring monosaccharide and a stereoisomer of D-fructose. It is a white crystalline powder with a sweetness approximately 90% that of sucrose in a 10% aqueous solution. D-Tagatose is produced commercially from lactose in a process involving enzymatic hydrolysis of lactose to D-galactose, followed by chemical isomerisation under basic conditions, then purification by mineralisation, ion exchange chromatography and re-crystallisation.

D-Tagatose has technological properties similar to traditional sugars and can be used as a reducing sugar as it caramelises at elevated temperatures. However, in contrast to traditional sugars, it is only partially absorbed by the body resulting in reduced energy value. The major fraction of D-tagatose reaches the large intestine unabsorbed, where it undergoes fermentation.

Toxicological assessment

The safety of D-tagatose has been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). FSANZ has reviewed these evaluations as well as reviewing the more recent studies.

In 2001, JECFA allocated an Acceptable Daily Intake (ADI) of '0-80 mg/kg bw/day' for D-tagatose on the basis that there was some concern regarding the potential of D-tagatose to induce glycogen deposition and hypertrophy in the liver, and to increase the concentrations of uric acid in serum.

In 2003, D-tagatose was re-evaluated by JECFA to consider two new toxicity studies conducted in rats, and two new studies of plasma uric acid levels in human volunteers[1]. On the basis of the results from those studies, the Committee concluded that their previous concerns, in relation to liver glycogen deposition and hypertrophy in rats, as well as plasma uric acid levels in humans, had been adequately addressed. The new studies, however, raised new concerns arising from the results of a two-year rat study – which demonstrated increased adrenal, kidney and testes weights in rats receiving high doses of D-tagatose. The Committee concluded the toxicological significance of this finding could not be assessed, as histopathological examination of these tissues had not been undertaken. The Committee therefore allocated a temporary ADI of 0-125 mg/kg bw/day based on results from human studies which indicate no adverse effects at doses of D-tagatose up to 45 g per day (in three divided doses). In establishing the temporary ADI for D-tagatose, the Committee stated that the ADI did not apply to individuals with hereditary fructose intolerance.

FSANZ evaluated the more recent data provided to JECFA as well new information in relation to histopathological examination of tissues from the two-year rat study. Results from the histopathological examinations indicate the increased organ weights observed in rats are of no toxicological significance to humans.

On the basis of these assessments, it was concluded there is no evidence of any public health and safety concern associated with consumption of D-tagatose up to 15 g/day (0.25 g/kg bw/day for a 60 kg adult), even in susceptible individuals (those who have gout or

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[1] The JECFA report and toxicological monograph are in press

are hyperuricaemic). There is some evidence that mild gastrointestinal effects such as nausea, diarrhoea and flatulence may occur in some individuals at doses of 30 g/day and above. D-Tagatose is not considered suitable for consumption by individuals with hereditary fructose intolerance.

Dietary exposure

Based on US food consumption data (1994-1996, 1998) JECFA reported the mean estimated intake for D-tagatose from a range of food uses (excluding chewing-gum, dietary supplements and meal replacements) to be approximately 9 g/day for consumers with mean intakes, and 18 g/day for the 90th percentile group. Intake from chewing gum was predicted to be 4 g/day for consumers with mean intakes and 8 g/day for those with intakes at the 90th percentile. These estimates are based on the use of D-tagatose at the maximum technological levels, and therefore the actual dietary intake would be expected to be significantly lower. An analysis based on the same assumptions, combined with available data on food consumption from Australia and the European Union, showed that the predicted intake of D-tagatose would be similar in these regions.

Estimated dietary exposures to D-tagatose were calculated on a chronic and single eating occasion basis for the Australian and New Zealand populations using National Nutrition Survey (NNS) data. Exposure to D-tagatose is estimated to be below 15 g/person/day for all population groups. Estimated mean chronic dietary exposures to D-tagatose for consumers were 2.7 g/d for the Australian population (2 years and above), 1.9 g/d for Australian toddlers 2-4 years, 3.0 g/d for Australian children aged 5-12 years and 1.9 g/d for the New Zealand population (15 years and above). Estimated 95th percentile exposures to D-tagatose for consumers were 9.0 g/d for the Australian population, 6.9 g/d for children 2-4 years, 10.5 g/d for children 5-12 years and 7.4 g/d for the New Zealand population. Ninety-fifth percentile exposures are overestimated as 24-hour food consumption data exaggerates habitual exposures.

Estimated short-term exposures were calculated for individual food groups to assess whether consumption reached levels where gastrointestinal effects were likely. Most estimated short-term exposures were below 24 g for any population group. However, the modelling indicated that individuals consuming large amounts of formulated dietary foods or formulated supplementary sports foods potentially have higher infrequent levels of exposure.

Energy value for D-tagatose

About 20-25% of ingested D-tagatose is absorbed from the small intestine. The absorbed fraction is metabolised via the same pathway as fructose, with the remaining unabsorbed fraction being available for fermentation in the large bowel. The metabolisable energy factor for D-tagatose is best reflected by a value of 11 kJ/g.

Risk characterisation

On the basis of the available animal and human safety data on D-tagatose, there are no public health and safety concerns associated with use of D-tagatose for the general population at low to moderate levels of exposure. Mild gastrointestinal effects and effects on uric acid levels have been observed only following relatively high-level exposure (15-30 g/day).

On the basis of the proposed use of D-tagatose in a range of foods at the maximum levels proposed, the highest potential exposure by age group was Australian children 5-12 years – 10.5 g/day at the 95th percentile exposure level, which is below the level at which mild, gastrointestinal effects have be observed. This dietary exposure estimate is likely to be an over-estimate since it is based on conservative assumptions.

D-Tagatose is not considered suitable for consumption by individuals with hereditary fructose intolerance. This is a rare condition and most individuals with this condition would receive professional advice in relation to dietary matters.

In conclusion, there are no public health and safety concerns associated with the use of D-tagatose in foods as proposed.

D-TAGATOSE

A Human Health Risk Assessment

INTRODUCTION

D-Tagatose is a naturally occurring sweet-tasting monosaccharide that may be used as a sweetener in foods to replace sucrose. It has a sweetness approximately 90% that of sucrose in a 10% aqueous solution. D-Tagatose is an odourless white crystalline powder with an appearance very similar to sucrose, and is identified by the CAS (Chemical Abstract Service) number 87-81-0.

D-Tagatose occurs naturally in *Sterculia setigera* gum, a partially acetylated acidic polysaccharide. D-Tagatose is also found in small quantities (2 to 800 ppm) in sterilised cow's milk and milk powder as well as in other dairy products (Troyano et al., 1996).

Structure & Properties

D-Tagatose is a stereoisomer (epimer) of D-fructose, differing in the spatial configuration of the hydroxyl group at C-4. D-Tagatose is a ketohexose in which the fourth carbon is chiral, a mirror image of the respective carbon atom of the common sugar, D-fructose. The empirical formula for D-tagatose is $C_6H_{12}O_6$ (see Figure 1); it has a molecular weight of 180.16.

Figure 1: The structural formula of tagatose

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\begin{array}{c}
\text{CH}_2\text{OH} \\
| \\
\text{C=O} \\
| \\
\text{HO-C-H} \\
| \\
\text{HO-C-H} \\
| \\
\text{H-C-OH} \\
| \\
\text{CH}_2\text{OH}
\end{array}
$$
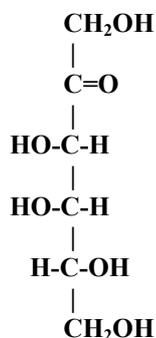
Physical properties

The crystal form of D-tagatose is a tetragonal bipyramid. Crystallisation from aqueous solution results in anhydrous crystals in an α-pyranose form, with a melting point of 134-137 °C.

The solubility in water of D-tagatose is similar to sucrose. It is non-hygroscopic; D-tagatose will not absorb water from its surrounding atmosphere under normal conditions. Compared with sugar alcohols (polyols), tagatose is more soluble than erythritol and less soluble than sorbitol.

D-Tagatose solutions are lower in viscosity (180 cP at 70% w/w and 20 °C) than sucrose solutions at the same concentrations, but slightly higher than fructose and sorbitol. D-Tagatose exerts a greater osmotic pressure, and hence a lower water activity than does sucrose at equivalent concentrations.

Chemical properties

D-Tagatose is a reducing saccharide and takes part in Maillard reactions, which leads to a distinct browning effect in food. It also decomposes (caramelizing) more readily than sucrose at high temperatures. At low and high pH, D-tagatose is less stable and is converted to various compounds.

Production

The production process of D-tagatose occurs in a stepwise manner starting from the raw material lactose (a disaccharide). Food-grade lactose is initially enzyme hydrolysed to galactose and glucose, by passing the solution through an immobilised lactase column, and the resulting sugar mixture is fractionated by chromatography. The chromatographic separation of glucose and galactose is essential at this stage and is similar to the normal industrial separation of glucose and fructose, using calcium-based cationic resins.

The galactose chromatographic fraction is converted to D-tagatose under alkaline conditions by adding a suspension of $Ca(OH)_2$ and, optionally, a catalyst $CaCl_2$. Adding sulphuric acid stops the reaction. D-Tagatose is stable under the conditions of the isomerisation process. The resulting filtrate is further purified by means of demineralisation and chromatography. The purified D-tagatose solution is then concentrated and crystallised to give a white crystalline product that is more than 99% pure.

Applications in food

D-Tagatose is well suited for confectionery products such as chocolate and hard boiled candies, fondants, fudges, and caramels due to its similar sweetness to sucrose, good ability to crystallise and low caloric value. It could also have applications in ice cream, soft drinks, and breakfast cereals.

REVIEW OF TOXICOLOGICAL DATA

Evaluations by JECFA

D-Tagatose was evaluated in 2000 and 2001 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) which allocated an ADI of 0-80 mg/kg bw/day based on a NOEL of 0.75 g/kg bw/day and a safety factor of 10. The NOEL was derived from a 28-day study in humans (FAO/WHO, 2001, 2002).

Following the submission of new data from animal and human studies, D-tagatose was re-evaluated by JECFA at their 61st meeting in June 2003. The Committee determined that the previous NOEL of 0.75 g/kg bw/day was still applicable and applied a safety factor of 3 to allow for inter-individual variation. The Committee applied an additional safety factor of 2 to account for uncertainty regarding new findings in a 2-year toxicity study in rats. The previous ADI was replaced with an ADI of 0-125 mg D-tagatose/kg bw. The new ADI is temporary, pending provision of further information regarding findings in the recent rat study.   The temporary ADI does not apply to individuals with hereditary fructose intolerance caused by 1-phosphofructoaldolase (aldolase B) deficiency or fructose 1,6-diphosphatase deficiency.

The reports from the JECFA evaluation are provided below.

Report from JECFA 55

D-Tagatose was tested in Sprague-Dawley rats in a series of short-term toxicity studies. The observed increases in liver weights and liver hypertrophy were found to be due, at least in part, to glycogen accumulation. The hepatic changes were partially reversed after exclusion of D-tagatose from the diet. Recovery from the induced liver hypertrophy took longer than recovery from glycogen accumulation. Data from short-term studies of the mechanism of glycogen accumulation suggest that the hepatic changes are due to physiological changes in Sprague-Dawley rats and that Wistar rats are less sensitive to expression of these effects.

The precise metabolic pathway of D-tagatose that leads to gluconeogenesis has not been established. D-Tagatose is metabolised more slowly than fructose. A similar biochemical effect characterised by glycogen accumulation occurs in patients with hereditary fructose intolerance, and this reaction can increase the rates of purine breakdown and accumulation of uric acid. D-Tagatose is more effective than fructose in increasing the concentration of uric acid in serum.

In two studies of developmental toxicity in Sprague-Dawley rats, minimal effects were observed in dams, including reduced food consumption at doses greater than 12 g/kg bw/day and initial depression of weight gain, which returned to normal later in the study. A dose-related, statistically significant increase in liver weight was found, but histological examination of the livers revealed no abnormalities. No effects were found in either study on reproductive or developmental parameters.

The results of tests for genotoxicity *in vitro* and *in vivo* were consistently negative.

A number of studies of gastrointestinal effects have been conducted in healthy human

volunteers and in patients with type 2 diabetes (Donner *et al*., 1999). Nausea and adverse gastrointestinal effects were reported in healthy adults given D-tagatose at high doses. Studies in which baseline serum concentrations of insulin and glucose were investigated showed no effect following administration of single or multiple doses, but a decreased glycaemic response was observed when D-tagatose was given before a glucose tolerance test.

Elevated serum uric acid concentrations were reported in three out of six studies in which this parameter was measured; in two of these studies, the values exceeded the normal range.

In the three studies in which parameters indicative of liver function or hepatic changes were measured, no effects were observed.

On the basis of the available data, the Committee concluded that D-tagatose is not genotoxic, embryotoxic or teratogenic. The Committee noted that the increased liver weights and hepatocellular hypertrophy seen in Sprague-Dawley rats occurred concurrently with increased glycogen deposition; however, the reversal of increased glycogen storage after removal of D-tagatose from the feed occurred more rapidly than regression of the liver hypertrophy.

Although the gastrointestinal symptoms seen in adult humans with the expected daily intake of D-tagatose were minor, the Committee was concerned about the increased serum uric acid concentrations observed in a number of studies in humans following administration of either single or repeated doses of D-tagatose. Similar increases are seen with other sugars, such as fructose, but D-tagatose appears to be a more potent inducer of this effect. The Committee noted that the effect of D-tagatose has not been studied in people prone to high serum uric acid concentrations.

The Committee concluded that an ADI could not be allocated to D-tagatose because of concern about its potential to induce liver glycogen deposition and hypertrophy and to increase serum uric acid concentrations. Two studies in Sprague-Dawley and Wistar rats were submitted that might help to resolve the relevance of the induction of liver glycogen deposition and hypertrophy, but the reports were received in draft form and were not suitable for consideration at the present meeting. Before reviewing the compound again, the Committee wished to evaluate the final reports of these studies and data to clarify the extent, mechanism and toxicological consequences of the increased serum uric acid concentrations observed in humans exposed to D-tagatose.

Report from JECFA 57

The Committee reviewed the reports of two studies in rats, the results of a study in volunteers (on the relevance of the glycogen deposition and liver hypertrophy) and some published studies on the increased uric acid concentrations in serum after intake of D-tagatose, other sugars and other food components.

Review of the results of the studies considered by the Committee at its fifty-fifth meeting and comparisons with the data reviewed at the present meeting revealed a difference in sensitivity between Wistar and Sprague-Dawley rats. Sprague-Dawley rats given D-tagatose at a concentration of 50 g/kg of diet for 28 days showed increased hepatic glycogen only when they had not been fasted the night before necropsy, and this effect was not associated with any microscopic changes in the liver. In a 90-day study in which Sprague-Dawley rats were

killed after fasting overnight, administration of D-tagatose at a concentration of 50 g/kg of diet had no adverse effect on the liver. In a 6-month study in Wistar rats in which the animals were killed after fasting 7, 14 and 28 days and 3 and 5 months after treatment, administration of D-tagatose at concentrations of up to 100 g/kg of diet had no adverse effects. Wistar rats are therefore less susceptible to the hepatic effects of D-tagatose than Sprague-Dawley rats. As D-tagatose stimulated glycogen deposition to a similar degree in the two rat strains in short-term studies, the difference is likely to occur at a later stage, during glycogen-induced or other stimulation of liver growth.

The authors suggested that the increase in normal liver mass seen in fasted rats fed diets containing 100 or 200 g/kg D-tagatose is triggered by increased postprandial storage of liver glycogen resulting from simultaneous feeding of D-tagatose and glucose equivalents. In order to test this hypothesis, the effects of separate and simultaneous administration of D-tagatose and glycogen precursors on liver weight and glycogen level were investigated in Wistar and Sprague-Dawley rats. The results neither supported nor invalidated the hypothesis.

As several studies have been performed in healthy volunteers and in patients with diabetes, the number of persons varying from 4 to 73, the Committee based its toxicological evaluation on the data from these studies. The length of these studies varied from several days to several weeks; one study of 12 months' duration included only a limited number of patients with type 2 diabetes. The toxicological aspects investigated included gastrointestinal effects, increased serum uric acid concentrations and hepatic effects.

Mild gastrointestinal symptoms were reported in only one study, in 3 of 10 patients with type 2 diabetes receiving D-tagatose at 10 g/day for several days, whereas in other studies diarrhoea was observed only in patients receiving 25 g three times daily for 8 weeks. In healthy individuals, administration of a single dose of 30 g induced diarrhoea in some individuals only, whereas other studies showed no laxative effect of single doses of D-tagatose as high as 75 g.

The serum or plasma concentration of uric acid was increased transiently in some studies, but the increased uric acid concentration was above the normal range for a number of days in only one study of persons receiving 75 g/day. The other studies showed either no increase or a transient increase in serum uric acid concentrations within the normal range.

In a 28-day study in which 15 g of D-tagatose or 15 g of sucrose were given three times daily to volunteers, magnetic resonance imaging was used to determine liver volume, and glycogen concentrations and several clinical chemical parameters were measured (Boesch *et al*, 2001). The results did not reveal any relevant effect on the liver. In addition, no diarrhoea and no increase in serum uric acid concentration were observed. Therefore, the NOEL was 45 g/person per day, equivalent to 0.75 g/kg of body weight per day (for a person weighing 60 kg).

The Committee considered the 28-day study in which humans received a daily dose of 45 g of D-tagatose or sucrose in three divided doses as most representative of human dietary intake and therefore most relevant for assessing the acceptable intake of D-tagatose accurately. While effects were observed after administration of a single dose of 75 g, no effects were seen following administration of three daily doses of 15 g of D-tagatose, equivalent 0.75 g/kg of body weight per day. The Committee established an ADI of 0-80 mg/ kg of body weight on the basis of this NOEL and a safety factor of 10.

Report from JECFA 61

At the sixty-first meeting, the Committee reviewed the results of two new toxicity studies conducted in rats, and two new studies of plasma uric acid levels in human volunteers, which were submitted by the sponsor with a request for a re-evaluation.

Studies in rats previously reviewed by the Committee had focused on the hepatic effects of D-tagatose, in particular, increased liver weight and hypertrophy. These studies indicated that these effects were due, at least in part, to glycogen accumulation, and that the Sprague-Dawley strain of rats was more sensitive to these effects than Wistar rats. The new 28-day study investigating the effects of 20% D-tagatose in the diet has shown that, of six rat strains, the largest increase in liver weight occurred in Sprague-Dawley rats, and the smallest increase in Wistar rats, confirming previous observations of strain differences. The role of glycogen however was not specifically investigated.

In a two-year study in Wistar rats, the administration of diets containing 2.5, 5 or 10% D-tagatose and 10% D-tagatose + 10% fructose did not result in histological changes in the liver, although increased liver weights were reported in male and female rats fed on 10% D-tagatose. In addition to this, increased absolute and relative adrenal weights were observed in female rats at all doses of D-tagatose, but not in those receiving fructose alone. Increased adrenal weights were reported in male rats fed on 5% and 10% D-tagatose. The weights of the kidneys in females, the testes in males and the caecums in both sexes were also increased at 10% D-tagatose, and in some cases at 5% D-tagatose. The Committee concluded that in the absence of histopathological confirmation of the nature of the changes induced by D-tagatose in the adrenals, kidneys and testes, it is not possible to assess the toxicological significance of these changes to humans.

Two new human studies showed that a single dose of 30 g D-tagatose to small numbers of healthy volunteers, or 15 g D-tagatose to hyperuricemic individuals, had no biologically significant effects on uric acid production or excretion, and no recorded gastrointestinal effects. At its forty-eighth meeting, the Committee noted that D-fructose increases uric acid production by accelerating the degradation of purine nucleotides, probably by hepatocellular depletion of inorganic phosphate resulting from accumulation of ketohexose-1-phosphate. Degradation of D-tagatose-1-phosphate is slower than that of D-fructose-1-phosphate, and therefore the hyperuricemic effect of D-tagatose may be greater than that of D-fructose, and hyperuricemic individuals are a potentially vulnerable group for adverse effects of D-tagatose. The new study demonstrated no increase in serum uric acid within four hours of consumption of 15 g of D-tagatose by this vulnerable group.

In studies reviewed previously by the Committee, the maximum increases in serum uric acid and D-tagatose, and the maximum decrease in serum ATP, were seen within one hour of ingesting D-tagatose. It is therefore anticipated that no effect would be expected in hyperuricemic individuals following repeated consumption of 15 g of D-tagatose at subsequent meals.

The Committee concluded that the two-year study in rats demonstrated that the previously reported liver glycogen deposition and hypertrophy did not result in histopathological changes following long term administration of D-tagatose, and thus addressed concerns expressed at the fifty-fifth meeting. However, the study identified new findings, namely

increased adrenal, kidney and testes weights. The Committee considered that these changes might have been due to high osmotic load resulting from the high dietary doses administered, but as histopathological examination of the tissues had not been done, this could not be confirmed. Pending provision of the histopathology data, the Committee confirmed that the human data provided the most relevant basis for assessing the acceptable intake of D-tagatose.

At the fifty-seventh meeting, the Committee identified a NOEL for healthy individuals of 45 g D-tagatose per day in three divided doses. The study on hyperuricemic individuals discussed at the current meeting indicated that the NOEL is also applicable to this vulnerable group. The Committee considered that a safety factor of 3 would be appropriate to allow for inter-individual variation. In view of the additional uncertainty regarding the nature of the effects observed in the adrenals, kidneys and testes in the two-year study in rats, the Committee concluded that the ADI should be temporary and applied an additional safety factor of 2. The previous ADI was removed, and on the basis of the NOEL of 0.75 g/kg bw/day, and a safety factor of 6, the Committee allocated a temporary ADI of 0-125 mg D-tagatose/kg bw.

The temporary ADI does not apply to individuals with hereditary fructose intolerance due to a deficiency in either 1-phosphofructoaldolase (aldolase B) or fructose 1,6-diphosphatase. The Committee requested information on the histological examination of the adrenals, kidneys and testes of the rats from the two-year study by 2006.

Evaluation by FSANZ

Chronic toxicity and carcinogenicity in rats

A chronic (24 months) toxicity and carcinogenicity study was conducted in young Wistar albino rats (5-6 weeks old) fed a diet of 2.5, 5 or 10% D-tagatose, 20% fructose, and 10% D-tagatose + 10% fructose w/w (50 rats/sex/group). D-Tagatose and fructose were obtained as crystalline white powders with > 99% purity. The control substance was a pre-gelatinised potato starch as a coarse white powder.

Study methodology

Four groups of rats (50/sex/group) were treated with D-tagatose in the diet at 2.5, 5, 10 or 10% D-tagatose + 10% fructose (equivalent to 1.0, 2.0 or 4.0 g/kg bw/day for males and 1.2, 2.5 and 4.9 g/kg bw/day for females). The test substance was incorporated in the feed at the expense of 20% barley. The control, low-dose and mid-dose diets were compensated by adding respectively 20%, 17.5%, 15% and 10% pre-gelatinised potato starch.

Clinical observations were recorded daily and bodyweight were measured weekly for the first 13 weeks and subsequently once every month. From 6 months after the start of the study until the end of the study, the animals were palpated weekly to detect palpable masses. Food consumption was assessed on a cage basis, by weighing the feeders, over each 1-week period during the first 13 weeks and subsequently over 1-week periods every month. Blood samples for haematology were taken from all rats after 12 and 24 months. At the end of the study, all animals were sacrificed and a complete necropsy performed (gross examination, organ weights and tissue sampling). Histopathology was performed on liver only.

Results

The highest average intake of the test substance was 4.0 g/kg bw/day in males and
5.0 g/kg bw/day in females of the highest dose group. There were no dose related differences
in appearance, general condition or behaviour among the groups. The incidence of grossly
visible or palpable masses was not considered a treatment-related effect. The test substance
did not affect mortality rate.

Mean body weights decreased in males and females at the highest dose. The decrease was
statistically significant but there was no dose-response relationship. There were no noticeable
differences in overall food intake.

Haematology showed that haemoglobin concentration and packed cell volume tended to be
decreased in females fed 10% D-tagatose throughout the study. There were no significant
differences in clinical chemistry variables among the groups.

There was a statistically significant increase in the relative weight of the liver in females
given the highest doses of D-tagatose and fructose (10% D-tagatose, 20% fructose and 10%
D-tagatose + 10% fructose groups), and in males in the 10% D-tagatose + 10% fructose
group. This liver enlargement however was not associated with any histopathological
changes. There were no observable differences between the treated and control animals in
terms of liver tumour incidence.

In addition, increased absolute and relative adrenal weights were observed in female rats at
all doses of D-tagatose, but not in those receiving fructose alone. The effect was not clearly
dose-related, with the largest increase (79%) being in the group receiving a dietary
concentration of 5%. Increased adrenal weights were reported in male rats fed on 5% and
10% D-tagatose. Macroscopic examination revealed a relatively high incidence of
enlargement of the adrenals in some treatment groups, which was generally consistent with
the increased adrenal weights. The weights of the kidneys in females, the testes in males, and
the caecums in both sexes were also increased at 10% D-tagatose, and in some cases at 5% D-
tagatose.

Discussion and conclusion

There was no evidence in this study of liver carcinogenicity in rats administered D-tagatose in
the diet up to 20%, over a period of two years. Histopathological examination of the livers
did not reveal any treatment-related changes. The increases in the relative liver weights in
both D-tagatose and fructose groups could be ascribed to additional growth of hepatocytes in
reaction to increased glycogen deposition.

Likewise, the increased weight seen in the adrenals, kidneys and testes could be ascribed to
the high osmotic load resulting from the large dietary doses administered. Previous studies
have reported that these organs have been affected by high doses of low solubility
carbohydrates in the rat. Such effects are particularly well documented in the adrenals of rats
but do not appear to be reproduced to the same extent in human subjects. Also, gross dietary
imbalance due to high doses of polyols may result in metabolic and physiological
disturbances in the rat, which have been associated with changes in calcium uptake and
excretion, accompanied by nephrocalcinosis and adrenal medullary hyperplasia.

Histopathological examination of the adrenals, kidneys and testes would enable confirmation of these effects. All other clinical and haematological parameters remained normal.

Based on the above findings, long-term exposure to D-tagatose at dietary levels of up to 10% (4 g/kg bw for males and 5 g/kg bw for females) does not lead to adverse health effects in rats. Based on this study a NOEL of 4 g/kg bw/day is derived.

Histopathological examination of tissues from chronic and carcinogenicity study

The adrenals, kidneys and testes tissues from this 2-year study were embedded in paraffin for possible future histopathological examination. During a re-evaluation of D-tagatose by JECFA, the committee considered that the toxicological significance of the increased relative kidney, adrenal and testes weights found in this study could not be properly determined in the absence of histopathological examination. TNO (Zeist, the Netherlands) was commissioned to undertake the histopathological examinations of the preserved tissues.

Results

Testes
No treatment related histopathological changes were observed in the testes of male rats. Leydig cell hyperplasia and neoplasia occurred with similar frequency in all groups.

Kidneys
The treatment related histopathological changes consisted of:

- increased pelvic mineralisation in males in the 5 and 10% D-tagatose treatment groups, as well as the 10% D-tagatose + 10% fructose group; and

- increased medullary & corticomedullary mineralisation in males in the 10% D-tagatose as well as the 10% D-tagatose + 10% fructose group.

There was a high spontaneous incidence of pelvic and medullary mineralisation in the control group females - 88% exhibiting pelvic mineralisation and 48% exhibiting medullary mineralisation.

Adrenals
The observed treatment related histopathological changes consisted of:

- increased focal medullary hyperplasia in females in the 5 and 10% D-tagatose groups, as well as the 10% D-tagatose + 10% fructose group; and

- increased phaeochromocytomas[2] in males in the 5 and 10% D-tagatose treatment groups, as well as the 10% D-tagatose + 10% fructose group, and in females in the 10% D-tagatose as well as the 10% D-tagatose + 10% fructose groups.

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[2] a tumour of the adrenal gland that causes excessive release of epinephrine and norepinephrine, hormones that regulate heart rate and blood pressure.

There was a high spontaneous incidence of medullary hyperplasia and phaeochromocytomas in control group males – 60% exhibiting medullary hyperplasia and 30% exhibiting phaeochromocytomas.

Discussion and conclusion

The only treatment related histopathological changes observed were in the adrenals and kidneys from the medium (5%) and high (10%) dose groups. The effects observed consisted of increased adrenal medullary proliferation and neoplasia and increased pelvic and corticomedullary nephrocalcinosis. The increase in relative testes weight was not associated with any significant histopathological changes.

The histopathological changes observed are identical to those seen following the long-term administration to rats of other slowly digestible carbohydrates (sugars, polyols) (Bär 1985, Bär 1988, Roe 1989, Lynch *et al* 1996). In this respect, the changes observed in the adrenals and kidneys following administration of D-tagatose are unremarkable.

The increased occurrence of nephrocalcinosis and adrenomedullary proliferative lesions in response to the ingestion of high doses of low digestible sugars and polyols is a well-known phenomenon which appears to be secondary to the treatment-induced hyper-absorption of dietary calcium. The mechanism by which slowly digestible carbohydrates stimulate calcium absorption is largely unknown but it has been suggested that their slow absorption from the gatrointestinal tract and subsequent intestinal fermentation may play a central role in disturbing calcium homeostasis, at least in the rat (Bär 1988).

The link between increased calcium absorption and nephrocalcinosis is well understood. The increased absorption leads to a several fold increase in urinary calcium excretion (hypercalciuria), which, in turn, is manifest as deposits of calcium phosphate near the corticomedullary junction (cortico-medullary nephrocalcinosis) or in the renal pelvis (pelvic nephrocalcinosis) (Roe 1989).   The link between increased calcium absorption and proliferative changes in the adrenal medulla is less well understood. It has been hypothesised that increased intestinal absorption of calcium and subsequent disturbance of the calcium regulating hormonal system influences the functional status of the adrenal medulla in rats and that a prolonged stimulation of the chromaffin cells leads ultimately to a hyperplastic and neoplastic response (Roe & Bar 1985).

There is widespread agreement in the literature that the histopathological changes observed in the rat following long term administration of high doses of low digestible carbohydrates occurs through a common mechanism that has no direct relevance to human safety. In the rat, the occurrence of these effects involves three factors: (1) A high genetic susceptibility to these effects, as evidenced by the high spontaneous incidence of these lesions in the controls and the fact that such lesions are not observed in mice or dogs following ingestion of large amounts of slowly digestible carbohydrates (Lynch et al 1996); (2) an intestinal hyper-absorption of calcium, followed by a disturbance of the calcium homeostatic hormonal system; and (3) a functional and proliferative responsiveness of the adrenal gland to changes in calcium homeostasis. In humans, none of these conditions is fulfilled. The genetic susceptibility in humans is very low as shown by the rare occurrence of phaeochromocytomas (0.005-0.1%) (Manger et al 1985). The ingestion of slow digestible carbohydrates does not measurably affect calcium uptake or homeostasis (Lynch et al 1996) and, there is no known association between hypercalciuria and a higher risk for

phaeochromocytomas in humans (Bär 1988). Therefore, the increased urinary calcium excretion and associated proliferative effects on the adrenal medullary tissue seen in rats is likely a species-specific phenomenon, occurring through a mechanism, or mechanisms, that are not operative in humans (Lynch et al 1996).

There is nothing in the results of the histopathological examination of adrenals, kidneys and testes from the 2-year chronic toxicity and carcinogenicity study to suggest that the effects observed with D-tagatose are anything other than the well-described and studied general phenomenon that occurs in rats following high intakes of other low digestible carbohydrates. It seems reasonable to conclude therefore that the effects observed are peculiar to the rat and are of no toxicological significance to humans. Studies on the effect of D-tagatose ingestion on calcium homeostasis in humans would be useful to confirm this conclusion.

Effect of D-tagatose in humans

Six male volunteers between 22 and 24 years of age, and with body weights of 62-65 kg, were administered a single oral 30 g dose of D-tagatose incorporated into breakfast marmalade. D-Tagatose was obtained as a powder with > 99% purity. Marmalade was prepared with a D-tagatose content of about 40% and a fruit content of about 60%.

Study methodology

Blood samples were collected from the volunteers 1 hour prior to providing them with a light breakfast and blood collection continued just before and up to 4 hours after breakfast. The subjects were required to avoid alcohol the previous night. Six hours after the breakfast, clinical examination was performed on the subjects. Urine was collected over a 24-hour period starting from intake of the test breakfast.

Plasma and urine uric acid concentrations were determined. Plasma glucose and plasma inorganic phosphate were also measured within one hour of blood and urine collection.

Results

All 6 subjects completed the study according to protocol. Plasma glucose concentrations showed statistically significant increase at 1 hour after test breakfast. Plasma uric acid concentrations showed statistically significant increase in all 6 subjects at all postprandial times but no correlation to effect of time (1h to 4h) and they remained within the normal range at all times. Postprandial plasma phosphate levels showed statistically significant decrease 1-2 hour after breakfast, but increased towards the end of the observation period. The levels remained within the normal range throughout the observation period.

The 24-hour urinary excretion of uric acid was within the normal range. No side effects (flatulence, bloated feeling and laxation) were reported up to 24-hour post treatment observation period.

Conclusions

This study confirms earlier findings using animal models that D-tagatose produces an increase in the plasma uric acid concentrations in humans, however the uric acid levels remain well within the normal range after ingestion of 30 g D-tagatose.

Effect of an oral dose of D-tagatose on the plasma uric acid levels of hyperuricemic male volunteers

In a study by Diamentis and Bar (2002), twelve male volunteers (7 hyperuricemic and 5 with gout) between 57 and 64 years of age, and with body weights of 72-84 kg, were administered a single 15 g dose of D-tagatose incorporated into breakfast marmalade[3]. D-Tagatose was obtained as a powder with > 99% purity. Marmalade was prepared with D-tagatose content of about 40% and a fruit content of about 60%.

Study methodology

Blood samples were collected from the volunteers 1 hour prior to providing them with a light breakfast and blood collection continued just before and up to 4 hours after breakfast. The subjects were required to avoid alcohol the previous night. Six hours after the breakfast, clinical examination was performed on the subjects. Urine was collected over a 24-hour period starting from intake of the test breakfast.

Plasma and urine uric acid concentrations were determined. Plasma glucose, plasma inorganic phosphate, creatinine and lactate were also measured within one hour of blood and urine collection.

Results

All 12 subjects completed the study according to protocol. Plasma glucose concentrations showed statistically significant increase from 0.5 to 2 hours after test breakfast. Plasma uric acid concentrations showed statistically significant increase in all 12 subjects at all postprandial times but they remained small and not different from that observed during the pre-treatment period at all times. Postprandial plasma phosphate levels did not vary in response to the intake of test breakfast.

The 24-hour urinary excretion of uric acid was above the normal value in three subjects. No side effects (flatulence, bloated feeling and laxation) were reported up to 24-hour post treatment observation period.

Conclusions

This study shows that a single dose of 15 g D-tagatose does not produce a clinically significant increase of plasma uric acid concentrations in hyperuricemic and gouty subjects.

Additional information

Summaries of other published literature on the safety and metabolism of D-tagatose in humans is presented in Table 1.

Table 1: Additional Published Literature on the Safety/Metabolism of D-Tagatose in Humans

Normen L, Laerke HN, Jensen BB,	The aims of this study were to measure the excretion of

Langkilde AM, Andersson H, *Small-bowel absorption of D-tagatose and related effects on carbohydrate digestibility: an ileostomy study,* Am J Clin Nutr 2001 Jan;73(1):105-10.	D-tagatose from the human small bowel, to calculate the apparent absorption of D-tagatose, and to study the effects of D-tagatose on the small-bowel excretion of other carbohydrates. It was concluded that *!% of a 15 g dose of D-tagatose/d was absorbed. D-Tagatose had only a minor influence on the apparent absorption of other nutrients.
Buemann B, Gesmar H, Astrup A, Quistorff B, *Effects of oral D-tagatose, a stereoisomer of D-fructose, on liver metabolism in man as examined by 31P-magnetic resonance spectroscopy,* Metabolism 2000 Oct;49(10):1335-9.	The effect of 30 g D-tagatose or D-fructose administered orally on ketohexose-1-phosphates, ATP, and inorganic phosphate (Pi) levels in the liver was studied by 31P-magnetic resonance spectroscopy (PMRS) in 5 young male volunteers. Blood and urine were collected to detect a possible increased uric acid production. The results suggest that a moderate intake of D-tagatose may affect liver metabolism by phosphate trapping despite the fact that the sugar may only be incompletely absorbed in the gut.
Buemann B, Toubro S, Astrup A., *Human gastrointestinal tolerance to D-tagatose,* Regul Toxicol Pharmacol 1999 Apr;29(2 Pt 2):S71-7	The gastrointestinal effects of D-tagatose on after the consumption of 29 or 30 g of D-tagatose was investigated. Nausea and diarrhea were reported with an incidence of 15.1 and 31.5%, respectively, in 73 healthy young male subjects in a screening study. Increased flatulence after D-tagatose was frequently reported in all the studies and the flatulence did not decline during a 15-day period with intake of 30g in one dose daily. In most cases, symptoms were reported as light or moderate. The results suggest that 30g taken at one time may be above the recommended dose for ordinary use.
Buemann B, Toubro S, Raben A, Blundell J, Astrup A, *The acute effect of D-tagatose on food intake in human subjects,* Br J Nutr 2000 Aug;84(2):227-31.	A double-blind randomized crossover study was performed with nineteen normal-weight men to investigate the effect on subsequent ad libitum food intake of replacing 29 g sucrose with 29 g D-tagatose as sweetener to a breakfast meal. The results from this study suggest that D-tagatose may contribute to a reduced energy intake.

Buemann B, Toubro S, Holst JJ, Rehfeld JF, Bibby BM, Astrup A, *D-tagatose, a stereoisomer of D-fructose, increases blood uric acid concentration,* Metabolism 2000 Aug;49(8):969-76.	The effect of 30 g oral D-tagatose versus D-fructose on plasma uric acid and other metabolic parameters was tested in 8 male subjects by a double-blind crossover design. Serum uric acid concentration was significantly higher after D-tagatose compared with either 30 g D-fructose or plain water. D-Tagatose attenuated the glycaemic and insulinemic responses to a meal that was consumed 255 minutes after its administration. Moreover, both fructose and D-tagatose increased plasma concentrations of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1). The metabolic effects of D-tagatose occurred despite its putative poor absorption.
Laerke HN, Jensen BB, Hojsgaard S, *In vitro fermentation pattern of D-tagatose is affected by adaptation of the microbiota from the gastrointestinal tract of pigs,* J Nutr 2000 Jul;130(7):1772-9.	*In vitro* fermentation experiments with pig intestinal contents and bacteria harvested from the gastrointestinal tract of pigs were used to investigate the degradation of D-tagatose and the formation of fermentation products. Two groups of eight pigs were fed either a control diet containing 150 g/kg sucrose or a diet which had 100 g/kg of the sucrose replaced by D-tagatose. After 18 d the pigs were killed and the gastrointestinal contents collected for in vitro studies. No microbial fermentation of D-tagatose occurred in the stomach or in the small intestine, whereas the sugar was fermented in the caecum and colon. The authors suggest that D-tagatose is not fermented in the upper gastrointestinal tract, and the ability of the large intestinal microbiota to ferment D-tagatose is dependent on adaptation.

Hereditary fructose intolerance

Hereditary fructose intolerance (HFI) is an inherited condition in which affected individuals develop hypoglycaemia and severe abdominal symptoms after ingesting foods containing fructose and its cognate sugars (e.g. sucrose and sorbitol). The condition is considered to be quite rare, the incidence falling somewhere between 1 in 12,000 to 1 in 130,000 live births (James *et al*. 1996).

In establishing a temporary ADI for D-tagatose of 0-125 mg/kg bw, JECFA noted that the ADI did not apply to individuals with HFI due to 1-phosphofructoaldolase (aldolase B) deficiency or fructose 1,6-diphosphatase deficiency.

There is no published information on the suitability of D-tagatose for individuals with HFI, nor is there any direct scientific evidence establishing that individuals with HFI are also intolerant to D-tagatose. However, as D-tagatose is metabolised via the same biochemical pathway as fructose, using the same enzymes, it is likely that D-tagatose would produce the same effects as fructose in individuals with HFI. As D-tagatose is incompletely absorbed (20%), it is expected nevertheless that such individuals may tolerate several-fold higher intakes of D-tagatose.

HFI is usually detected in early childhood when infants are weaned from breast milk or infant formula. If the condition remains undiagnosed, continued ingestion of these sugars may lead to severe and irreversible liver and kidney damage as well as growth retardation. Once a diagnosis has been established, and provided the tissue damage has not been extensive, the introduction of a fructose-free diet results in rapid alleviation of the acute symptoms followed by recovery. Individuals with HFI typically develop a strong aversion to sweet foods, which serves to protect them from further exposure to the harmful sugars. In addition, diagnosed individuals typically receive dietary counselling to assist them to correctly identify and avoid problem foods.

Discussion of the toxicology data

A number of issues were raised in relation to the potential for D-tagatose to induce glycogen deposition and hypertrophy in the liver and to increase the concentrations of uric acid in serum (55th JECFA). Subsequent studies conducted in both humans and animals adequately addressed the concerns but raised new questions in relation to organ weights in the two-year rat study (57th JECFA), the toxicological significance of which could not be determined because of the absence of histopathology data on the relevant tissues.

FSANZ has subsequently examined the histopathology data from adrenal, kidney and testes in the high dose groups of the rat study and concluded that the results do not indicate any unusual findings of toxicological significance to humans. No significant toxicological effects have thus been observed in the human or animal studies conducted to date.

A temporary ADI of 0–125 mg/kg bw was established by JECFA on the basis of a NOEL of 45 g D-tagatose/day (in three divided doses) from human studies and using a safety factor of six. However, the application of a safety factor in this case may not be appropriate since bulk ingredients such as D-tagatose cannot be tested above 40-60 g/day due to osmotic effects in the gastrointestinal tract leading to laxation.

There is some evidence that mild gastrointestinal effects such as nausea, diarrhoea and flatulence can occur in some individuals at doses 30 g/day and above. Effects on uric acid were not observed in a sensitive population at 15 g/day or in a normal population at 30 g/day.

D-Tagatose is not considered suitable for consumption by individuals with hereditary fructose intolerance.

DIETARY EXPOSURE

D-Tagatose can be used in foods such as ready-to-eat breakfast cereals, chewing gum, ice creams, gum (soft) type sweets, confectionery and beverages.

USA data

A detailed dietary exposure assessment for D-tagatose based on United States food consumption data (US Department of Agriculture Continuing Survey of Food Intakes by Individuals, 1994-96) indicated that mean daily exposure to D-tagatose for consumers of foods containing added D-tagatose (excluding chewing gum and formulated diet foods) was 5 g/day and 11 g/day for the 90th percentile consumer.

Expressed in terms of grams per kilogram body weight per day (g/kg bw/d), the highest exposure is projected for preschool children aged 2-5 years with mean and 90th percentile exposures of 0.17 and 0.33 g/kg bw/d respectively. For consumers of all age groups combined, the estimated exposure is 0.08 and 0.18 g/kg bw/d for the mean and 90th percentile respectively. The additional exposure from chewing gum is estimated at 0.06 – 0.07 g/kg bw/d for the average consumer (assuming a use level of D-tagatose of 60% in the gum).

The average daily exposure to D-tagatose from formulated diet foods was considered separately as these products are intended as meal replacements or as a snack in the context of weight loss or weight control diets. The average daily exposure to D-tagatose from this use is estimated at 0.08 – 0.16 g/kg bw/d for the 20+-year-old consumer from one or two servings of a formulated diet food per day. US food consumption data are based on two days of food records. Use of multiple day records tends to significantly reduce the predicted high consumer exposure (Rutishauser, 2000).

The USFDA estimated the dietary exposure to D-tagatose under the intended conditions of use to be 7.5 g/day for the mean consumer and 15 g/day for the 90th percentile.

Australian and New Zealand data

Exposure to D-tagatose was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with proposed levels of use of D-tagatose in foods. Dietary survey data for both Australia and New Zealand are derived from the 1995 NNS from Australia that surveyed 13 858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4 636 people aged 15 years and above. Both of these surveys used a 24-hour food recall methodology.

Naturally occurring sources of D-tagatose were not included in the exposure assessment as it was assumed that they would not contribute substantially to the total exposure. The concentration of D-tagatose used in the exposure assessment is shown in Table 2.

Table 2: Food groups and concentrations of D-tagatose used in the dietary exposure assessment

Food Name	Concentration Level (mg/kg)	Foods included	Assumptions
Low fat / fat reduced ice cream	30 000	Carbohydrate modified ice cream products, ice confection	
Bubble gum and chewing gum	600 000		
Gum (soft) type sweets confectionery	100 000	Jubes, jellies, Turkish delight, marshmallow	
Hard boil sugar confectionery	150 000		
Icings and Frostings	300 000		
Processed cereal and meal products	22 000	"Healthy" breakfast cereals	DEA assumed market share of 11%
Formula dietary foods	250 000	Diet bars / biscuits	Based on 80 g serve size and 20 g D-tagatose / serve
Dietetic formulae for slimming and weight loss	53 000		13.4-13.6 Based on a 375g serve size and 20 g D-tagatose / serve
Supplementary foods for dietetic uses	53 000	Meal replacement drinks	
Formulated supplementary sports foods	53 000	Sports drinks, high protein drinks	
Water based flavoured drinks, artificially sweetened	10 000	Cordial, soft drink	
Dairy desserts, artificially sweetened	30 000	Mousse, puddings	Fat reduced chilled yogurt products were not included
Confectionery	100 000	Health bars, muesli bars, chocolate coated health bars, breakfast bars	

Limitations of the dietary modelling

Only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile exposures are likely to be higher than actual high percentile exposures over a lifetime.

Results

Estimated dietary exposures to D-tagatose

The estimated dietary exposures to D-tagatose are shown in Table 3. The estimated mean exposure for consumers of D-tagatose for the Australian population aged 2 years and above is 2.7 g/day. The estimated mean exposure for consumers of D-tagatose for Australian toddlers aged 2 – 4 years is 1.9 g/day and for children aged 5 – 12 years it is 3.0 g/day. The estimated mean exposure for consumers of D-tagatose for the New Zealand population aged 15 years and above is 1.9 g/day. The estimated 95th percentile exposures for consumers of D-tagatose for the total Australian and New Zealand populations are 9.0 g/day and 7.4 g/day respectively. The estimated 95th percentile exposures for consumers of D-tagatose for Australian toddlers aged 2 – 4 years and children aged 5 – 12 years are 6.9 g/day and 10.5 g/day respectively.

Table 3:   Estimated dietary exposures to D-tagatose

Country	Age group	Number of consumers of D-tagatose	Consumers as a % of total respondents[#]	Grams per day		
				Mean all respondents	Mean consumers only	95th percentile consumers only
Australia	Whole population (2 years+)	7341	53	1.4	2.7	9.0
	2-4 years	472	81	1.6	1.9	6.9
	5-12 years	1166	78	2.4	3.0	10.5
New Zealand	Whole population (15 years+)	2311	50	0.9	1.9	7.4

Total number of respondents for Australia: whole population = 13 858, 2-4 years = 583, 5-12 years = 1 496;
 New Zealand: whole population = 4 636.

Comparison of estimated dietary exposures

The estimated dietary exposures calculated from the Australian data are slightly lower on a population basis than those calculated from the US data. Differences in methodology of calculating the exposures (including a different population, consumer consumption patterns, different NNS methodology and calculations) can result in differences in estimated exposures.

JECFA estimates exposure to D-tagatose at 3-9 g/d from a number of countries. High consumers were estimated to be exposed to up to 18 g/d (WHO 2002). These estimates are slightly higher in grams per day than the Australian/New Zealand exposure estimates.

All estimated short-term exposures from single eating occasion, for any population group, for any food, are 24 grams or lower, except for the Australian population for formulated dietary foods (59.3 g) and formulated supplementary sports foods (55.9 g). These products are likely to be infrequently consumed, and would mostly be consumed by adults.

Dietary exposure

Estimated dietary exposures were calculated on a chronic and single eating occasion basis for the Australian and New Zealand populations and Australian children aged 2-4 years and 5-12 years.

Estimated mean chronic dietary exposures to D-tagatose for consumers were 2.7 g/d for the Australian population (2 years and above), 1.9 g/d for Australian toddlers 2-4 years, 3.0 g/d for Australian children aged 5-12 years and 1.9 g/d for the New Zealand population (15 years and above). Estimated 95th percentile exposures to D-tagatose for consumers were 9.0 g/d for the Australian population, 6.9 g/d for children 2-4 years, 10.5 g/d for children 5-12 years and 7.4 g/d for the New Zealand population. Ninety-fifth percentile exposures are overestimated as 24-hour food consumption data exaggerates habitual exposures. These exposures are also based on maximum proposed use levels, also producing an overestimate of exposure.

Estimated short-term exposures for high consumers in a single eating occasion were calculated for individual food groups to assess whether gastro-intestinal effects were likely. All estimated short-term exposures were 24 grams or less for any food for any population group, except for the Australian population consuming large amounts of formulated dietary foods (59 g D-tagatose/day), or formulated supplementary sports foods (56 g D-tagatose/day).

ENERGY FACTOR FOR D-TAGATOSE

Energy factors are used in the calculation of a food's energy content, and components that are recognised as contributing significantly to the energy content of a food (e.g. macronutrients) are assigned values for this purpose. Other food components can contribute to energy intake in a more moderate way, and may be assigned an energy factor where there is sufficient supporting evidence. As D-tagatose may be used as a sugar substitute, and is not an unavailable carbohydrate, the default energy factor is 17 kJ/g. However, data are available that enable a more accurate calculation.

Energy factors may be derived using the following formula for metabolisable energy (ME):

$$ME = GE - FE - UE - GaE - SE$$

Where   GE = gross energy
          FE = energy lost in faeces
          UE = energy lost in urine
          GaE = energy lost in gases from large intestine fermentation
          SE = energy content of waste products lost from surface areas

Calculating the metabolisable energy of D-tagatose

Three studies on pigs and nine studies on humans were relevant. The totality of the evidence in these twelve studies provides a consistent set of results for each species. Each of the components that comprise ME (GE, FE, UE, GaE and SE) requires a separate assessment and calculation. An assessment of the evidence for these components is provided below, with the subsequent derivation of the ME for D-tagatose.

Gross Energy (GE)

GE or heat of combustion is the total quantity of energy available within a substance. This value is best measured by adiabatic bomb calorimetry, which provides very precise estimates.

A value 15.7 kJ/g can be obtained for the GE of D-tagatose (Levin et. al. 1995; Livesey 1999). None of this information is based on published bomb-calorimetry results, however 15.7 kJ/g is similar to known GE values for glucose and fructose (15.5 kJ/g and 15.2 kJ/g respectively).

> A value of **15.7 kJ/g ingested D-tagatose** was assigned to GE.

Percentage of Gross Energy completely absorbed in the upper intestine

Intestinal absorption is an important consideration, as it determines the energy directly available to the human body from D-tagatose, and the amount of D-tagatose remaining in the large bowel for further fermentation. The percentage of D-tagatose available for fermentation will influence the values obtained for FE and GaE.

Two of the three studies on pigs (Jensen and Laue 1998; Lærke and Jensen 1999) supply information on intestinal absorption. These studies indicate that approximately 25% of ingested D-tagatose is absorbed from the small intestine, leaving approximately 75% available for further fermentation in the large bowel.

Unlike studies on pigs, human studies cannot ethically undertake invasive portal vein measurements to directly determine the intestinal absorption of D-tagatose. Indirect measurements from urinary excretion values are also unsuitable, as D-tagatose is readily metabolised by the human liver upon absorption from the gastrointestinal tract (Buemann et. al. 1998; Livesey 1999). L-Rhamnose, however, is incompletely metabolised in humans and has a similar chemical structure to D-tagatose. Therefore, seven of the nine human studies (Bjarnason et. al. 1991; Bjarnason et al. 1991; Delahunty and Hollander 1986; Howden et al. 1991; Maxton et. al. 1986; Menzies et al. 1990; Mooradian et. al. 1986) were used as a means of deriving the intestinal absorption of D-tagatose in humans from the urinary excretion of ingested L-rhamnose.

In using data on ingested L-rhamnose, consideration has been given to the influence of lipophilicity and molecular size on intestinal absorption (Hamilton et. al. 1987). L-Rhamnose will thus have a higher (unknown) rate of intestinal absorption compared to D-tagatose.

The seven human studies indicated that approximately 8-17% of ingested L-rhamnose was excreted in the urine. This information was used to assign a value of 17-24% to the intestinal absorption of L-rhamnose, and subsequently inferred that no more than 20% of D-tagatose will be absorbed from the gastrointestinal tract in humans (i.e. 80% available for fermentation).

As a number of assumptions are required when using the human studies on L-rhamnose, results from both pigs and humans have been given equal weighting in the determination of intestinal absorption values.

> The range of **20-25%** of ingested D-tagatose is to be assigned to intestinal absorption. Consequently, **75-80%** of ingested D-tagatose will be assigned to the percentage of D-tagatose available for fermentation.

Energy Lost in Faeces (FE)

FE can be calculated by separating into three components:

- uFE – the energy lost through excretion of the ingested substance in faeces unchanged,
- mFE – the energy lost in microbial mass through fermentation, and
- oFE – the energy lost through short chain fatty acids that escape large intestinal absorption.

As D-tagatose is completely fermented in humans and appears unchanged in the faeces in minute quantities (Livesey, 1999), uFE can be assigned a value of 0% of fermented D-tagatose. In addition, oFE can be set at 0% of fermented D-tagatose. No data were available on mFE; instead the default value of mFE has been used. This value is stated as 30% of fermented D-tagatose.

> With no energy losses from uFE and oFE, the total value for FE is equivalent to mFE, or
> **30% of fermented D-tagatose**.

Percentage of D-Tagatose Excreted into Urine

The UE is derived using the percentage of ingested D-tagatose excreted into the urine, multiplied by GE. Using results from studies on pigs (Jensen and Laue 1998; Jørgensen and Lærke 1998), the excretion of 3-7% of ingested D-tagatose into urine has been observed. A slightly lower range was obtained from two human studies (Buemann et. al. 1998; Buemann et. al. 1999), with the observation that approximately 1-5% of ingested D-tagatose is excreted into the urine.

As the results from pigs align closely with those from humans, the human values have been preferentially used as the basis for calculating UE.

> The range of **1-5% of ingested D-tagatose** is to be assigned to the percentage of D-tagatose excreted into the urine.

Energy Lost in Gases from Large Intestine Fermentation (GaE) and Energy Content of Waste Products Lost from Surface Areas (SE)

The default values for GaE or SE are considered appropriate in this case.

> GaE and SE will be assigned values of **5% of fermented D-tagatose and 0 kJ/g of ingested D-tagatose** respectively.

Calculation of the metabolisable energy for D-tagatose

Each of the separate components in the equation for ME are derived as follows:

GE = 15.7 kJ/g ingested D-tagatose
FE = % ingested D-tagatose available for fermentation x 0.3 (30%) x GE
UE = % ingested D-tagatose excreted into the urine x GE
GaE = % ingested D-tagatose available for fermentation x 0.05 (5%) x GE
SE = 0 kJ /g ingested D-tagatose

As a range of values can be obtained for both the percentage of D-tagatose available for fermentation (75-80%) and the percentage of ingested D-tagatose excreted into the urine (1-5%), the calculation of ME produces a range of values as listed in Table 4.

Table 4:   Calculation of ME using the range of percentages for UE and availability of D-tagatose for fermentation

Combination of Different Percentages	GE	FE	UE	GaE	SE	ME
Available for Fermentation = 80% Urinary Excretion = 1%	15.7	3.77	0.16	0.63	0	11.15
Available for Fermentation = 75% Urinary Excretion = 1%	15.7	3.5	0.16	0.6	0	11.42
Available for Fermentation = 80% Urinary Excretion = 5%	15.7	3.77	0.8	0.63	0	10.52
Available for Fermentation = 75% Urinary Excretion = 5%	15.7	3.5	0.8	0.6	0	10.79

All values are in kJ/g ingested D-tagatose

Despite the variation in percentages for intestinal absorption and urinary excretion of D-tagatose, the overall impact on the calculation of ME is small. Therefore, the ME of D-tagatose falls within the range of 10.52 – 11.42 kJ/g, with 11 kJ/g being the most appropriate value when rounded to a whole number.

RISK CHARACTERISATION

On the basis of the available animal and human safety data on D-tagatose, there are no public health and safety concerns associated with use of D-tagatose for the general population at low to moderate levels of exposure. Mild gastrointestinal effects and effects on uric acid levels have been observed only following relatively high-level exposure (15-30 g/day).

On the basis of the proposed use of D-tagatose in a range of foods at the maximum levels proposed, the highest potential exposure by age group was Australian children 5-12 years – 10.5 g/day at the 95th percentile exposure level, which is below the level at which mild, gastrointestinal effects have be observed.

The dietary exposure estimates for all age groups are likely to be over-estimates due to the conservative nature of the assumptions used in the modelling, namely:

*   D-tagatose is used in all the foods proposed – this is very unlikely;

*   all foods contain D-tagatose at the maximum level proposed;

*   food containing D-tagatose is consumed every day - this tends to overestimate food consumption for high consumers.

D-Tagatose is not considered suitable for consumption by individuals with hereditary fructose intolerance. This is a rare condition and most individuals with this condition would receive professional advice in relation to dietary matters.

In conclusion, there are no public health and safety concerns associated with the use of D-tagatose in foods as proposed.

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