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FOOD STANDARDS AGENCY

Mr Andreas Klepsch
European Commission
By email

15 August 2005 Reference: NFU 537

Dear Mr Klepsch,

INITIAL OPINION: D-TAGATOSE

On 1 March 2005, the UK Competent Authority accepted an application from Bioresco Ltd, on behalf of Arla Food Ingredients (Denmark) for D-Tagatose as a novel food ingredient, in accordance with Article 4 of regulation (EC) 258/97. The Advisory Committee on Novel Foods and Processes (ACNFP) reviewed this application and their opinion is attached. I apologise for the delay in submitting this opinion as the ACNFP's evaluation was extended while we obtained additional information from the applicant.

In view of the ACNFP's opinion, the UK Competent Authority considers that D-Tagatose meets the criteria for acceptance of a novel food defined in Article 3(1) of Regulation (EC) 258/97.

I am copying this letter and the ACNFP's opinion to the applicant.

Yours sincerely,

Dr Chris Jones
For the UK Competent Authority

INVESTOR IN PEOPLE

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POSITIVE ABOUT DISABLED PEOPLE

ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR D-TAGATOSE

UK OPINION

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

OPINION ON AN APPLICATION UNDER THE NOVEL FOODS REGULATION FOR D-TAGATOSE

Applicant	**Bioresco, on behalf of Arla Foods, Denmark**
Responsible Person	**Dr Albert Bär**
EC Classification	**2.1**

Introduction

1. An application has been submitted by Bioresco, acting on behalf of Arla Food Ingredients, Denmark for authorisation of D-tagatose as a novel ingredient in the EU.

2. D-tagatose is a monosaccharide, an enantiomer of D-fructose (inversion at C-4), which is not commonly found in food, although it is found at low levels in heat-treated dairy products such as sterilised and dried milk. D-tagatose has 75-92% the sweetness of sucrose and behaves like other sugars in terms of hygroscopicity, and stability under low pH and raised temperature. Its principal purpose is as a carbohydrate source, with purported nutritional effects of non-cariogenicity and as a prebiotic. During preliminary discussions with the applicant, the Secretariat noted that the use of D-tagatose in foods could fall within the legal definition of a sweetener, requiring authorisation under food additive legislation rather than the regulation on novel foods. This issue has been resolved following discussion with the Commission and other MS and the consensus view is that tagatose should be regarded as a novel food ingredient and not as a food additive.

3. This opinion details the safety of this novel ingredient and does not investigate or comment on the perceived nutritional effects that the applicant attributes to its consumption.

I. Specification of the novel food

Information on this aspect is provided on pp 14-16 and pp25-27, Annexes 1, 3 and 4 of the application dossier

4. As an enantiomer of D-fructose, D-tagatose has the empirical formula $C_6H_{12}O_6$ (see Figure 1). An overview of the compositional analyses of D-tagatose and the raw materials used in its production are given in Annex 1, sections 3 and 5. Detailed information on the specifications of raw materials, process chemicals and ion exchange resins are listed in Annex 1.

5. The novel ingredient (NI) is synthesised by enzymatic hydrolysis from lactose with a purity of ≥99%. All chemicals used in the production process are high purity and have low levels of heavy metals (Annex 1). The resulting D-tagatose has a purity of no less than 98%, a lead content no greater than 1 ppm and an ash content of no more than 0.1%.

6. D-tagatose is produced from lactose using a two-step process. In the first instance lactose is enzymically hydrolysed to galactose and glucose. The galactose is then isomerised to D-tagatose at a high pH using calcium hydroxide as a complexing agent.

7. Batch-on-batch variation has been determined by analysis of 6 batches of D-tagatose, produced by the applicant at pilot scale (Annex 4). These indicate a high degree of reproducibility. HPLC data (Annex 4) show that the only detectable impurity in the final product is galactose, which is present as a by-product of the production process.

8. D-tagatose has been evaluated by JECFA[1] on three occasions, most recently in 2004 when it allocated an ADI "not specified"[2]. The detail of the toxicological evaluation by JECFA is discussed later in this paper. The JECFA specification for D-tagatose is given in Annex 3.

Discussion *Members were satisfied with the specification of the novel food.*

II. Effect of the production process applied to the novel food

Information on this aspect is provided on pp 17 – 24 of the application dossier

9. D-tagatose is produced from food-grade lactose by a two-stage process involving enzymatic hydrolysis of food-grade lactose to form galactose, which then isomerises to D-tagatose under alkaline conditions. The applicant has summarised the process on p17 and included a detailed flow diagram (Figure 2).

10. All chemicals used in the production process including the raw material (lactose) and the immobilised lactase (obtained from *Aspergillus oryzae*) are food grade, as are all anti-microbials and column regeneration chemicals.

11. **Process**

    Lactose is first dissolved in hot water and the pH is adjusted, by addition of lactose solution that has been passed through an ion exchange column, to obtain a mildly acidic solution. This solution is then pasteurised before being passed through a column that contains immobilised lactase. This enzyme preparation is widely used throughout the EU. To avoid contamination, the column is regularly treated with a defined anti-microbial solution.

[1] **JECFA**: Joint FAO/WHO Expert Group on Food Additives.

[2] **ADI Not Specified**: Used by JECFA to refer to a food substance of low toxicity which on the basis of the available data, the total dietary exposure necessary to achieve the desired effect, and acceptable background levels in food does not represent a hazard to health.

12. The resultant hydrolysed lactose solution is concentrated by evaporation before being fractionated using a cation exchange resin. The resultant fractions are collected and the galactose-rich fraction retained. This fraction is cooled and the galactose is converted to D-tagatose by addition of a defined amount of $Ca(OH)_2$, which moves the isomerisation equilibrium in favour of the D-tagatose. D-tagatose is precipitated as an insoluble complex with calcium Once this stage is completed the NI is removed and re-dissolved by addition of CO_2 which neutralises the mixture and causes precipitation of the calcium as $CaCO_3$.

13. **Purification**
    The NI is purified by filtration, evaporation, demineralisation, and fractionation. These are described in detail on pages 20-22 of the application dossier.

14. The applicant notes that the conditions used to produce the NI are relatively benign and do not favour other reactions that could potentially occur, particularly during the isomerisation of D-galactose. A brief discussion of the potential impurities that could arise as a result of the occurrence of these 'side reactions' is detailed on page 25. None of the compounds described were found in detectable quantities in the end product (Annex 4).

Discussion *Members were content that the production process employed by the applicant does not give rise to concern*

IX. Anticipated intake/extent of use of the novel food

Information on this aspect is provided on pp 33-46 and Annex 6 of the application dossier

15. The applicant intends the NI to be used as a nutritive ingredient in a variety of products. The availability of these products will not be restricted geographically and there are no plans to target these products at particular consumer groups. A list of products and the levels at which D-tagatose is typically expected to be added can be found in the table below:

Food Category	**Proposed food use**	**Added Tagatose (g per 100g of food)**
Baked goods	Cookies	2
	Quick breads	2
	Muffins	2
	Quick bread type	2
	Coffee cakes	2
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)	1
Coffee drinks	Such as cappuccino and latte	1
Frozen milk based desserts, reduced/low fat	Light ice cream	3
	Frozen milk desserts	3
	Low fat and non fat frozen yoghurts	3

	Related frozen novelties	3
Hard candies	Hard candies including regular and dietetic candies	15
Health bars and diet soft candies	Low fat, reduced fat, diet meal, energy or nutrient fortified bars, dietetic soft candies	10
Icings	Icings (or glazes), such as those used on cookies, pastries, brownies, and angel food, chiffon, and pound cakes	30
Meal Replacement / supplement Beverages	Meal replacement beverages, diet meal beverages, nutrient supplement beverages (ready- to- drink, prepared from mix, and dry mix forms)	5g per 240 ml serving (2.08g per 100g)
	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	3
Ready-to-eat cereals	All ready-to-eat cereals	3g per 5-55g serving (5-20g per 100g)
Smoothies	Fruit and dairy “smoothie” type beverages	1
Soft/chewy candies	Soft/ chewy candies such as caramels, toffees, taffies, nougats, Creams, fudges, fondant, and fruit- based confectionery (excluding Marshmallows, soft jellies, gummies, panned candies, and liquorice)	3
Chewing gum	Tooth friendly (non-cariogenic) chewing gum	30
Table top sweeteners, low calorie	Sugar substitutes/replacements	1g per serving
Yoghurt	Yoghurt	2

16. The applicant has used dietary survey data to estimate the likely consumption of tagatose in the United States population. These data were taken from the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII) on US households and from the 1998 CSFII on children aged 0-9. The data were collected using 24-hour recall interviews for two non-consecutive days and defined according to time and eating occasions. In all cases, it was assumed that all foods or ingredients in each category would contain the NI at the level stated in the table above. A more detailed breakdown and discussion is given in Annex 6 of the dossier. The table below provides a summary of the estimated intake of the NI for US population older than 2 years old:

Summary of the estimated intake of D-tagatose from its proposed food use (excluding chewing gum and food supplements)					
Population	**Age**	**2-day average intake of D-tagatose**			
		g/person/day		**g/kg bw/day**	
		Mean	**90th Percentile**	**Mean**	**90th Percentile**
Children	2-5	3.2	6.2	0.19	0.37
Young schoolchildren	6-12	4.3	8.5	0.14	0.28
Teenagers	13-19	4.7	9.5	0.08	0.16
Adults	> 20	4.8	10.5	0.06	0.14
Total population	> 2	4.6	9.8	0.08	0.19

17. The intake of the NI from sugarless chewing gum was based on the results from a separate US survey carried out in 1995. The results of this survey indicate that the average gum consumption in the US population was 2.5 pieces per day. The equivalent figures for pre-school children and teenagers were 1.6 and 3.0 per day. (Annex 6 Table 26).

18. The applicant states that, for technological reasons related to the production of tablets, the intake of the NI via the consumption of food supplements is unlikely to exceed 3g/person/day. The applicant has not explained the derivation of this figure.

19. In response to a request from the Committee, the Secretariat compared the data obtained from the US dietary survey data with the UK NDNS data. The results, calculated using the closest matching food categories are detailed below. These data show comparable levels of consumption would be seen in the UK population.

Comparison of intake estimates based on US and UK dietary survey data					
US Data (g/person/d)			UK data (g/person/d)		
Age Group	Mean	90th %ile	Age Group	Mean	90(97.5) th %ile
Pre-school (2-5 years)	3.2	6.2	Pre-School (1½ - 4½ years)	2.8	6.9 (10.3)
School Children (6-12 years)	4.3	8.5	School Children (4-18 years)	5.6	11.9 (17.7)
Teenagers (13-19 years	4.7	9.5			
Adults (> 20)	4.8	10.5	Adults (18- 64)	3.7	9.7 (11.6)

Discussion *Estimates of D-tagatose intake for the US and British populations are similar, based on the list of expected uses provided by the applicant. Members noted that higher levels of intake could result in future if the range of uses was expanded or if D-tagatose is incorporated at higher levels.*

XI. Nutritional information on the novel food

Information on this aspect is provided on pp 28-34 of the application dossier

20. **Reduced Energy Value.** Studies described by the applicant indicate that D-tagatose is incompletely absorbed and therefore has a lower energy value compared with sucrose. The applicant refers a number of studies that indicate that the NI has an energy value of 1.5kcal/g. This figure is significantly lower than the value of 4kcal/g that currently applies for the labelling all sugars as specified in the Nutritional Labelling Directive (90/496/EC).

21. **Lower glycaemic impact and prebiotic activity.** A number of studies were described by the applicant in the dossier. These do not have any bearing on the safety assessment of the novel ingredient.

Discussion *Members agreed that the studies provided by the applicant in relation to the efficacy of the novel ingredient were not relevant to the safety assessment. It was noted that current European Community nutrition labelling rules require that sugars are labelled to indicate that they supply 4 kcalories/g. A more appropriate value can only be applied for D-tagatose if the applicant seeks an amendment to the Nutrition Labelling Directive (90/496/EEC).*

XII. Microbiological information on the novel food

Information on this aspect is provided in Annex 4 of the application dossier

22. The production of the NI does not involve the use of micro-organisms. The microbiological purity of D-tagatose is detailed in tables 1 and 2 of Annex 4. These data indicate that the final product is essentially free from microbial contamination

Discussion *Members agreed that the production does not involve the use of a micro-organism and were content that the production process employed by the applicant does not give rise to concern.*

XIII. Toxicological information on the novel food

Information on this aspect is provided on pp p 44-111 of the application dossier

23. **Biochemical Aspects (Absorption, distribution and excretion)**
    The applicant presents a number of studies that indicate a variable and incomplete absorption of D-tagatose. One study also details a pronounced increase in the short chain fatty acids in the blood. SCFA's are produced by bacterial fermentation of the unabsorbed NI in the large intestine. The applicant refers to this 'prebiotic' effect as a tangible benefit that can be attributed to the consumption of the NI.

24. Several studies carried out on humans indicate that intestinal side effects, including stool softening, may occur in susceptible individuals after the consumption of more than 15g D-tagatose (ingested in a single sitting). The

tolerable daily dose is a multiple of the tolerable single dose as the intestinal effects are not cumulative over time.

25. **Metabolism**

The applicant has referred to a number of scientific studies that demonstrate that the metabolism of D-tagatose takes place along well defined biochemical pathways. Following an initial phosphorylation step, the metabolism converges with the pathway seen for fructose.

26. **Toxicological studies**

The applicant includes reports from a number of animal studies, which are listed below. The applicant has also conducted four studies indicating a lack of genotoxicity. These studies have also been reviewed by JECFA, which considered D-tagatose three times during 2001-2004. The initial JECFA evaluation of D-tagatose highlighted a number of questions concerning, glycogen deposition and hypertrophy in the liver, and increased serum levels of uric acid.

27. The applicant commissioned a number of additional studies that paid particular attention to these parameters, and following a detailed evaluation JECFA allocated an ADI "not specified" for D-tagatose at its 63rd Meeting in June 2004. The applicant has submitted the same data for novel food approval.

Genotoxicity studies				
Test	**Test system**	**Concentration**	**Results**	**Reference**
Bacterial gene mutation [a]	*S.typhimurium* (TA 1535, TA 1537, TA1538, TA98, TA100); E.coli (WP2uvrA)	100-5000 mg/plate	Negative	Lawlor, 1993; Kruger, 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/bw (p.o.)	Negative	Murli, 1994a; Kruger et al., 1999a
TK-locus mutation [a, c]			Negative	

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

Animal studies					
Type of study	**Species (N)**	**Dose level (% of diet or g/kg bw)**	**Results**	**NOAEL (% of diet and/or g/kg bw/d)**	**References**
acute toxicity test	Rats (5M, 5F) Mice (5M)	10g/kg bw (single dose)	no mortality or reaction to treatment	10g/kg bw	Trimmer, 1989
Subchronic (90-d) toxicity study	S-D rats (20M 20F / group)	0,5,10,20% 10% fru + 10% cellulose	soft stool (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 (F) and 4.1 (F) g/kg bw/d]	Trimmer et al., 1993 Kruger et al., 1999c
Subchronic (29-31 d) study on liver parameters [d]	S-D rats (20M / group)	0,5,10,20% tag	Dose dependent increase of liver glycogen and lower weight [b)]. No ultrastructural (EM) changes of liver tissue except increased glycogen deposition. Slight increased ALAT, ASAT in 20% tag group probably in response	n.d [d)]	Lina et al., 1998 Bar et al., 1999
Subchronic (6-month) toxicity study	Wistar rats (60 F/group)	0, 5, 10% tag, 20% fru, 10% tag + 10% fru Interim kills on day 3, 7, 14, 28, 94, 128 (10F / 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 1-28)]	Lina & de Bie, 2000d
Chronic (24-month) toxicity/carcinogenicity 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 tests (cecum: weight only). Liver enlargement in 10% tag (M), 20% Fru (M), 10% tag +fru (M&F) but no morphological changes. Increased nephrocalcinosis in females of all tag dose groups and in 10% tag (M) and 10% + 10% fru (M). increased incidence of adrenomedullary proliferative	2.5% of diet [< 1 g/kg bw/d]	Lina & Kuper, 2002 Lina & Bar, 2003

			disease in 2.5% tag (M), 5% tag (M & F), 10% (M & F) and 10% + 10% fru (M&F)		
Energy balance study (33-d)	Pigs (2 / group)	0, 20% tag, 20% suc, 10% tag + 10 % suc	No ultrastructural (EM) changes of liver tissues	5 g/kg bw/d	Mann, 1997
Embryotoxicity / teratogenicity study (range finding)	S-D rats (5M / 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 effect otherwise).	20 g/kg bw/d (11 g/kg bw/d)	Schroeder, 1994a
Embryotoxicity / teratogenicity study	S-D rats (24M / 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

Key: M = Male, F = Female

Abbreviations: tag, D- tagatose; fru, fructose; suc, sucrose; ALAT, alanine aminotransferase; ASAT, aspartate minotransferase; 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.

Discussion *The novel ingredient has been subject to a number of toxicological studies. The Committee noted the toxicological assessment by JECFA in 2004 and agreed with the expert group that the data did not highlight any toxicologically significant findings, and exhibited properties that were similar to other carbohydrates of other low digestibility.*

Allergenicity and Labelling

Information on this aspect is provided in p 109-110 and Annex 4 of the application dossier

28. The NI is manufactured from crystalline lactose, obtained from cheese whey, which contains protein at levels of up to 0.2%. Recognising the known allergenic potential of milk and derived products, the applicant has demonstrated the absence of whey protein in the NI using an ELISA method. (<10µg protein equivalent / g NI, see Annex 4). The same assay detected protein in 2 (of 3) lactose samples tested.

29. The applicant speculates that the absence of whey protein is to be expected due to the production process, which involves the use of heat-treatment, high pH, ion-exchange resins and activated carbons.

30. In their consideration of the product JECFA concluded that ingestion of 30g or more of the NI may cause gastrointestinal effects in humans. The applicant has also suggested that no warning on laxative effects is necessary for foods listed in the table containing D-tagatose because the maximum intake of D-tagatose would be extremely unlikely to exceed 10g per eating occasion for consumers of any age group (see Table 3 of application dossier). This statement is based on high level US consumption data using figures at the 90th percentile. Estimates using UK NDNS data are similar. The applicant has also acknowledged that the products described in the table are indicative of intended use only, and it would be appropriate to label any foods containing more than 15g of D-tagatose per serving with the statement “excessive consumption may produce laxative effects”. This text is in line with the current requirement for polyols (Directive 96/21/EC) which applies to foods containing more than 10% polyols. The applicant’s proposal will cover all food categories and is based on the intolerance being induced by the amount, rather than concentration. Unlike polyols, tagatose is proposed for certain beverages, where higher levels of intake may be achieved at a lower concentration of D-tagatose.

31. Following a specific request by the Committee, the applicant submitted additional data to demonstrate that the proposed labelling described above was equally applicable to children as well as adults.

Discussion *Members noted that although the applicant provides evidence that the NI is unlikely to contain whey proteins, the product is derived from a milk source. A new amendment (2003/89/EC) to the food labelling directive (2000/13/EC) requires specified food allergens and their derived ingredients to be included in ingredients listing. Milk is a specified allergen and this requirement therefore applies to the novel ingredient, irrespective of the manufacturing process, unless the applicant applies to the Commission for a formal exemption. Members wished to note that it was their*

view that the data provided to demonstrate that the product was free from milk proteins was unlikely to offer sufficient grounds to qualify for an exemption.

Concerning the potential for exerting a laxative effect, the Committee noted the proposal for labelling on the basis of consumption of more than 15g of the NI in a single serving, similar to the labelling requirement for polyols set out in Directive 96/21/EC. There are no data on the effects of tagatose consumption amongst children although young children are known to be generally more prone to diarrhoea, probably because they have a less developed GI tract. The limited data available on other poorly absorbed compounds, such as sorbitol, indicate that pre-school children may be more sensitive than adults and older children. The applicant does not intend the ingredient to be used in foods specially manufactured for young children but it is likely that they will consume general foods that contain D-tagatose, particularly soft drinks. The Committee therefore considered that the labelling criterion proposed by the applicant is appropriate for solid foods, but proposed that all beverages containing more than 1% D-tagatose should also carry the same advisory labelling.

General discussion

32. Members noted that D-tagatose has been subjected to thorough toxicological testing and agreed with the conclusion of JECFA that it is a substance of low toxicity and does not represent a hazard to health.

33. Like other poorly absorbed compounds, D-tagatose may cause mild gastrointestinal effects in high level consumers. The individual doses of D-tagatose associated with these effects is in the range 15-30 grams which is unlikely to be achieved from consumption of the tagatose-containing foods described by the applicant. Nevertheless, the range of uses may be extended in future and Members supported the applicant's proposal to include advisory labelling on any food product that contained in excess of 15g D-tagatose per serving as being adequate to ensure that consumers were advised of the effect of potential gastrointestinal intolerance. To take account of consumption by young children, and because of evidence that poorly-absorbed compounds may exert a greater laxative effect when taken in liquid form, this advisory labelling should also be applied to all beverages containing more than 1% D-tagatose.

34. Members also noted that allergen labelling as defined in amendment 2003/89/EC to the food labelling directive (2000/13/EC*)* will apply to all products that contain the NI, unless the applicant applies to the Commission for a specific exemption to be incorporated into the relevant directive.

Conclusion

35. The Advisory Committee on Novel Foods and Processes is satisfied by the evidence provided by Bioresco on behalf of Arla Foods that D-tagatose is acceptable, subject to the applicant’s adherence to the proposed specification and the labelling requirements described above.

9 August 2005