COMMITTEE PAPER FOR DISCUSSION **ACNFP/113/5**

ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

D-RIBOSE

ISSUE

A revised application has been submitted to the UK Competent Authority for authorisation of D-ribose under the novel foods regulation (EC) No 258/97. The Committee is asked to advise whether the available data provide an adequate basis for a safety assessment, and if it recommends authorisation of the product.

Background

1. An application has been submitted by Bioenergy Life Science, Inc. for authorisation of D-Ribose as a novel ingredient in the EU. The application was originally accepted by the UK Competent Authority on 17 March 2008 and was discussed by the Committee at meetings during 2008-2009, culminating in a request from the Committee for additional information to resolve uncertainties arising from a study on reproductive toxicity.

2. The applicant sought further advice from the Committee in April 2013 (ACNFP/110/2) on what additional information should be provided. The Committee advised that it did not see, at this stage, the need for additional animal studies and it would consider D-ribose in the light of a revised dossier in which the applicant should incorporate the additional information provided during the 2008/9 discussions, along with any new data that is relevant to the safety of D-ribose.

3. The revised dossier is attached at Annex 1. The main changes to the original document are:
    - Updated intake estimates;
    - Updated post-market monitoring information;
    - More detailed discussion of findings from the reproductive toxicity study;
    - Additional commentary on the potential effects of D-ribose on glucose metabolism and on uric acid levels.

4. Given the time that has elapsed since the original application, and the resulting turnover in membership of the Committee, this paper summarises the complete dataset as if it was a new application.

5. D-Ribose is a naturally occurring 5-carbon sugar that is present in all living cells. Phosphate forms of ribose have been detected in numerous human and animal tissues and cells. Ribose is a component of RNA, which is used for genetic transcription, adenosine triphosphate (ATP), the reduced form of nicotinamide

diadenine dinucleotide (NADH), and other chemicals important to cellular metabolism. Ribose is also a precursor for the synthesis of purine nucleotides. Endogenously, ribose is synthesised from the conversion of glucose *via* the pentose phosphate pathway (PPP). The applicant states that exogenous supplementation of D-Ribose allows the cell to bypass the rate-limiting steps of the PPP, providing a precursor for ATP and nucleotide synthesis.

6. D-Ribose is produced by the applicant by fermentation, using a non-genetically modified strain of *Bacillus subtilis* (*B. subtilis*) that is unable to utilise D-Ribose for downstream metabolism due to a disruption in the D-Ribose metabolic pathway.

7. Ribose has a sweet taste, approximately equivalent to that of glucose The applicant states that D-Ribose is intended for use as an energy source in a range of foods, including food supplements and foods for special medical uses..

8. The present application for authorisation of D-Ribose was prepared pursuant to Commission Recommendation (97/618/EC) of 29 July 1997 concerning the scientific aspects and presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients. D-Ribose has been classified as a pure chemical or simple mixture from a non-GM source (class 1.2). The requirements for a submission for this class are as follows:

I	**Specification of the NF**	**X**
II	**Effect of the production process applied to the NF**	**X**
III	**History of the organism used as the source of the NF**	**X**
IV	*Effect of the genetic modification on the properties of the host organism*	**-**
V	*Genetic stability of the GMO*	**-**
VI	*Specificity of expression of novel genetic material*	**-**
VII	*Transfer of genetic material from GM microorganisms*	**-**

VIII	*Ability to survive in and colonise the human gut*	**-**
IX	**Anticipated intake/extent of use of the NF**	**X**
X	*Information from previous human exposure to the NF or its source*	**-**
XI	**Nutritional information on the NF**	**X**
XII	**Microbiological information on the NF**	**X**
XIII	**Toxicological information on the NF**	**X**

The information presented in the dossier is structured accordingly and is considered below under these schemes.

9. A non-confidential version of the original application dossier was placed on the FSA website to allow the public to input into the UK assessment. No comments were received.

I. Specification of the novel food

Annex 1, p 5-8 and p 16-17

10. D-Ribose is a dry powdery product that is white to slightly yellow in colour, of high purity (minimum 97%), and containing low levels of arsenic (<1 mg/kg) and lead (<0.1 mg/kg). Other heavy metals were not analysed.

11. Batch on batch variation was assessed by chemical and physical analyses of five different lots of D-Ribose, as shown in Annex 1, page 8. Certificates of analysis for the different lots can be found in Annex 1, Appendix B. The results of these analyses indicated a narrow range of variation in composition and contaminants. The stability of D-Ribose has been studied in different packaging (Annex 1, p 16-17) and the shelf-life of D-Ribose was determined to be 24 months at room temperature. The melting point for D-Ribose in the specification is reported over a wide range (80-90°C), although analyses of five different lots of D-Ribose showed the range of melting points to be narrow (84-85°C), as might be expected for a product of high chemical purity.

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

Annex 1, p 9-16; PROTECT - COMMERCIAL

12. D-Ribose is produced by fermentation using a transketolase-deficient strain of *B. subtilis*. The metabolic pathway for D-ribose production in transketolase-deficient strains of *Bacillus* spp is illustrated in Figure II.2-1 (Annex 1 p 13).

13. The production strain is unable to utilise D-Ribose, which accumulates in the fermentation medium. The medium is centrifuged to remove bacteria and solid particles and then filtered, decolourised and desalted with successive steps using activated carbon and other absorbents. Purified D-ribose is concentrated by evaporation and crystallised using ethanol. D-ribose crystals are recovered by centrifugation before being dried and packaged. The raw materials used in the production of D-ribose and components of the fermentation media are listed in Annex 1 p 10-11. Specifications of the raw materials are provided in Annex 1, Appendix C.

14. The applicant states that D-Ribose is unlikely to contain the production organism and is free from any bacteria (including the production organism) or bacterial components. No protein was detected using the Bradford assay (detection limit 0.1 µg/ml) in samples of 20% D-Ribose solution from six randomly selected lots of Bioenergy D-Ribose (Annex 1, Appendix E) and residual ethanol levels were in the range 0.01% – 0.02%. Tests on three batches showed only low levels of bacterial endotoxins (polyliposaccharides) at 20% or less of the specified upper limit of 25 Endotoxin Units per gram.

III. History of the organism used as a source of the novel food

Annex 1, p 21-24

15. *Bacillus spp*. are Gram positive rod-shaped, spore forming bacteria. *B.subtilis* is found in soil and naturally present in foods such as spices and milk powder. A long history of safe food use has been established for *B.subtilis*. For example, it is involved in the production of some traditional fermented foods (soy sauce, soybean paste, tofu) and in the production of certain chemicals (ribose, riboflavin and other metabolites).

16. A number of D-Ribose producing *Bacillus spp*. are listed in the American Type Culture Collection (ATCC). These strains, developed by chemical or UV mutation, are unable to utilise D-Ribose as a result of a lack of transketolase activity. D-Ribose producing *Bacillus spp*. are asporeogenous as they are unable to convert ribose into ribitol and teichoic acids, which are required for spore formation. Bioenergy D-Ribose is produced using *B*. *subtilis* ATCC 21951, which was originally deposited as *Bacillus pumilus* but later re-classified.

17. The *B*. *subtilis* group of the genus *Bacillus* is genotypically and phenotypically distinct from the *B*. *cereus* group, which contains the pathogens *B. anthracis* and *B*. *cereus*. The complete genome sequence for *B.subtilis* is established and the genes encoding virulence factors in *B*. *anthracis* and *B*. *cereus* are not present and there are no known plasmids encoding for antibiotic resistance. Further information can be found in Annex 1, Appendix F.

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

Annex 1, p 25-36

18. The applicant intends to use their D-Ribose product as an ingredient in a variety of foods. A list of products and the proposed food uses and levels can be found below.

Summary of the Individual Proposed Food Uses and Use-Levels for D-Ribose in the EU				
Food Category	**Proposed Food Use**	**Maximum use-level (%)**	**Serving Size (g) (1)**	**D-Ribose per Serving (g)**
1 Dairy products and analogues	1.4 Flavoured fermented milk products including heat treated products	2.2	125	2.75
3 Edible ices	3 Edible ices	0.4	75	0.3
5.1 Cocoa and chocolate products (as covered by Directive 2000/36/EC)	5.1 Cocoa and chocolate products (as covered by Directive 2000/36/EC) (3)	1.7	40	0.68
5.2 Other confectionery including breath freshening microsweets	5.2.1 Other confectionery with added sugar	2.0	35	0.7
	5.2.2 Other confectionery without added sugar	2.0	35	0.7
7 Bakery wares	7.2 Fine bakery wares (2)	2.0	15 – 110	0.3 – 2.2
13 Foods intended for particular nutritional uses (as defined by Directive 2009/39/EC)	13.2 Dietary foods for special medical purposes (defined in Directive 1999/21/EC), excluding products in category 13.1.5	2.2	250 – 500	5.5
	13.3 Dietary foods for weight control diets intended to replace total daily food intake of an individual meal	5.0	250 (bars) 60 (beverages)	1.0 (bars) 3.0 (beverages)
14.1 Non-alcoholic beverages (4)	14.1.2.1 Fruit juices (as defined by Council Directive 1001/11/EC)	1.2	180	1.92
	14.1.2.2 Vegetable juices	1.0	240	2.4
	14.1.3 Fruit nectars (as defined by Council Directive 2001/112/EC) and vegetable nectars and related products	1.2	4160	1.9
	14.1.4.1 Flavoured drinks with sugar	0.4	250	1.0
	14.1.4.2 Flavoured drinks with sweeteners	0.4	250	1.0
	14.1.5 Coffee, tea, herbal and fruit infusions; chicory, tea, herbal and fruit infusions; and chicory extracts (5)	0.7	190	1.33

(1) Serving sizes are based on the UK Food Portion Sizes handbook (FSA, 2002) and manufacturers' websites
(2) Excludes buns, cornets, pies, crumpets, gingerbread and croissants
(3) Includes chocolate confectionery such as chocolate coated nuts, fruit, caramels, chocolate eggs, bonbons etc. These are all the chocolate products that are not the chocolate bar type.
(4) Flavoured drinks do not include any sugar-free or very low calorie varieties as these beverages were found to have very low carbohydrate content and therefore not a target for D-ribose
(5) Reduced calorie beverages are only included if they have a carbohydrate content >0.2g/100ml.

19. The applicant also proposes that D-Ribose will be available as a food supplement at a recommended dosage of up to 10 g per day. This use is not included in the intake estimates presented below, as the applicant considers that D-Ribose used as a food supplement would typically be taken as an alternative to the foods listed in the above table.

20. Intakes were estimated for a range of population groups using information from the most recent data from the NDNS rolling programme, representing the first 3 years of data collection (2008/09, 2009/10, 2010/11). These estimates are summarised in the tables on page 35 of the dossier (Annex 1).

21. On an all-user basis, the highest estimates of mean and 97.5th percentile intakes of D-Ribose by the UK population were observed in male teenagers at 3.1 and 11.3 g/person/day, respectively. On a body weight basis, toddlers (age 1-3) consumed the greatest amount of D-Ribose with the highest mean and 97.5th percentile all-user intakes of 123 and 385 mg/kg body weight/day, respectively. These are highly conservative estimates based on a worst-case scenario where all possible foods contain D-Ribose at the maximum levels given in the table above.

X. Information from previous exposure to the novel food

Annex 1, p 37-38, 73

22. The applicant notes that ribose is a natural component of the diet. There are no estimates of dietary intake, but endogenous production in humans is of the order of 3-15 grams/day.

23. Adverse reaction records have been maintained by Bioenergy, Inc. for D-Ribose sold in the US since 2004. Approximately 1900 metric tonnes have been sold in the US, equivalent to approximately 1.3 billion servings.. Thirty-three adverse reactions reports have been received from customers in relation to D-Ribose supplements and no reports have been received for foods with added D-Ribose. The adverse reports are summarised in the following table, categorised according to severity (life threatening, major, minor or nuisance) and type.

24. D-Ribose has a calorific value of 4 kcal/g, which is equivalent to that of other carbohydrates.

Summary of the type and severity of adverse reactions for D-Ribose (1 January 2005 to present)

Severity	Type	Description (Number of Reports)
Nuisance	CNS	Headache (1); Light-headedness (1)
Nuisance	MS	Felt funny (1); weakness/fatigue (1), Swollen leg (1)
Nuisance	Derm	Rash (3), Itching (3)
Nuisance	Endo	Transient elevated blood sugar in diabetic customers (4)
Nuisance	GI	Diarrhoea (2); upset stomach (5); excess gas (1); constipation (2)
Minor	CV	Transient racing heart, edgy (3)
Minor	MS	Muscle tightness (1)

Type of adverse reactions:

Cardiovascular (CV), Respiratory, Central Nervous System (CNS), Urinary,

Musculoskeletal (MS), Immunological, Endocrine (Endo), Oncologic, Gastrointestinal (GI), Dermatological (Derm) and Other.

XI. Nutritional information on the novel food

Annex 1, p 39-40

25. D-Ribose has a calorific value of 4 kcal/g, which is equivalent to that of other carbohydrates.

26. The applicant has provided a description of the metabolic fate of ingested D-Ribose (Annex 1, p 45). Ingested D-Ribose enters the pentose phosphate pathway downstream of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and is made available for flux down the purine nucleotide pathway of purine and pyrimidine synthesis. When pentose phosphates are not required for purine nucleotide synthesis in muscle or when ribose is present in excess amounts, they are recycled through glycolysis mainly in the liver via conversion into fructose-6-phosphate, fructose 1,6 bisphosphate and glyceraldehyde 3-phosphate and eventually form carbon dioxide and water. yielding energy via ATP turnover. Fructose-6-phosphate is further converted to glucose-6-phosphate, which in turn is converted to glucose-1-phosphate and incorporated into glycogen for storage.

27. Significant decreases in blood glucose levels have been reported in some clinical studies (Annex 1, p 69, 71-72, 74-76) but this has only been observed where subjects were given a bolus of D-Ribose of 10 g or greater with no additional source of energy after an overnight fast. For the intended uses proposed by the applicant, D-Ribose per serving size would not exceed 5.3 g and an additional carbohydrate energy source would be included with D-Ribose which the applicant states will compensate for any blood glucose lowering effects. The applicant acknowledges that the proposed use in reduced calorie drinks will not provide additional carbohydrate energy sources but these beverages will contain only 1 g of D-Ribose per serving, below the level where glucose lowering effects were observed. The applicant has concluded that D-Ribose consumption should not be a risk for diabetics.

28. The mechanisms of the alterations in glucose levels following large bolus doses of D-Ribose cannot be explained fully by an increase in insulin levels. Other possible mechanisms include a reduction in glycogenolysis, in response either to a ribose-induced increase in intracellular ATP levels or to an inhibitory effect of ribose on phosphoglucomutase, one of the enzymes involved in glycogenolysis.

XII. Microbiological information on the novel food

Annex 1, p 41-42

29. The microbiological specification for the novel ingredient is as follows:

- Total microbial count $\leq$100 CFU/g
- *Salmonella* spp. absent in 25 g
- Coliforms $\leq$10 CFU/g
- Yeast and mould $\leq$ 100 CFU/g

30. Microbiological analysis of five lots of D-Ribose confirmed that they all met these specifications.

XIII. Toxicological information on the novel food

Annex 1, p 43-78

31. A number of toxicological studies have been described in the dossier as demonstrating the safety of D-Ribose. Animal studies, mutagenicity studies and genotoxicity studies are summarised in the tables below.

(a) Animal Studies

Source of D-Ribose	**Tests**	**Result**
Bioenergy	Sub-chronic toxicity (13 weeks) at doses up to 20% in the diet, equivalent to 0-15 and 0-15.7 g/kg bodyweight per day for males and females, respectively	NOAEL was established to be the highest dose, equivalent to a mean daily intake of 15 and 15.7 g/kg bodyweight per day for males and females, respectively
Bioenergy	Developmental toxicity at doses equivalent to 0-9.91 g/kg bodyweight per day	NOAEL for teratogenicity was established at 9.91 g/kg bodyweight per day, but a "clearly undisputable NOAEL for teratogenicity was at a D-ribose intake of between 3.64 and 4.61 g/kg bodyweight per day"

(b) Mutagenicity and genotoxicity studies

Source of D-Ribose	**Tests**	**Result**
Bioenergy	Bacterial reverse mutation assay using 4 strains of histidine-requiring *Salmonella typhimurium* and 1 strain of tryptophan-requiring *Escherichia coli* (with and without metabolic activation)	Negative on all strains
Bioenergy	*In vitro* chromosomal aberration assay in Chinese hamster ovary cells (with and without metabolic activation)	Negative
Bioenergy	Gene mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells	Negative
Bioenergy	*In vivo* rat bone marrow micronucleus assay	Negative
Other sources	Bacterial reverse mutation assay using 2 strains of histidine-requiring *Salmonella typhimurium*	Negative

32. In the developmental study, offspring were examined at 21 days of age and the only potentially adverse findings were that the offspring of rats fed the two

highest doses (10% and 20% ribose in the diet) showed an increased incidence of a condition called “wavy rib” and some signs of delayed ossification. The Committee has previously discussed these findings at length and concluded that further research is unlikely to provide useful information. The applicant discusses these results in pages 56-58 of Annex 1 and concludes that these are transient variations that are related to maternal stress associated with the very large amounts of monosaccharide in the D-Ribose diets.

33. Human studies investigating effects of D-Ribose (seventeen oral administration studies and four intravenous administration studies) are described in Annex 1 p 51-63. The majority of these are efficacy studies with the recording of side effects. Doses ranged from 2 to 270 g/day. The applicant has stated that overall, D-Ribose has been shown to be well tolerated with no adverse effects reported other than diarrhoea at daily doses of 60 g or greater. These doses are five times higher than the estimated 97.5th percentile intake of D-Ribose from the applicant’s proposed food uses (11 g/person/day) so the applicant does not envisage any safety concerns for the proposed uses.

34. D-Ribose administration can be associated with blood glucose lowering effects. For example, asymptomatic and transient hypoglycaemic effects (returning to baseline levels approximately 2 hours after D-Ribose ingestion) were observed with bolus doses of 10 g or greater in healthy individuals, where D-Ribose was administered with no other added source of energy after an overnight fast (Annex 1 p 55-56, study by Fenstad *et al*., 2000). The applicant has explained that this should not pose a problem for their proposed uses of D-Ribose (see Section XI above).

35. Single oral doses of 5 and 10 g of D-Ribose resulted in a significant increase in blood uric acid levels at 30 and 60 minutes after administration compared to a 2 g dose in healthy individuals (Annex 1 p 65-66, study by Fenstad *et al*., 2000). However, this response was not dose dependent as uric acid levels were lower after ingestion of 10 g of D-Ribose than after ingestion of 5 g. The authors suggest that this may be a result of D-Ribose-induced purine synthesis, with uric acid being a catabolic end product of adenosine and inosine. The applicant notes that, while very high doses of D-Ribose have been linked with increased uric acid levels in some studies, the intake of D-Ribose at the levels described in Section IX above will not be a concern for individuals with hyperuricemia

Allergenicity

Annex 1, p 79

36. The applicant notes that *B.subtilis* is not likely to be present in the final D-Ribose preparation due to the purification process employed during manufacture. Additionally, the Bradford assay did not detect any proteins in

samples of a D-Ribose solution (LOD 0.1 µg/ml) so the applicant suggests that the allergenic potential of D-Ribose is limited.

Labelling

Annex 1, p 36

37. The applicant has provided the following labelling suggestion:

‘The designation 'D-ribose' shall be displayed on the labelling of the product in the list of ingredients of foodstuffs containing it. The food product may also incorporate on the label the words “contains an additional source of D-Ribose” in a typeface that is at least the same size as the list of ingredients itself.’

Consumer access and choice

38. The Secretariat has considered the issues of access and choice in relation to D-Ribose. If authorised, D-Ribose would be available for use in products across the UK and subsequently in other EU Member States. In practical terms, access to products containing D-Ribose could be limited by a high price or by limited geographic distribution, which are both driven by commercial considerations that cannot be predicted at this stage.

39. It is envisaged that the introduction of products containing D-Ribose will increase existing consumer choice. The consumer would be aware of the presence of D-Ribose through the ingredient list and, most likely through special marketing that highlights its contribution to the nutrient composition of foods.

COMMITTEE ACTION REQUIRED

40. The Committee is asked whether the available data provide satisfactory basis for evaluating the safety of this novel food ingredient.

41. If so the Committee is asked whether it is content, pending its review of any public comments on the application, to recommend approval of D-Ribose as an ingredient in the foodstuffs listed in paragraph 18.

42. If not, the Committee is asked to indicate what additional data would be required.

Secretariat
November 2013

Annex attached:

Annex 1- PROTECT: COMMERCIAL Revised application for the approval of D-Ribose as an ingredient in foods and food supplements (Confidential version)

(Appendices to the dossier are available to Members on request.)