Food Standards Agency
food.gov.uk

Mr Andreas Klepsch
European Commission
By email

31 August 2012

INITIAL OPINION: **UV TREATED BAKER'S YEAST**

Dear Mr Klepsch,

On 26 April, the UK Competent Authority accepted an application from Lallemand for an ultraviolet (UV) treated baker's yeast (*Saccharomyces cerevisiae),* which contains enhanced levels of vitamin D2 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 this UV treated baker's yeast, at levels not exceeding the maximum use levels described, meets the criteria for acceptance of a novel food defined in Article 3(1) of regulation 258/97.

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

Yours sincerely,

(By e-mail only)

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For the UK Competent Authority

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ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

OPINION ON A UV TREATED BAKER'S YEAST

Applicant **Lallemand**

Responsible Person **Celia Martin**

EC Classification **2.2**

Background

1. An application was submitted by Lallemand, for the use of ultraviolet (UV) treated baker's yeast (*Saccharomyces cerevisiae),* which contains enhanced levels of vitamin D2, as a novel food ingredient.

2. The applicant intends that the novel food, referred to as in this opinion **vitamin D2 yeast concentrate**, will be used for the leavening of bread, as a food supplement and in other foods which typically contain baker's yeast. The same yeast is approved for use in bread products in Canada and the US and the applicant reports the amount of vitamin D2 as either micrograms or International Units and this is reflected in this paper. 1mg vitamin D is equivalent to 40,000 IU.

3. Vitamin D2 (ergocalciferol) is produced photochemically from the precursor ergosterol in plants and fungi and is chemically distinct from vitamin D3 (cholecalciferol), which is synthesised in human skin from the precursor 7-dehydrocholesterol following exposure to sunlight. There is relatively little vitamin D present naturally in food (chiefly oily fish, eggs and liver) but a number of foods are routinely fortified with vitamin D e.g. breakfast cereals and margarine.

4. Both vitamin D2 and D3 are listed in Annexes 2 and 3 of regulation (EC) 1170/2009, which permits their addition to both foods and food supplements . In the EU, Vitamin D2 is currently found in food supplements by the UV treatment of purified ergosterol that is extracted from baker's yeast. This vitamin D2 yeast concentrate is to be marketed as an alternative to other vitamin D sources which are already permitted to be added to foods, including (in theory) bread.

5. In accordance with the Novel Foods Regulation, vitamin D2 yeast concentrate has been classified as a complex novel food from non-GM sources source (class 2.2).

I Specification of the Novel Ingredient (NI)

Dossier, p 7-15

6. The applicant has provided a specification for vitamin D2 yeast concentrate which is detailed in the Dossier (Appendix I.6.1) and summarised below. Other than containing significant levels of vitamin D2 the yeast is described as being no different to conventional, untreated yeast, which is widely used for in baking of bread.

Proposed Specification of vitamin D2 yeast concentrate	
Parameter	**Specification**
Appearance	Tan coloured, free flowing granules
Vitamin D2[1]	1,800,000 – 3,500,000 IU vitamin D /100g yeast
Coliforms[2]	<1000 / gram
E. coli[2]	<10 / gram
Salmonella[2]	Absent / 25 grams
Shelf life	3 years (sealed package)

[1]AOAC analysis [2] FDA Bacteriological Analytical Manual

7. The production of vitamin D3 *in vivo* by humans following exposure to sunlight produces two related sterols – tachysterol and lumisterol – and the applicant has carried out chromatographic analysis (HPLC) to determine whether the UV treatment of their yeast results in the production of these or other, related, sterols. The detailed results of these studies, carried out using HPLC, are available in the Dossier (Appendix I.5.1 A,B&C) and are summarised in Section 1.5.1. The results indicate that, prior to UV treatment, the only sterol present in the yeast in significant quantities was ergosterol (7.05mg/g). Following exposure to UV, in addition to the internal standard, three sterols were observed. These were identified to be ergosterol (6.56mg/g), ergocalciferol (vitamin D2, 1.06mg/g) and tachysterol.

8. The applicant was unable to quantify the level of tachysterol present but noted that it was significantly smaller than the equivalent vitamin D2 peak (4.27% compared with 16.03%, as a proportion of total peak area). A separate exercise to quantify the levels present in two commercial lots was carried out by another laboratory, which found tachysterol levels to be 140 & 145mg/kg and vitamin D2 672 & 825mg/kg (Dossier, Appendix I.5.1 A). All other minor peaks were also present prior to UV treatment.

9. In order to determine the accuracy of the HPLC method, the level of vitamin D2 present in a single batch was analysed in duplicate on three occasions over a 24 day period. This analysis showed little variation over the study period and gave

an average of 3,290,000 IU vitamin D/100g (Dossier Table 1). The same batch was also assessed using an alternative, modified, AOAC detection method giving a similar result (3,230,000 IU/100g). (Dossier, Appendices I.5.1.E,F & G).

Vitamin D2 in bread. The applicant also investigated the level of vitamin D2 present in three loaves baked using vitamin D2 yeast concentrate. In this study vitamin D2 yeast concentrate was blended with standard yeast to give 400 IU /100g bread. The levels of vitamin D in both the vitamin D2 yeast concentrate and the bread were analysed and found to be 3,440,000 and 489 IU / 100g respectively.

Discussion *The Committee agreed that the analytical data provided by the applicant were particularly thorough and was satisfied that the novel ingredient can be produced reproducibly by the applicant.*

II Effect of the production process applied to the NI

Dossier p16-26

10. Lallemand is a well established company who have been producing baker's yeast for almost 100 years. In addition to producing yeast for bread baking they also produce yeast for a range of other industrial applications e.g. brewing, oenology, animal health, and bioethanol production. They have provided extensive details of the procedures used to produce commercial quantities of yeast and the methods that they employ to ensure purity. These are detailed in the Dossier in Section II.2.1 (Confidential).

11. The yeast that is subjected to UV treatment is produced in the same manner as Lallemand's conventional baker's yeast product. To enhance the vitamin D content, the yeast (known as yeast cream) is continually pumped past UV lamps, wavelength 254nm, for 96 hours at 4°C prior to drying using a fluid bed dryer (yeast for baking) or a spray dryer or roller dryer (yeast for supplements). The resulting product, which contains between 1,800,000 and 3,500,000 IU vitamin D/100g, is a concentrated form of vitamin D2. When used for baking, it and would be blended with conventional yeast to ensure that the end product contains the required amount of vitamin D2, as detailed in Section IX below.

12. Lallemand analyse every production lot to determine the vitamin D2 content (in triplicate) and to check compliance with the microbiological specification. All Lallemand manufacturing sites are ISO certified and operate under GMP conditions.

13. **Genetic stability of vitamin D2 yeast concentrate.** UV is widely used for sanitation or sterilisation purposes and the applicant acknowledges that non-fatal, intensive doses of UV can induce mutations in microorganisms, particularly at the wavelength that they use for vitamin D2 production (254mn). In line with its

sanitising properties, the use of UV is particularly effective in inducing mutations that result in cells being unable to multiply. However, the applicant reports that the system they employ has little detrimental effect on the viability of the cells due to the constant circulation of the yeast cream, which contains concentrated amounts of cells, and the relatively poor transmission power of UV.

14. Scientific studies investigating the nature of mutations seen in yeast cells indicate that there are no gross chromosomal rearrangements and there are reports of an increase in mobile element TY transposition[1]. The applicant reports that the UV dosage that the yeast cells receive is much lower than the doses used for sanitation purposes or to induce mutations for research purposes (e.g. strain development). To demonstrate this, the applicant used RAPD-PCR and RFLP DNA fingerprinting techniques which they regard to be sufficient to identify both chromosomal rearrangements and point mutations. These techniques have previously been used to detect mutants in other organisms and, to increase the likelihood of inducing mutations, the applicant extended the time of exposure to UV from 96h to 160h. The results of the analyses can be seen (in the form of gels) on p24-5 of the Dossier. Based on these results the applicant concludes that each of the colonies subjected to UV treatment had an identical genetic profile to the control strain.

15. **Stability of the vitamin D2 yeast concentrate** Analysis of three lots of vacuum packed vitamin D2 yeast concentrate indicated that there was no significant reduction in the level of vitamin D2 over a three year period. The applicant does not indicate whether there is any reduction in the level of vitamin D2 if the yeast is incorporated into commercial products with an extended shelf life (e.g. food supplements).

Discussion *The Committee accepted that there were appropriate controls in place on the production of the NI to ensure the safety of the final product. The Committee did not regard the methods employed by the applicant to be adequate to identify potential mutants, noting that alternative methods such as RT-PCR[2] would be more appropriate. However the Committee accepted that, although the number of mutants may increase as a result of the UV treatment, the subsequent use of the vitamin D2 yeast concentrate could not lead to a mutant becoming the dominant strain in the final product.*

[1] A **transposable element** (TE) is a DNA sequence which can change its position within the genome. In *S.cerevisiae* there are 5 distinct families (TY1-TY5) which are all 'retrotransposon ' type TE's

[2] **RT-PCR** Reverse transcription polymerase chain reaction

III History of the organism used as the source of the NI

Dossier pp 27-30

16. *Saccharomyces cerevisiae* is extensively used by the both baking and brewing industries. It has a long history of safe food use across the world. EFSA has categorised *S. cerevisiae* as a microorganism that is proposed for QPS status[3]. Although EFSA acknowledges concerns in relation to invasive infection in certain compromised individuals, this appears to be related to a particular sub-species commonly referred to as *Saccharomyces boulardii*, which is not used in brewing or baking and is not a concern for the wider population.

17. The applicant also reports a number of uses of vitamin D2 rich yeast in the treatment of rickets in the early decades of the twentieth century and refers to approvals for their vitamin D2 yeast concentrate in the US and Canada (Dossier, Appendices B and C)

Discussion *The Committee accepted that baker's yeast and vitamin D2 have a long history of safe food use. Although there were reports of invasive infection of a related species, which was not used for food production purposes, this was not a cause for concern.*

IX Anticipated intake and extent of use of the NI

Dossier p 31-36

18. As detailed in paragraph 4 above, the applicant intends their vitamin D2 yeast concentrate to be an alternative to existing vitamin D2 ingredients that can be added to a range of foods and food supplements. However as vitamin D2 yeast concentrate requires assessment as a novel food the applicant has acknowledged that there is a requirement to indicate the intended uses in order to estimate likely intake and has done this based on use in bread, which is the major perceived use of the novel ingredient.

19. The applicant proposes that bread will be formulated to ensure that, irrespective of the level of vitamin D2 present in individual batches of the yeast concentrate, it would contain a maximum of 5µg vitamin D2 (200IU) per 100g[4]. 100g of this bread will provide the recommended daily allowance (RDA) for Vitamin D2.[5] The applicant indicates that proposed level of incorporation into food supplements will be in line with the same 5µg/day RDA figure. Based on the specification in

[3] EFSA Scientific Committee (2007) "Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA" http://www.efsa.europa.eu/en/efsajournal/doc/587.pdf

[4] One slice of large loaf of white bread weighs between 25 and 38 g, dependent on thickness (MAFF, 1988).

Section II above, 5µg of vitamin D2 is equivalent to between 5.7 and 11mg of the UV-treated yeast.

20. The applicant used published data from the UK 2010 National Diet and Nutrition Survey (NDNS) to estimate intake from a number of different bread types. As the NDNS publication only provides mean consumption figures, the applicant has assumed a high level consumption (97.5th percentile) of three times the mean value. The applicant has presented the mean and high level consumption figures for each individual bread category separately, assuming that all products on the market contain 5µg vitamin D2. The highest estimates of mean and high level daily intake of vitamin D2 were 3.85µg and 11.55µg in adult males, 3.75µg (high level 11.25µg) male teenagers (age 11-18) and 2.4µg (7.2µg) in boys (age 4-10).

21. Experts in food chemical intake for the Food Standards Agency have reviewed the intake assessment data provided and noted that, although the approach used by the applicant is only an approximation of high level consumption, the estimates that the applicant provided were consistent with their own analysis. The Food Standards Agency analysis provided estimates based on the consumption of all types of bread (see table below) and would appear to confirm that individuals are unlikely to consume large amounts of different breads over the course of a day, a point noted by the applicant. The Food Standards Agency officials also noted that the differences seen in the applicant's data could, in part, be due to an acknowledged skew in the published 2010 figures (caused by over-reporting of weekend consumption patterns) which is compensated for in the FSA figures.

FSA: Estimated intake of vitamin D2 from bread baked with vitamin D2 yeast concentrate and assuming that all bread contains the vitamins D yeast concentrate (based on 2010 and 2011 NDNS data)

	Mean intake (µg/day)	97.5th%ile Intake (µg/day)
Children(4-18)	3.6	8.6
Adults (19-64)	4.5	10.8
Adults (65+)	4.0	10.2

22. The vitamin D2 yeast concentrate is to be used in food supplements as a direct "like for like" replacement for existing ingredients and, as such, will not significantly add to the level of vitamin D consumed by individuals via supplements.

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[5] Commission Directive 2008/100/EC. This RDA for vitamin D is based on the earlier recommendations of the FAO/WHO (1988)

23. Data for estimating the current intake of vitamin D from food supplements are not available but the applicant highlights three separate surveys which provide estimates of dietary vitamin D intake, all of which are significantly lower than the EU RDA figure (see para 21 above).

- In 2003 the UK Expert Group on Vitamins and Minerals reported the intake of vitamin D from dietary sources to be in the region of 3-4µg/day (based on NDNS 2003 consumption data)[6]. These figures are slightly higher than figures reported using the 2010 NDNS data set (Dossier p42-43) which indicate that mean dietary intake of vitamin D is 1.9-3.1µg/day for males and 2.0-2.7µg/day for females.

- The applicant also reports the findings of a 2007 dietary study investigating the nutritional and energy intake of 15,000 Germans between the age of 14 and 80, which indicated that the median level of vitamin D2 intake is around 2-3µg/day (Dossier p40-41) and that 82% of men and 91% of women consume less than the 5µg RDA (see para 20 above). The German figures and the 2010 NDNS data both record young men and women as having the lowest intake of vitamin D.

- In 2007 the Scientific Advisory Committee on Nutrition reported that a significant proportion of the UK population does not consume sufficient vitamin D to meet the UK 1998 Reference Nutrient Intake values of between 7 and 10µg/day[7].

24. The applicant notes that both the mean and high level intake estimates are less than the RDA set by the US Institute of Medicine in 2010 (15µg/day) and, even if background intake from other dietary sources is taken into account, significantly less than the upper limits set by the same organisation and by EFSA in 2006 (See section XIII below).

Discussion *The Committee noted the shortcomings in the approach used by the applicant to estimate intake, but agreed that it did provide a reasonable estimate of the mean and high level consumption of vitamin D2 yeast concentrate. The Committee thanked the Food Standards Agency officials for validating the approach taken by the applicant and noted that it should be the responsibility of the applicants to provide a rigorous assessment of the likely intake of novel foods.*

[6] http://cot.food.gov.uk/cotreports/cotjointreps/evmreport/
[7] LINK NEEDED

XI Nutritional information on the Novel Food
Dossier p37-45

25. The applicant comments on the intake of vitamin D from dietary sources using information presented in EFSA's 2006 report on tolerable upper intake levels for vitamins and minerals. The UK and German surveys report that vitamin D consumption is below recommended daily intake levels (see previous section). The applicant also notes that the there is extensive consumption of *S. cerevisiae.* Allergenic potential and bioavailability are covered in Section XIII below.

Discussion *The Committee noted that there is increasing evidence of vitamin D deficiency in the EU, and that increasing dietary intake is one way to address this concern.*

XII Microbiological Information
Dossier p48

26. The applicant tests every batch of their yeast to ensure that it meets its microbiological standards (see specification para 6.). Information on the reference methods is available in Dossier, Appendix I.6.1.

Discussion*: Members accepted that the production process did not give cause for microbiological concern, and that compliance with the specification would ensure that the NI is free from pathogenic microorganisms.*

XIII Toxicological information
Dossier p.48-56

27. **Bioavailability.** The applicant acknowledges that there is an ongoing debate regarding the comparative effectiveness of vitamin D2 and vitamin D3, with a number of scientific studies reporting D3 as being the more 'potent' form. The uncertainty appears to be in relation to the effectiveness of the two forms in maintaining serum levels of 25-hydroxyvitamin D (25(OH)D) the pro-hormone that is measured to assess vitamin D bioavailability and status. Although both vitamin D2 and D3 are converted to the respective forms of 25(OH)D *in vivo,* measurement techniques appear unable to distinguish between 25(OH)D2 and 25(OH)D3. A number of studies point to the slow rate of conversion from vitamin D2 to 25(OH)D2 and a propensity for 5(OH)D2 to be metabolised quicker than the D3 variant. The applicant points to a number of recent studies, carried out in both humans and rats, which point to little or no difference in bioavailability between the two forms and also points to the use of vitamin D2 as the primary form for the prevention of vitamin D deficiency in children over the past 50 years.

28. The applicant has not carried out any toxicity studies on its vitamin D2 yeast concentrate but refers to a number of reviews which have set safe upper limits for

vitamin D, which are summarised in the table below. As a variant of an existing source of vitamin D2, the applicant considers that these limits apply equally to their vitamin D2 yeast concentrate. These limits are higher than the anticipated intake of vitamin D2 from the novel ingredient (see para 19 above) which give a figure of around 15µg per day for high level consumers when the highest background sources are taken into account, plus an additional 5µg if both bread and supplements are consumed.

Published upper limits for vitamin D (µg/day)

	EFSA[1]	IoM[2]	EVM[3]
Adult	50	100	25
Child (14-18)	-	100	-
Child (11-17)	50	-	-
Child (9-13)	-	100	-
Child (3-10)	25	-	-
Child (4-8)	-	75	-

[1] EFSA 2006 [http://www.slv.se/upload/dokument/efsa/upper_level_opinions_full-part33,0.pdf]
[2] US Institute of Medicine 2010 report [http://www.iom.edu/Reports/2010/Dietary-Reference-Intakes-for-Calcium-and-Vitamin-D.aspx]
[3] UK Expert Group on Vitamins and Minerals report. Figure refers to supplementation and the general population. [http://cot.food.gov.uk/pdfs/vitmin2003.pdf]

29. The applicant has also reviewed a number of recent (since 2007) studies which confirm that long term exposure to relatively high levels of vitamin D does not, in the main, give rise to any adverse health effects. These are not detailed in this paper as they are summarised in the Dossier, pages 54-62.

Discussion. *The Committee noted that there have been a number of reviews into the safety of vitamin D and although, these give differing upper limits, they are all higher than the maximum high level of intake for vitamin D2 yeast concentrate, even if background sources are taken into account.*

30. **Tachysterol.** The applicant noted that the tachysterol is regarded to be both inert and non-toxic (Horlick, 1981; Gilchrest, 2006). However the Committee queried whether this view applied if it was taken orally. The Committee also noted that the presence of tachysterol could potentially reduce or block absorption of vitamin D *in vivo*.

31. The applicant's response noted that there is little published information available investigating the effect of tachysterol on the metabolism of other nutrients. A study by Holick *et al,* (1981) reports no effect on calcium absorption in rats which were given 0.25µg vitamin D3 and injected with 1µg tachysterol, but a small (insignificant) effect on calcium metabolism when 10µg was injected. The applicant highlighted the author's view that vitamin D binding protein has little affinity for tachysterol. The applicant also noted that tachysterol would be present in the final food at particularly low levels. To produce bread containing

200IU/100g vitamin D2 requires 6.67mg of the vitamin D2 yeast concentrate and at this level of incorporation the resulting intake of tachysterol would be 0.93µg/100g bread. As detailed in para 19 above the applicant proposes a daily intake of 200IU vitamin D2 from the novel ingredient. For high level consumers of bread who may also consume a supplement this would equate to around 15 µg of vitamin D2 and 2.8µg tachysterol.

Discussion. *The Committee reviewed the response from the applicant and was satisfied that it provided sufficient evidence that the vitamin D2 yeast concentrate (containing tachysterol) had a similar bioavailability to existing sources of vitamin D2. The Committee also accepted that the levels of tachysterol in the novel ingredient were very low, equating to a maximum of 2.7µg tachysterol per day and noted that this figure is below EFSA's threshold of toxicological concern*[8].

32. **Allergy.** The applicant advised that no allergens are used in the production of vitamin D2 yeast concentrate. The Food Standards Agency advises that food allergy to *S. cerevisiae* is extremely rare and, although inhalant allergies to fungi and yeast such as *S. cerevisiae* are more common, they are rare when compared to other allergic conditions.

Discussion. *The Committee accepted that the allergenic risk of the vitamin D2 yeast concentrate was no greater than for other foods containing S. cerevisiae and although there is a risk of an individual with an inhalant allergy to S. cerevisiae having a severe systemic reaction after consuming the yeast, this would apply equally to other (non-vitamin D enriched) S. cerevisiae preparations.*

Overall Discussion

The Committee considered that information provided by the applicant in regard to their concern about tachysterol was sufficient to demonstrate that its low level presence was not a cause for concern. With regard to potential intake, the Committee noted the simplistic approach used by the applicant, but accepted the view of Food Standards Agency officials that this approach was a reasonable estimate of the likely consumption of the novel ingredient. The Committee also accepted that, based on these figures, there was an adequate margin of safety for all population groups.

Conclusion

The Advisory Committee on Novel Foods and Processes is satisfied by the evidence

[8] http://www.efsa.europa.eu/en/consultationsclosed/call/110712a.htm

provided by the applicant, Lallemand, that the range of uses for the novel ingredient (vitamin D2 yeast concentrate) is acceptable

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