Position papers

2’-Fucosyllactose (2’-FL), 3’-Sialyllactose Sodium Salt (3’-SL) and 6’-Sialyllactose (6’-SL) – Additional Information Discussion Paper

Committee Paper for Discussion - ACNFP/165/03

Advisory Committee for Novel Foods and Processes

Application for authorisation as a novel food for 2’-Fucosyllactose (2’-FL), 3’-Sialyllactose Sodium Salt (3’-SL) and 6’-Sialyllactose (6’-SL) – Additional Information for review.

Application numbers RP1476, RP1477 and RP1478

Issue

The Committee first reviewed these three applications in September 2023 and requested further information from the applicant. The Committee is invited to consider the response from the applicant and whether this addresses the request for clarification satisfactorily or if further information is required.

In light of the previous discussion on having a Committee Advise Document (CAD) to support review, a CAD for each novel food has been prepared. The Committee is also invited to consider each CAD and provide comments with a view of finalising the assessments for these novel foods.

Background

1. On the 10th March 2022, the FSA received the submissions for 2’-fucosyllactose (2’-FL), 3’-sialyllactose (3’-SL) and 6’-sialyllactose (6’-SL) as novel foods from Kyowa Hakko Bio Company. 2’-FL, 3’-SL and 6’-SL are manufactured by microbial fermentation using a genetically modified strains of *E. coli* W, and then refined to yield the purified novel foods. 2’-FL, 3’-SL and 6’-SL are intended to be used as an ingredient in a number of food products and as food supplements.

2. The Committee first reviewed these dossiers at the 162nd meeting. Following discussion at the last meeting the Secretariat sought further information from the applicant in the following areas:

- Identity of the novel food
- Production Process
- Compositional information
- Specification (2’-FL only)
- Stability
- Nutritional Information (3’-SL and 6’-SL only)
- Toxicological information
- Allergenicity

3. The Committee is asked whether the applicant’s response addresses the outstanding questions from their request for information. To inform the discussion and further development of a CAD, the FSA’s requested further for information and the applicant’s response are provided:

- 2’-FL request for information (Annex A) and applicant’s response (Annex B)
- 3’-SL request for information (Annex D) and applicant’s response (Annex E)
- 6’-SL request for information (Annex G) and applicant’s response (Annex H)

4. The Secretariat has also drafted separate Committee Advice Documents for 3’-SL and 6’-SL as novel foods which can be found in Annexes C, F, and I, respectively. Members are asked to comment on the text and whether this represents an accurate summary of the assessment of each novel food.

Applicant’s response to request for further information on 2’-FL

Identity of the novel food

5. The Committee requested the applicant provide further discussion on the NMR and LC-MS/MS analytical data for 2’-FL. The applicant has provided a detailed explanation using the mass spectra and NMR spectra to support their conclusion that the novel food is 2’-FL (Annex B: p1 – 5 Response to RFI Letter; Annex B: Annex – NMR and MS data [confidential]).

Production Process

6. The Committee requested clarification on the differences in the manufacturing process between the applicant’s product and the current authorised forms of 2’-FL. The applicant has provided a summary comparison based on publicly available information and concluded the processes are similar (Annex B: p6 – 9 Response to RFI Letter).

7. Members sought further information on the food safety management plan and critical control points (CCPs) for the novel production process. The applicant has updated the process flow chart, the HACCP plan and provided further details on CCPs (Annex B: p10 – 12 Response to RFI Letter; Annex B: Annex – HACCP plan [confidential]).

8. The Committee requested additional information concerning different steps of the production process. The applicant has provided further details on the cell banks, the purification steps, and the spray drying of the novel food (Annex B: p12 – 17 Response to RFI Letter).

9. Members sought further information on the genetic modifications of the production organism and clarification on any safety concerns from the potential expression of secondary metabolites. In response, the applicant has provided a more detailed explanation of the genetic modifications in the microorganism (Annex B: p17 – 21 Response to RFI Letter; Annex B: Annex – ORF analysis [confidential]).

Compositional information

10. The Committee requested the applicant provide clarification on the unidentified components and potential presence of contaminants in the novel food. The applicant has proposed the identity of saccharides that may be present as unidentified components. The applicant has also provided a rationale for the inclusion of aflatoxin M1 in the analysis and the choice of method for determining the protein content in the novel food (Annex B: p22 – 23 Response to RFI Letter).

Specification

11. Members noted that the specification parameter for 2’-FL is ≥ 82% w/w, yet the compositional batch analysis of the novel food indicates the mean content of 2’-FL is ≥ 93% w/w. The applicant has agreed to amend the specification limit for 2’-FL to ≥ 90% w/w (Annex B: p24 Response to RFI Letter).

Stability

12. The Committee reviewed the data from the stability study and highlighted that the pH range appeared to be vary and was greater than the specification limit. The applicant states that 2’-FL is a neutral sugar with no buffering capacity. The specification limit has been changed to 4.5 – 8.5 (Annex B: p25 Response to RFI Letter).

Toxicological information

13. Members noted that an *in vivo* micronucleus test (OECD 474) was provided with the application. However, as no information had provided on the target tissue exposure, this test was not considered valid. The Committee requested the applicant conduct an *in vitro* micronucleus test (OECD 487).

14. The applicant has conducted an *in vitro* micronucleus test which confirms results that the novel food is not clastogenic nor aneugenic and did not have micronucleus-inducing potential at concentrations up to 2,000 µg/mL in the absence or presence of metabolic activation (Annex B: p26 – 27 Response to RFI Letter; Annex B: Annex – *in vitro* micronucleus test report [confidential]).

Allergenicity

15. The Committee requested the applicant provide further discussion on the results from the ELISA tests for milk and soy protein. The applicant has confirmed that both proteins were below the limit of quantification of 1.0 µg/g (Annex B: p28 Response to RFI Letter).

16. Members sought clarification on the use of the term “allergen free” in the application dossier. The applicant has removed this term and replaced with statements that milk protein and soy protein were not present above the detection limit (Annex B: p28 Response to RFI Letter).

Applicant’s response to request for further information on 3’-SL

Identity of the novel food

17. The Committee requested the applicant provide further discussion on the NMR and LC-MS/MS analytical data for 3’-SL. The applicant has provided a detailed explanation using the mass spectra and NMR spectra to support their conclusion that the novel food is 3’-SL (Annex D: p1 – 5 Response to RFI Letter; Annex D: Annex – NMR and MS data [confidential]).

Production Process

18. Members sought further information on the food safety management plan and critical control points (CCPs) for the novel production process. The applicant has updated the process flow chart, the HACCP plan and provided further details on CCPs (Annex D: p6 – 8 Response to RFI Letter; Annex D: Annex – HACCP plan [confidential]).

19. The Committee requested additional information concerning different steps of the production process. The applicant has provided further details on the cell banks, the purification steps, and the spray drying of the novel food (Annex D: p8 – 12 Response to RFI Letter).

20. Members noted that the applicant used a gel-based method to confirm the absence of the genetically modified microorganism in the novel food. Confirmation was sought using a quantitative method. The applicant conducted an assessment for the presence of viable cells and residual DNA in three batches of novel food. Based on the results, the applicant concludes no viable cells or DNA from the production microorganism are present (Annex D: p12 – 15 Response to RFI Letter; Annex D: Annex – absence of cells and DNA report [confidential])

21. The Committee sought further information on the genetic modifications of the production organism and clarification on any safety concerns from the potential expression of secondary metabolites. In response, the applicant has provided a more detailed explanation of the genetic modifications in the microorganism (Annex D: p15 – 20 Response to RFI Letter; Annex D: Annex – ORF analysis [confidential]).

Compositional information

22. The Committee requested the applicant provide clarification on the unidentified components in the novel food. The applicant has proposed the identity of saccharides that may be present as unidentified components (Annex D: p21 Response to RFI Letter).

23. Members queried the variation in water content between the first five batches of novel food and subsequent batches. The applicant has stated that the difference in water content reflects changes in the production equipment and scale of production. The applicant further states that these changes still meet the specification limit (Annex D: p22 Response to RFI Letter).

24. The Committee sought clarification on potential presence of contaminants in the novel food. The applicant provided a rationale for the inclusion of aflatoxin M1 in the analysis and the choice of method for determining the protein content in the novel food (Annex D: p21 and 23 Response to RFI Letter).

Nutritional information

25. Members requested that the applicant evaluate the sodium intakes from consumption of novel food in consumers. The applicant has conducted an exposure assessment and concluded that the sodium intake from the novel food is negligible compared to dietary sources (Annex D: p24 Response to RFI Letter).

Toxicological information

26. Members noted that an *in vivo* micronucleus test (OECD 474) was provided with the application. However, as no information had provided on the target tissue exposure, this test was not considered valid. The Committee requested the applicant conduct an *in vitro* micronucleus test (OECD 487).

27. The applicant has conducted *in vitro* micronucleus test which confirms that the novel food is not clastogenic nor aneugenic and did not have micronucleus-inducing potential at concentrations up to 2,000 µg/mL in the absence or presence of metabolic activation (Annex D: p25 – 26 Response to RFI Letter; Annex D: Annex – *in vitro* micronucleus test report [confidential]).

28. The Committee requested the applicant provide an explanation for the changes in the urinary parameters observed during the *in vivo* 90-day feeding study. The applicant states that these alterations are not toxic effects and are fluctuations in the normal homeostasis (Annex D: p26 Response to RFI Letter; Annex D: Annex – historical control data [confidential]).

Allergenicity

29. The Committee requested the applicant provide further discussion on the results from the ELISA tests for milk and soy protein. The applicant has confirmed that both proteins were below the limit of quantification of 1.0 µg/g (Annex D: p27 Response to RFI Letter).

30. Members sought clarification on the use of the term “allergen free” in the application dossier. The applicant has removed this term and replaced with statements that milk protein and soy protein were not present above the detection limit (Annex D: p28 Response to RFI Letter).

Applicant’s response to request for further information on 6’-SL

Identity of the novel food

31. The Committee requested the applicant provide further discussion on the NMR and LC-MS/MS analytical data for 6’-SL. The applicant has provided a detailed explanation using the mass spectra and NMR spectra to support their conclusion that the novel food is 6’-SL (Annex G: p1 – 6 Response to RFI Letter; Annex D: Annex – NMR and MS data [confidential]).

Production Process

32. Members sought further information on the food safety management plan and critical control points (CCPs) for the novel production process. The applicant has updated the process flow chart, the HACCP plan and provided further details on CCPs (Annex G: p7 – 9 Response to RFI Letter; Annex D: Annex – HACCP plan [confidential]).

33. The Committee requested additional information concerning different steps of the production process. The applicant has provided further details on the cell banks, the purification steps, and the spray drying of the novel food (Annex G: p9 – 13 Response to RFI Letter).

34. Members noted that the applicant used a gel-based method to confirm the absence of the genetically modified microorganism in the novel food. Confirmation was sought using a quantitative method. The applicant conducted an assessment for the presence of viable cells and residual DNA in three batches

of novel food. Based on the results, the applicant concludes no viable cells or DNA from the production microorganism are present (Annex G: p13 – 16 Response to RFI Letter; Annex D: Annex – absence of cells and DNA report [confidential])

35. The Committee sought further information on the genetic modifications of the production organism and clarification on any safety concerns from the potential expression of secondary metabolites. In response, the applicant has provided a more detailed explanation of the genetic modifications in the microorganism (Annex G: p16 – 20 Response to RFI Letter; Annex D: Annex – ORF analysis [confidential]).

Compositional information

36. The Committee requested the applicant provide clarification on the unidentified components in the novel food. The applicant has proposed the identity of saccharides that may be present as unidentified components (Annex G: p21 Response to RFI Letter).

37. Members queried the variation in water content between the first five batches of novel food and subsequent batches. The applicant has stated that the difference in water content reflects changes in the production equipment and scale of production. The applicant further states that these changes still meet the specification limit (Annex G: p22 Response to RFI Letter).

38. The Committee sought clarification on potential presence of contaminants in the novel food. The applicant provided a rationale for the inclusion of aflatoxin M1 in the analysis and the choice of method for determining the protein content in the novel food (Annex G: p21 – 23 Response to RFI Letter).

Nutritional information

39. Members requested that the applicant evaluate the sodium intakes from consumption of novel food in consumers. The applicant has conducted an exposure assessment and concluded that the sodium intake from the novel food is negligible compared to dietary sources (Annex G: p24 Response to RFI Letter).

Toxicological information

40. Members noted that an *in vivo* micronucleus test (OECD 474) was provided with the application. However, as no information had provided on the target tissue exposure, this test was not considered valid. The Committee requested the

applicant conduct an *in vitro* micronucleus test (OECD 487).

41. The applicant has conducted *in vitro* micronucleus test which confirms that the novel food is not clastogenic nor aneugenic and did not have micronucleus-inducing potential at concentrations up to 2,000 µg/mL in the absence or presence of metabolic activation (Annex G: p25 – 26 Response to RFI Letter; Annex D: Annex – *in vitro* micronucleus test report [confidential]).

Allergenicity

42. The Committee requested the applicant provide further discussion on the results from the ELISA tests for milk and soy protein. The applicant has confirmed that both proteins were below the limit of quantification of 1.0 µg/g (Annex G: p27 Response to RFI Letter).

43. Members sought clarification on the use of the term “allergen free” in the application dossier. The applicant has removed this term and replaced with statements that milk protein and soy protein were not present above the detection limit (Annex G: p28 Response to RFI Letter).

Committee Action Required

- The Committee is asked whether the response from the applicant for each novel food is sufficient to address the data gaps identified at the last meeting.
- If not, the Committee is asked to indicate what further data is required and the feedback that should be given to the applicant.
- The Committee is asked to review the Committee Advice Document for these novel foods and comment on whether this accurately reflects the conclusions of the assessments.

ACNFP Secretariat

April 2024

Annexes

Annex A – Request for Information for 2’-FL

Annex B – Applicant’s Response to RFI letter and Annex for 2’-FL

Annex C – Draft Committee Advice Document for 2’-FL

Annex D – Request for Information for 3’-SL

Annex E – Applicant’s Response to RFI letter and Annex for 3’-SL

Annex F – Draft Committee Advice Document for 3’-SL

Annex G – Request for Information for 6’-SL

Annex H – Applicant’s Response to RFI letter and Annex for 6’-SL

Annex I – Draft Committee Advice Document for 6’-SL