Meeting

# 3’-Sialyllactose Sodium Salt Discussion Paper

**Committee Paper for Discussion - ACNFP/162/10**

**Advisory Committee For Novel Foods and Processes.**

**Application for Authorisation as a Novel Food for 3’-Sialyllactose Sodium Salt, from Kyowa Hakko Bio Co. Ltd**

**Application number RP1477.**

## Issue

1. An application has been received under the novel food authorisation process (regulation 2015/2283) for 3’-Sialyllactose sodium salt (3’-SL), a Human Milk Oligosaccharide (HMO).

2. The Committee is asked to advise whether the available data provides an adequate basis for a risk assessment, and whether the novel food is safe and not nutritionally disadvantageous under the proposed use and use levels.

## Background

3. On the 10$^{th}$ March 2022 the FSA and FSS received the submission for 3’-SL from Kyowa Hakko Bio Co., Ltd.

4. The novel food ingredient is produced via fermentation using a genetically modified strain of E. coli W followed by a series of purification steps involving isolation, concentration, and spray-drying. The applicant proposes to use the novel food in conventional foods (dairy products and analogues and cereal bars), beverages, foods for specific groups (including Foods for Special Medical Purposes, total diet replacement for weight control, infant formulas, follow-on formula and baby foods for infants and young children) and in food supplements

as an alternative source of 3’-SL in the absence of other exposure sources.

5. Since the submission of the present application, an authorisation for 3’-SL as a novel food on the GB market was granted in June 2022 for another applicant who sought and received data protection. In accordance with Article 26 of Regulation 2015/2283 as retained in UK law, a subsequent applicant can obtain authorisation for 3’-SL as a novel food on the condition that no reference is made to the protected proprietary scientific evidence or data.

6. This application is seeking an authorisation for the same uses as the previous application for 3’SL authorised in 2022. A higher dose has been sought for food supplements in those above 3 years of age and this is presented in the relevant section. As this is a separate applicant there are differences in the strain of microbe used, their internal production, systems and the testing used as this was a proprietary method.  The present application is submitted as a full application with newly provided scientific data and evidence to support an authorisation.

7. The application dossier is attached as Annex A. The request for further information letters (with responses) are attached as Annex B. All supporting documents for all sections of the application, which have been updated following the request for further information, can be found in Annex C. All annexes contain confidential information.

8. As part of a trial for a new way of working for some applications, a draft Committee Advice Document for this application has been prepared for members consideration, alongside this introductory paper. This reflects that there have been several reviews for breast milk sugars and as such the key considerations have been discussed by the Committee in the past. Members are invited to review and comment on the draft found in Annex D.

# This application

# Identification

9. The novel food ingredient, 3’-SL, is a sialylated oligosaccharide composed of three molecules N acetylneuraminic acid (NeuAc), D galactose and D glucose and is identical in structure to 3’-SL found in human milk (Annex A; p11 – 16 of dossier).  Purity by specification is ≥82% (dry weight basis (dwb)) and the final novel food product includes low levels of related saccharide molecules N acetylneuraminic acid (≤9% w/w), 3'-Sialyllactulose (≤5% w/w), D-glucose (≤3%

w/w), D-lactose (≤3% w/w) and 6'-Sialyllactose sodium salt (≤1% w/w). Identity confirmed by LC-MS spectrometry and 1H NMR and 13C NMR spectroscopy (Annex C [Annex A]). The final composition is verified by HPLC-CAD (Annex C [Annex G]).

10. The novel food final product is expected to contain 6'-Sialyllactose sodium salt (6’-SL) in low levels. An authorisation for 6’-SL as a novel food on the GB market exists but the application is held by another applicant who has received data protection. A separate application for authorisation for 6’-SL as a novel food was submitted by Kyowa Hakko bio co., ltd on March 10th 2022.

11. The applicant has provided a range of data that characterises the identity of the genetically modified fermentation microorganism E. coli W, including taxonomic classification (Annex A; p 14 , Table 2.a.2-1 of dossier) and information on the modification and donor organisms (Annex C [Annex B]). Identity verified by Whole Genome Sequence (WGS) and highly homologous to the E. coli W strain per the American Type Culture Collection (ATCC) ATCC 9637 (Annex C [Annex C]).

# Production Process

12. The production method is described in detail (Annex A; p19 of dossier) and a schematic overview is provided (Annex A; p 20 of dossier and Annex C [Annex E]).

13. The production process involves a 2-step fermentation and purification process. The initial fermentation step involves culture of the production microorganism in a defined nutrient media under controlled conditions.  The fermentation involves inoculation of the cultured E. coli W into a nutrient media to synthesise 3'-SL in the presence of glucose and lactose.

14. The second processing step involves isolation and purification of 3’-SL using filtration and cationic and anionic exchange followed by concentration and spray-drying. Additional details on pH and controls were provided by the applicant (Annex B; p 3 of response letter).

15. The acceptance criteria and specifications for the raw materials and processing aids have been provided (Annex A; p 15 – 18 and Table 2.b.1.2-1 of dossier) (Annex C [Annex D]).

16. Initially the applicant used a media containing soy peptone for the fermentation step. This element of the production process has been updated to remove the presence of soy to minimise the risk for those allergic to this food.

The applicant has provided details to show the whether the novel food is altered by use of the alternative media and the potential impact on the risks for the novel food. The applicant states that the additional raw materials do not introduce new hazards or changes to parameters in the production process (Annex B; p 3 of response letter).

17. The applicant has provided a HACCP Plan (Annex C [Annex E]) to include critical control measures alongside justifications. Quality control is described (Annex A; p 20 of dossier).

# Composition

18. The applicant has reported analytical data for five independent batches of 3'-SL (Annex A; p 22 - 28 of dossier) (Annex C [Annex F]). Additional analyses for 3 batches were requested to provide comparable data for all parameters on batches representative of the final product and as intended to be manufactured following the change in culture media (Annex B; p 10 - 14 and Table 3.a-2 of response letter). The data indicates that the manufacturing process results in a consistent final ingredient that meets the proposed specifications for the novel ingredient– see Tables 1-6 below. The CoA's are provided in Annex C [Annex F]. The new media has little impact on the composition of the novel ingredient and as such this was accepted as justification for providing analysis for 3 further batches with the new media.

**Table 1. Compositional batch analysis of 3'-SL manufactured with a fermentation media containing soy peptone: physicochemical properties, carbohydrate content and purity.**

| | | Batch A | Batch B | Batch C | Batch D | Batch E |
|---|---|---|---|---|---|---|
| Parameter | Specification | | | | | |
| Physicochemical | | | | | | |
| pH | 4.0 – 9.0 | 6.5 | 6.5 | 6.4 | 6.3 | 6.4 |

| Composition | | | | | | |
|---|---|---|---|---|---|---|
| Purity (3’-SL) | ≥82 (% dwb) | 89 | 94 | 93 | 95 | 94 |
| NeuAc | ≤9 w/w % | 5.0 | 2.0 | 2.8 | 2.8 | 3.9 |
| D-glucose | ≤3 w/w % | ND | ND | ND | ND | ND |
| D-lactose | ≤3 w/w % | 0.1 | ≤0.05 | 0.1 | ≤0.05 | 0.1 |
| 3’-sialyllactulose | ≤5 w/w % | 0.5 | 0.4 | 0.4 | 0.4 | 0.5 |
| 6’-sialyllactose sodium salt | ≤1 w/w % | ≤0.2 | ND | ND | ND | ND |
| Sum of other carbohydrates (2) | Not established | 4.2 | 2.4 | 2.9 | 1.0 | 0.8 |
| Water | ≤9.0 w/w % | 5.4 | 5.2 | 5.0 | 5.5 | 5.7 |
| Residual protein (internal method) | ≤10 mg/kg (0.001%) | ≤1 (LOQ) | ≤1 (LOQ) | ≤1 (LOQ) | ≤1 (LOQ) | ≤1 (LOQ) |
| Residual protein (Bradford assay) | ≤100 mg/kg (1) | NT | ≤100 | ≤100 | NT | NT |

3’-SL: 3’-sialyllactose sodium salt; NeuAc: N acetylneuraminic acid; dwb: dry weight basis; NT: not tested; ND: not detected; LOQ: limit of quantification. (1) Evaluated using a test limit at 100 ppm; (2) Sum of carbohydrates such as NeuAc, D-glucose, D-lactose, 3’-sialyllactulose and 6’-sialyllactose sodium salt

**Table 2. Compositional batch analysis of 3’-SL manufactured with an updated fermentation media without soy peptone: physicochemical properties, carbohydrate content and purity.**

| | | Batch I | Batch J | Batch K |
|---|---|---|---|---|
| Parameter | Specification | | | |
| Physicochemical | | | | |
| pH | 4.0 – 9.0 | 5.9 | 5.7 | 5.8 |
| Composition | | | | |
| Purity (3’-SL) | ≥82 (% dwb) | 82 | 91 | 91 |
| NeuAc | ≤9 w/w % | 8.4 | 0.5 | 0.4 |
| D-glucose | ≤3 w/w % | 0.05 | 0.05 | 0.05 |
| D-lactose | ≤3 w/w % | 0.05 | 0.05 | 0.05 |
| 3’-sialyllactulose | ≤5 w/w % | NT | NT | NT |
| 6’-sialyllactose sodium salt | ≤1 w/w % | NT | NT | NT |
| Sum of other carbohydrates (2) | Not established | 6.9 | 7.5 | 7.2 |
| Water | ≤9.0 w/w % | 7.2 | 8.2 | 8.2 |

| | | | | |
|---|---|---|---|---|
| Residual protein (internal method) | ≤10 mg/kg (0.001%) | NT | NT | NT |
| Residual protein (Bradford assay) | ≤100 mg/kg (1) | ≤100 | ≤100 | ≤100 |

3’-SL: 3’-sialyllactose sodium salt; NeuAc: N acetylneuraminic acid; dwb: dry weight basis; NT: not tested; ND: not detected; LOQ: limit of quantification. (1) Evaluated using a test limit at 100 ppm; (2) Sum of carbohydrates such as NeuAc, D-glucose, D-lactose, 3’-sialyllactulose and 6’-sialyllactose sodium salt

**Table 3. Compositional batch analysis of 3’-SL manufactured with a fermentation media containing soy peptone: heavy metal contaminants.**

| | | Batch A | Batch B | Batch C | Batch D | Batch E |
|---|---|---|---|---|---|---|
| Parameter | Specification | | | | | |
| Heavy metals: | | | | | | |
| Arsenic | ≤0.2 mg/kg | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) |
| Cadmium | ≤0.2 mg/kg | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) |
| Lead | ≤0.2 mg/kg | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) |
| Mercury | ≤0.2 mg/kg | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) | ≤0.05 (LOQ) |
| Iron | ≤10 mg/kg | 0.2 | 0.2 | 0.3 | 1.1 | 0.4 |

LOQ: limit of quantification.

**Table 4. Compositional batch analysis of 3’-SL manufactured with an updated fermentation media without soy peptone: heavy metal contaminants.**

| | | Batch I | Batch J | Batch K |
|---|---|---|---|---|
| Parameter | Specification | | | |
| Heavy metals: | | | | |
| Arsenic | ≤0.2 mg/kg | 0.01 | 0.01 | 0.03 |
| Cadmium | ≤0.2 mg/kg | 0.01 | 0.01 | 0.01 |
| Lead | ≤0.2 mg/kg | 0.02 | 0.03 | 0.02 |
| Mercury | ≤0.2 mg/kg | 0.004 | 0.004 | 0.004 |
| Iron | ≤10 mg/kg | NT | NT | NT |

NT: not tested.

**Table 5. Compositional batch analysis of 3’-SL manufactured with a fermentation media containing soy peptone: microbial contaminants.**

| | | Batch A | Batch B | Batch C | Batch D | Batch E |
|---|---|---|---|---|---|---|
| Parameter | Specification | | | | | |
| Microbial parameters: | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Aerobic plate count | ≤1,000 CFU/g | 40 | 10 | 10 | 10 | 10 |
| Moulds and Yeasts | ≤100 CFU/g | 100 | 100 | 100 | 100 | 100 |
| *Salmonella* | Absent in 100 g | Negative | Negative | Negative | Negative | Negative |
| *Enterobacteriaceae* | Absent in 10 g | Negative | Negative | Negative | Negative | Negative |
| *Cronobacter spp*. (*Enterobacter sakazakii*) | Absent in 100 g | Negative | Negative | Negative | Negative | Negative |
| *Listeria monocytogenes* | Absent in 25 g | Negative | Negative | Negative | Negative | Negative |
| *Bacillus cereus* | ≤50 CFU/g | 10 | 10 | 10 | 10 | 10 |
| Residual endotoxins | ≤10 EU/mg | 0.044 | 0.006 | 0.036 | 0.011 | 0.019 |
| Aflatoxin M1 | ≤0.2 µg/kg | NT | NT | 0.02 (LOQ) | NT | 0.02 (LOQ) |

CFU: colony forming units; EU: endotoxin units; NT: not tested; LOQ: limit of quantification.

**Table 6. Compositional batch analysis of 2'-FL manufactured with an updated fermentation media without soy peptone: microbial contaminants.**

| | Batch I | Batch J | Batch K |
|---|---|---|---|

| Parameter | Specification | | | |
|---|---|---|---|---|
| Microbial parameters: | | | | |
| Aerobic plate count | ≤1,000 CFU/g | 10 | 270 | 10 |
| Moulds and Yeasts | ≤100 CFU/g | 10 (LOD) | 10 (LOD) | 10 (LOD) |
| *Salmonella* | Absent in 100 g | Negative | Negative | Negative |
| *Enterobacteriaceae* | Absent in 10 g | Negative | Negative | Negative |
| *Cronobacter spp*. (*Enterobacter sakazakii*) | Absent in 100 g | Negative | Negative | Negative |
| *Listeria monocytogenes* | Absent in 25 g | Negative | Negative | Negative |
| *Bacillus cereus* | ≤50 CFU/g | 10 | 10 | 10 |
| Residual endotoxins | ≤10 EU/mg | 0.0001563 (LOQ) | 0.0001563 (LOQ) | 0.0001563 (LOQ) |
| Aflatoxin M1 | ≤0.2 µg/kg | 0.02 (LOQ) | 0.02 (LOQ) | 0.02 (LOQ) |

CFU: colony forming units; EU: endotoxin units; NT: not tested; LOQ: limit of quantification; LOD limit of detection.

19. The analysis parameters included purity, other carbohydrates, residual proteins, microbial parameters, heavy metals, sodium, and physicochemical properties (Annex B; p 11 – 14, Table 3.a-2 of response letter). A purity of 82% to

95% (dry weight basis (dwb)) was demonstrated by the analysis. The applicant reports a lack of heavy metals and residual proteins (≤100 mg/kg) and microbial parameters were within limits. The applicant reports that the variation in aerobic plate count demonstrated in reference to lot J (270 CFU/g) remains under the 1,000 CFU/g (ISO 4833) limit and does not pose a safety concern with no evidence of bacterial contamination (Annex B; p 1 – 2 of response letter dated 02 February 2023).

20. Other saccharide molecules N acetylneuraminic acid (≤8.4% w/w), D lactose (≤0.1% w/w), D glucose (0.05% w/w), 3'-Sialyllactulose (≤0.5% w/w) and 6'-Sialyllactose sodium salt (≤ 0.2 %w/w) were detected in low levels. The applicant states that these molecules are natural components or break down products of human breast milk (Annex A; p 36 of dossier).

21. Absence of the genetically modified production organism and no detectable residual DNA in 3 batches of the final product is verified by PCR analysis (Annex A; p 29 – 30 of dossier). Results can be found in Annex C [Annex F].

22. All methods and validation are found in (Annex C [Annex G]). The applicant changed their analytical method for residual proteins from an internal method of the previous applicant to the Bradford assay (Annex B; p 10 of response letter). This has been used to characterise the novel ingredient for this application with the associated change in units in the specification.

# Stability

23. The applicant has provided a range of analytical data which characterises and describes the stability of 3’-SL. All 3’-SL batches analysed for stability were produced using a soy peptone media and due to little change in the composition of the novel food with soy peptone excluded from the fermentation media, the results from these stability studies were considered relevant.  All study reports are found in (Annex C [Annex J]).

24. The applicant reports the results from a 6-month accelerated storage conditions test (40 ± 2°C; 75 ± 5% Relative Humidity) on 5 batches (Annex A; p 30 – 33 and Table 2.c.3.1-1 of dossier). The parameters chosen for measurement and monitoring of stability includes physicochemical and biochemical (purity, carbohydrates and water content). The results show that parameters remained stable within specifications and the increase in the isomerisation product 3'-sialyllactulose remains at low levels within limits (Annex B; p 24 - 25 of response letter and Annex A; p 30 of dossier).

25. The applicant further demonstrates the stability of parameters of the novel food product under a 12-month normal conditions (25 ± 2°C; 60 ± 5% Relative Humidity) storage test on 1 batch. Microbiological stability is reported with regards to water activity values which remained stable under both accelerated and normal conditions (Annex A; p 33 – 34 of dossier). The applicant states that microbial growth and toxin formation is unlikely due to the low water content and low water activity of the final product. (Annex A; p 34 of dossier, and Annex B; p 27 of response letter).

26. Following request for further information on the data that supports the proposed 3-year shelf life, the applicant provided details of an Arrhenius equation used and further data (Annex B; p 24 - 27 of response letter). The applicant further reports results from a 24-month stability test on 1 batch under normal storage conditions (Annex B; p 24 - 26 and Table 3.c-4 of response letter) and results from a microbiological stability test at 24 months on 1 batch under normal storage conditions (Annex B; p 27 and Table 3.c-5 of response letter). Parameters remained stable and indicate that no microbial growth or toxin formation was detected.

# Specifications

27. The specification parameters for the novel food ingredient were assessed using internationally recognised methods or are otherwise determined using internally developed and validated methods – see Table 7 below for the specifications (Annex A; p 36 – 37 , Table 2.d-1  of dossier and Annex C [Annexes G and H]).

**Table 7. Specifications for 3’-SL.**

| | |
|---|---|
| Description: 3’-SL is a white to off-white powder that is produced by a microbial process. 3’-SL is isolated by spray-drying. | |
| Source: | Genetically modified strain of *Escherichia coli* W |
| Parameter | Specification |

| Physicochemical | |
|---|---|
| Appearance | Powder |
| Colour | White to off-white |
| pH | 4.0 – 9.0 |

| Composition | |
|---|---|
| Purity (3’-SL) | ≥82 (% dwb) |
| NeuAc | ≤9 w/w % |
| D-glucose | ≤3 w/w % |
| D-lactose | ≤3 w/w % |
| 3’-sialyllactulose | ≤5 w/w % |
| 6’-sialyllactose sodium salt | ≤1 w/w % |
| Water | ≤10.5 w/w % |
| Residual protein (internal method) | ≤10 mg/kg (0.001%) |
| Residual protein (Bradford assay) | ≤100 mg/kg (1) |

Heavy metals:

| | |
|---|---|
| Arsenic | ≤0.2 mg/kg |
| Cadmium | ≤0.2 mg/kg |
| Lead | ≤0.2 mg/kg |
| Mercury | ≤0.2 mg/kg |
| Iron | ≤10 mg/kg |

Microbial parameters:

| | |
|---|---|
| Aerobic plate count | ≤1,000 CFU/g |
| Moulds | ≤100 CFU/g |
| Yeasts | ≤100 CFU/g |
| *Salmonella* | Absent in 100 g |
| *Enterobacteriaceae* | Absent in 10 g |
| *Cronobacter spp.* *(Enterobacter sakazakii)* | Absent in 100 g |
| *Listeria monocytogenes* | Absent in 25 g |

| | |
|---|---|
| *Bacillus cereus* | ≤50 CFU/g |
| Residual endotoxins | ≤10 EU/mg |
| Aflatoxin M1 | ≤0.2 µg/kg |

3’-SL: 3’-sialyllactose sodium salt; NeuAc: N acetylneuraminic acid; CFU: colony forming units; dwb: dry weight basis; EU: endotoxin units. (1) Evaluated using a test limit at 100 ppm.

# History Of Use

28. The applicant conducted a literature search and review on the human biology of background exposure of 3’-SL from human breast milk and discusses likely intake levels based on the history for of consumption of human breast milk in infants (Annex A; p 38 – 40 dossier). The applicant reports three GRAS notifications in the USA for 3’-SL and one EU authorisation since 2021 (Annex A; p 41 - 42 of dossier).

29. The applicant also conducted a review on the human biology of background exposure of 6’-SL and N-acetylneuraminic acid (NeuAc) from human breast milk due to the presence of these at low levels in the novel food. They discussed intake levels based on the consumption of human breast milk (Annex A; p 42 of dossier). The applicant reports a GRAS notification in the USA for 6’-SL and one EU authorisation since 2021 (Annex A; p 42 of dossier). The applicant also reports that NeuAc is an EU authorised novel food on the Union List and reports a GRAS notification in the USA (Annex A; p 43 of dossier).

30. The applicant summarises the history regarding safety of the production microorganism (Annex A; p 38 of dossier). Details on the microorganism, applicable to a Category 1 food product within the GMO regulations are provided. As there is no presence of the microorganism in the final food it is not subject to review under the GMO regulations for food and feed Annex C [Annex B].

# Proposed Use and Intake

31. The applicant states that the novel food ingredient is intended to be used by the general population including infants, children and individuals on energy

restricted diets for weight reduction.

32. Proposed food uses and maximum use levels are presented in Table 8 below (Annex A; p 44 - 45 , Table 2.f.2-1 of dossier). The applicant states that the proposed food uses and maximum use levels are the same as an existing 2021 EU novel food authorisation (Annex C – References – EFSA NDA Panel 2020a), with the exception of food supplements for individuals >3 years of age where, compared to the 0.5 g/day EU authorisation, 1.0 g/day is proposed.

**Table 8. Proposed uses and maximum use levels for 3'-SL.**

| EU Category Number | Category Name | Proposed Maximum use Level |
|---|---|---|
| 1 | **Dairy products and analogues** | |
| 1.1 | Unflavoured pasteurised and unflavoured sterilised (including UHT) milk | 0.25 g/L |
| 1.2/1.3 | Unflavoured fermented milk-based products | 0.25 g/L beverages<br>0.5 g/kg for products other than beverages |
| 1.4 | Flavoured fermented milk-based products including heat treated products | 0.25 g/L beverages<br>2.5 g/kg for products other than beverages |
| 7 | **Bakery wares** | |
| 7.2 | Fine bakery wares (cereal bars only) | 2.5 g/kg |

| | | |
|---|---|---|
| 13 | **Foods for Special Groups (FSG)** | |
| 13.1 | **Foods for infants and young children** | |
| 13.1.1 | Infant formula as defined in Regulation (EU) No 609/2013 | 0.2 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer |
| 13.1.2 | Follow-on formula as defined in Regulation (EU) No 609/2013 | 0.15 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer |
| 13.1.3 | Processed cereal-based food and baby food for infants and young children as defined in Regulation (EU) No 609/2013 | 0.15 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer<br><br>1.25 g/kg for products other than beverages |
| 13.1.4 | Milk-based drinks and similar products intended for young children | 0.15 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer |
| 13.2 | **Foods for special medical purposes as defined in Regulation (EU) No 609/2013** | |

| | | |
|---|---|---|
| 13.2 | Foods for special medical purposes as defined in Regulation (EU) No 609/2013 | On a case-by-case basis |
| 13.3 | **Total diet replacement for weight control as defined in Regulation (EU) No 609/2013** | |
| 13.3 | Total diet replacement for weight control as defined in Regulation (EU) No 609/2013 | 0.5 g/L beverages (equivalent to 0.125 g/meal based on a standard 250 g meal replacement beverage)<br>5 g/kg for products other than beverages (equivalent to 0.15 g/meal based on a standard 30 g meal replacement bar) |
| 14 | **Beverages** | |
| 14.1.4 | Flavoured drinks (excluding cola-type drinks) | 0.25 g/L |
| 17 | **Food supplements as defined in Directive 2002/46/EC** | |
| 17 | Food supplements as defined in Directive 2002/46/EC | 1.0 g/day for individuals above 3 years of age<br>0.15 g/day for young children (12 to 35 months)<br>0.2 g/day for infants (≤11 months) |

3’-SL: 3’-sialyllactose sodium salt; UHT: ultra-high temperature.

33. The applicant refers to the estimations of mean and high anticipated intakes previously evaluated by EFSA's NDA Panel in 2020 (Annex A; p 45 - 46 of dossier). A summary is provided in Table 9 below (Annex A; p 46 , Table 2.f.3.1-1 of dossier).

**Table 9. Mean and High Anticipated Intakes of 3'-SL from Proposed Food Uses Based on Summary Statistics from the Individual Food Intake Data from the EFSA Comprehensive Food Consumption Database (EFSA NDA Panel, 2020a).**

| Age groups | Number of EU Dietary Surveys | Range of Means (lowest and highest) among EU Dietary Surveys (mg/kg bw) | Range of 95th Percentile (lowest and highest) among EU Dietary surveys (mg/kg bw) |
|---|---|---|---|
| Infants (up to 11 months) | 11 | 8 to 36 | 19 to 71 |
| Young children or toddlers (12 to 35 months) | 14 | 5 to 20 | 13 to 70 |
| Other children (3 to 9 years) | 19 | 2 to 8 | 5 to 14 |
| Adolescents (10 to 17 years) | 18 | 0 to 3 | 2 to 7 |
| Adults (18 to 64 years) | 19 | 0 to 2 | 1 to 4 |
| Elderly (≥65 years | 18 | 0 to 1 | 1 to 3 |
| Pregnant women | 2 | 1 to 2 | 2 to 4 |

| | | | |
|---|---|---|---|
| Lactating women | 2 | 1 to 2 | 3 to 4 |

3'-SL: 3'-sialyllactose; bw: body weight; EFSA: European Food Safety Authority; EU: European Union.

34. The applicant reports the highest estimated daily intake of 3’-SL amongst all population groups and all proposed uses, is in infants at a maximum intake level 0.2 g/L is 52 mg/kg body weight/day, with the assumption that only infant formula would be consumed (Annex A; p 45 of dossier). The applicant reports the highest estimated daily intake, at the 95th percentile, based on proposed uses and maximum use levels in foods combined and taken from Food Intake Data from the EFSA Comprehensive Food Consumption Database, is 70 and 71 mg/kg body weight/day in young children and infants, respectively (Annex C – References – EFSA NDA Panel 2020a).

35. The applicant reports the estimated highest daily intake of 3'-SL from food intake alone is in breastfed infants at 64 mg/kg body weight/day. The applicant refers to the 2020 EFSA NDA Panel evaluation and in consideration for conservative assumptions of the exposure assessment, that the highest estimated intakes in young children and infants are not likely to exceed 64 mg/kg body weight/day.

36. The applicant states that proposed intakes are substitutional alternatives to other sources (breast milk or authorised products containing 3’-SL) rather than combined.  Food supplements are not intended to be consumed with other sources on the same day. The applicant concludes from the assessment that the proposed intakes of 3’-SL are unlikely to exceed the estimated highest daily intake of 3'-SL in breastfed infants of 64 mg/kg body weight/day (Annex A; p 46 – 47 of dossier).

# Absorption, Distribution, Metabolism, Excretion (ADME)

37. The applicant reports that the novel food ingredient 3'-SL is structurally and chemically identical to the naturally occurring counterpart in human milk and is a non-digestible oligosaccharide. The applicant refers to the EFSA NDA Panel 2020 evaluation and reports limited digestion in the upper gastrointestinal tract, limited absorption, fermentation by intestinal microbiota in the colon and 40 – 97% is excreted unchanged in faeces and 2% in urine. The ADME of the novel food

ingredient is not expected to be different from the ADME of 3’SL from breast milk or the EU authorised novel food. (Annex A; p 48 - 49 of dossier).

38. The applicant states that the low levels of additional carbohydrate molecules in the final product consist of, those naturally present in breast milk, breakdown products of lactose and the isomerisation product 6’-SL whereby the overall exposure to these is expected to be insignificant. The applicant reports there are no laxative concerns regarding the low levels of 3'-sialyllactulose.

# Nutritional Information

39. 3'-SL is a dietary fibre and non-digestible carbohydrate (Annex A; p 39 of dossier). The applicant refers to nutrient composition with regards to the low levels of additional carbohydrates in the final product composition and that overall exposure to these is expected to be insignificant (Annex A; p 50 of dossier). The applicant reports that the low levels of exposure to 3'-sialyllactulose in the final product (0.5% w/w) is not expected to cause a laxative effect (Annex A; p 50 of dossier).

40. The novel food is expected to contribute to a low-level intake of sodium, with the final novel food product containing ≤5% sodium. The applicant reports on the highest estimated sodium intakes under proposed conditions of use for all population subgroups where the highest contribution was up to 8.88% of the considered safe daily intake in infants. The highest estimated intakes of sodium across all age groups from the proposed uses are presented in Table 2.h.2-1 (Annex A; p 51 – 52 , Table 2.h.2-1 of dossier).

41. Lactose is added into the production process as a fermentation aid and is also a resulting minor component of the novel ingredient produced by fermentation itself. The compositional batch analyses reported by the applicant detected D-lactose in low levels at ≤0.1% (dry weight basis (dwb)) and a specification limit of ≤3% is set by the applicant (Annex B; p 12, Table 3.a-2 of response letter). The potential for this to be a concern for those with Lactose intolerance was considered.

# Toxicological Information

42. The applicant conducted a bacterial reverse mutation assay (OECD 471) in accordance with GLP principles and reports that 3’-SL is non-mutagenic at concentrations of up to 5,000 µg/plate, in the absence or presence of metabolic

activation (Annex A; p 55 of dossier). The full study report is available in Annex C [Annex L].

43. The applicant conducted an in vivo micronucleus test in mice (OECD 474) in accordance with GLP principles and reports that 3'-SL is non-genotoxic and does not induce chromosomal aberrations in oral doses of up to 2,000 mg 3'-SL/kg body weight (Annex A; p 55 - 56 of dossier). The full study report is available in Annex C [Annex L].

44. The applicant conducted a 90-day oral toxicity study in rats (OECD 408) in accordance with GLP principles and reports a NOEL of 2,007 mg/kg body weight/day for 3’-SL (Annex A; p 57 - 59 of dossier). The full study report is available in Annex C [Annex L] and a summary of statistically significant observations can be found in Annex C [Appendix B.3].

45. The applicant refers to the results of toxicological studies identified in the literature on comparable 3’SL preparations (Annex A; p 64, Table 2.i.3.1-1 of dossier) reporting comparable genotoxicity (Annex A; p 66 of dossier, Table 2.i.3.2-1) and subchronic toxicity findings (Annex A; p 69 - 70 of dossier, Table 2.i.3.3-1). The applicant reports the results of two 21-day gastrointestinal developmental toxicity studies from literature of 3'-SL in piglets that report no compound-related adverse effects in oral doses of 3’-SL of up to 1,200 mg 3'-SL/kg body weight/day (Annex A; p 73 of dossier, Table 2.i.3.5-1).

46. The applicant highlights toxicity testing conducted by the applicant on 6’-SL for a separate application (Annex A; p 76- 92 of dossier). They suggest this indicates that there is not a concern from low levels of 6’-SL in the novel ingredient.

47. The applicant reports no safety concerns regarding any antimicrobial susceptibility or antimicrobial production with the proposed genetically modified culture microorganism (Annex A; p 103-105 of dossier). Results are reported from a phenotypic analysis (Annex C [Annex M]), genotypic analysis and genome search (Annex C [Annex C and Annex F]) and the applicant concludes absence of acquired antimicrobial resistance or potential for production of antimicrobial compounds.

# Allergenicity

48. The applicant states that the high purification steps (microfiltration and ultra-filtration) in the production process demonstrate the lack of allergenic potential

from any residual proteins ≤1 mg/kg (internal method) and ≤100 mg/kg (Bradford assay using a limit test at 100 ppm, Annex B; p 11 – 14 , Table 3.a.2 of response letter) and absence of the production strain or residual DNA in the final product (Annex A;  p 106 , p 29 - 30 of dossier).

49. Lactose is added into the production process as a fermentation aid and is also a resulting minor component of the novel ingredient produced by fermentation itself. To exclude the potential for contamination of milk protein allergens, the applicant reports the results of two ELISA tests that concluded milk proteins were not detected in the final 3'-SL product (Annex A; p 106 of dossier and Annex C [Annex F]). The applicant states that the altered nutrient media following the removal of soy peptone, removes the potential for the presence of soy allergens (Annex A; p 15 – 18 and Table 2.b.1.2-1 of dossier) (Annex C [Annex D]) (Annex B; p 3 of response letter).

50. The applicant conducted a literature search and found no reports of sensitisation, case reports of allergic reactions, or allergenicity studies for 3'-SL (Annex A; p 106 of dossier and Annex C [ Annex K])

51. The applicant reports that the allergenic potential of introduced proteins as a result of the genetic modification to the culture microorganism were assessed using the National Institute of Health Sciences (Japan) Allergen Database for Food Safety (ADFS) (Annex A; p 111 of dossier). The applicant concludes that no sequence alerts for potential allergenicity were identified - Annex C [Annex N]. The culture microorganism is not present in the final ingredient including absence of any residual DNA.

**Committee Action Required**

- The Committee is asked whether the available data provide a satisfactory basis for evaluating the safety of this novel food ingredient.
- If so, the Committee is asked whether it is content to recommend approval for 3'-SL as a novel food ingredient to be added to the range of foods specified. A draft Committee Advice Document has been prepared and members comments are sought on the draft.
- If not, the Committee is asked to indicate what additional data would be required.

ACNFP Secretariat

September 2023

# Annexes

Annex A – Dossier [Confidential]

Annex B – Request for information with applicant’s responses [Confidential]

Annex C – Supporting documents with annexes, references and appendices [Confidential]

Annex D – Draft Committee Advice Document