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Response to the 1st Call for submissions – Application A1155:

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Following the BASF comments to FSANZ on the findings of its assessment regarding Glycom's application for amending the Australia New Zealand Food Standards Code (the Code) to permit the voluntary addition of 2′-*O*-Fucosyllactose (2′-FL), either alone or in combination with Lacto-*N*-neotetraose (LNnT), to infant formula products and formulated supplementary foods for young children (FSFYC)***:***

1. ***FSANZ's preliminary position is to permit both 2'-FL and LNnT to be used as a nutritive substance, and as food produced using gene technology derived specifically from GM production strains E. coli SCR6 (for 2'-FL) and E. coli MP572 (for LNnT), for use in infant formula products and FSFYC*** *(Point 2.2.2 of the CFS report)*

We agree with and highly appreciate your proposal to permit both 2'-FL and LNnT to be used as nutritive substance, and as food which are important ingredients for infant nutrition and an important step to narrow the nutritional gap between infant nutrition and human milk and can provide benefits for many infants that cannot be breastfed. Sufficient availability on the Australian and New Zealand market is the prerequisite for offering these benefits. Only several providers together can meet the demand, seeing the global need for HMOs and the limited production capabilities of the individual companies producing HMOs by fermentation, all using genetically modified *E. coli* bacteria as production host.

The fermentation of genetically modified microorganisms itself is a standard process for producing highly purified specialty food and infant food ingredients, like vitamins or oligosaccharides. In the case of 2'-FL and LNnT, the production host is based on the well-known and safe strain *E.coli* K-12, a well characterized non-pathogenic lab strain. This lab-adapted strain is unable to colonize the human gut and is listed under the lowest biosafety level 1. The subsequent purification steps ensure chemically defined, highly purified and safe products, which is an essential prerequisite for the use in infant nutrition. The specification of the final product is met in each and any batch introduced into the market irrespective of the specific sub-strain used.

Therefore, we consider that it is not necessary (and contra productive to market coverage) to list also the strain subtypes as the host *E.coli* K-12 is a safe strain and any thereby developed strain subtypes by mutation do not lead to unsafe products. This is also verified by analytical testing on the absence of any bacterial residues, residual proteins, DNA, impurities, and endotoxins. This argument is also supported by the various HMO products which are already authorized and marketed overseas e.g. in Europe, USA and Singapore.

Europe:
In Europe 2'-FL and LNnT are permitted as novel foods with prescribed specifications for 2′-FL *micro* and LNnT *micro* produced with the GM strain *E. coli* K-12 as well as 2′-FL produced with the GM strain *E. coli* BL21[1]).

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With the latest update of the Unions list[1] all applications and notifications for 2'-FL based on *E. coli* K12 from different manufacturers have been consolidated into one authorisation with one corresponding specification. According to the new Novel food Regulation Regulation (EU) 2015/2283, which is applicable as of 1 January 2018, all authorizations are **generic** and the Union list serves as a reference for economic operators who wish to place in the market an authorised novel food (see https://ec.europa.eu/food/safety/novel_food/authorisations/union-list-novel-foods_en). This is also true for 2'-FL and LNnT which are generically authorized and business operators have to meet the established specifications for the two HMO-like substances. These specifications have been established on the level of the final product and the established safe production host which is *E. coli* K12 (for 2'-FL and LNnT) or *E. coli* B21 (for 2'-FL). No subtypes of the strain, which might be used by different companies, are mentioned in the specification of the European Union list (see also Table 1 with the parameter of the current version) indicating the equivalence of the products and the production methods and aiming to support an efficient and internationally competitive food industry.

USA:
In total five notifications for 2'-fucosyllactose have been submitted to FDA with the conclusion of being GRAS, which have not been objected by FDA. This means, although the products are based on different subtypes of *E. coli* K12 or even another strain of *E. coli* like BL 21 the safety of the products have not been questioned indicating that all notified product are substantial equivalent and can be used as alternative sources for 2'-FL in infant nutrition products.
In the following table 1, the different GRAS notification for 2'-FL based on *E. coli* K12 as well as the specification according to the **current** European Union list based on *E. coli* K12 and the proposed EU specification by Glycom (see Table 2.3 of the SD1_Risk Assessment) are assembled:

Table 1: Specifications of 2'-Fucosyllactose

Parameter	**US GRN 650 (*E. coli* K12)**	**US GRN 735 (*E. coli* K12)**	**US GRN 749 (*E. coli* K12)**	**EU product specifications proposed for 2'-FL by Glycom**	**Current European Union List specification for 2-FL (*E. coli* K12)** [e)]
Assay 2'-FL (waterfree)	Min 94.0% (HPLC)	min. 90% (HPAEC)	≥ 82 %	≥ 94 %	≥ 90 %
Assay (water free) for human-identical milk saccharides (HiMS)[a)]					
Identification					
Appearance, visual	Powder	Homogenous powder	Powder	Powder or agglomerates	Powder
Color, visual	white to off-white	White	White to off-white	White to off-white	White to off-white
Related substances					

[1] [https://ec.europa.eu/food/safety/novel_food/authorisations/union-list-novel-foods_en]

Parameter	US GRN 650 (*E. coli* K12)	US GRN 735 (*E. coli* K12)	US GRN 749 (*E. coli* K12)	EU product specifications proposed for 2'-FL by Glycom	Current European Union List specification for 2-FL (*E. coli* K12) [e]
D-Lactose	Max. 3.0 w/w%	Max 3 %	≤ 8 %	≤ 3.0 %	≤ 3.0 %
L-Fucose	Max. 1.0 w/w%	Max 2 %	≤ 6 % [d]	≤ 1.0 %	≤ 2.0 %
2'-Difucosyl-D-Lactose	Max. 1.0 w/w%	-	≤ 7 %	≤ 1.0 %	≤ 2.0 %
2'-Fucosyl-D-Lactulose	Max. 1.0 w/w%	-	≤ 6 % [d]	≤ 1.0 %	≤ 1.0 %
2'-Fucosyllactose			≤ 6 % [d]		
Fucosylgalactose	-	-	≤ 6 % [d]	-	-
Allo lactose	-	Max 2 %	-	-	-
Glucose	-	Max 2 %	≤ 6 % [d]	-	-
Galactose	-	Max 2 %	≤ 6 % [d]	-	-
Characteristic properties					
pH (20°C, 5% solution)	3.2 – 5.0	3.0 – 7.5 (10 % solution)	-	3.2 – 5.0	3.0 – 7.5
Sulfated Ash	Max. 1.5 %	Max. 0.2%	-	≤ 1.5 %	≤ 2.0 %
Acetic acid (as free acid and/or sodium acetate)	Max. 1.0 %	-	-	≤ 1.0 %	≤ 1.0 %
Water, Karl-Fischer	Max 5.0 %	Max 5.0 %	≤ 9.0 %	≤ 5.0 %	≤ 9.0 %
Heavy Metals/ Contaminants					
Pb	Max. 0.1 mg/kg	Max. 0.05 mg/kg	≤ 0.05 mg/kg	≤0.1 mg/kg	-
Cd	-	Max. 0.01 mg/kg	≤ 0.05 mg/kg	-	-
Hg	-	Max. 0.05 mg/kg	≤ 0.1 mg/kg	-	-
As	-	≤ 0.1 mg/kg	≤ 0.2 mg/kg	-	-
Endotoxin	-	Max. 10 EU/mg	-	≤ 10 EU/mg	≤ 10 EU/mg
Residual Protein (Bradford)	0.01 %	Max. 0.01 %	≤ 100 mg/kg	≤ 0.01 %	≤ 0.01 %
Microbiology					
Total microbial aerobic count	Max. 500 CFU/g [b]	Max. 3000 CFU/g	-	≤ 500 CFU/g	≤ 3000 CFU/g
Yeasts and Molds	Max. 10 CFU/g [c]	Max. 10 CFU/g [c]	-	Max. 10 CFU/g [c]	Max. 100 CFU/g [c]
Enterobacteria & other Gram-neg	absent in 10 g	absent in 10 g	-	Absent in 10 g	-
Cronobacter sakazakii	absent in 10 g	absent in 25 g	absent in 100 g	Absent in 10 g	-
Salmonella	absent in 25 g	absent in 25 g	absent in 100 g	Absent in 25 g	-
Bacillus cereus	Max. 50 CFU/g	Max. 100 CFU/g	-	Max. 50 CFU/g	-

Parameter	US GRN 650 (*E. coli* K12)	US GRN 735 (*E. coli* K12)	US GRN 749 (*E. coli* K12)	EU product specifications proposed for 2'-FL by Glycom	Current European Union List specification for 2-FL (*E. coli* K12) [e])
Listeria monocytogenes	absent in 25 g	-	-	Absent in 25 g	-
	[a]) Human-identical milk saccharides (HiMS) is defined as the sum of 2'-FL, lactose, difucosyllactose, and fucose [b]) Aerobic mesophilic total (plate) count [c]) Separate specifications for yeasts and moulds [d]) Limit of 6% for other carbohydrates that includes 3'-Fucosyllactose, 2'-Fucosyl-D-lactulose, Fucosylgalactose, Glucose, Galactose, Fucose, Sorbitol, Galactitol, Mannitol, and Trihexose [e]) https://ec.europa.eu/food/safety/novel_food/authorisations/union-list-novel-foods_en				

All GRAS notifications are based on the fact that the product (2'-FL) is chemically and structurally identical to naturally occurring oligosaccharides in human milk and to chemically synthesized oligosaccharides. The 2'-fucosyllactose is produced by the different manufacturers by fermentation followed by a downstream process that is effectively removing the biomass (specifically cell-walls incl. endotoxins and protein) and isolating a product of high purity with a low content of other related sugars independently of the strain subtype of *E. coli* used as production strain. In the safety, technical and health effects assessment – Application A1155, SD1 (hereinafter called "SD1"), point 3.1.3, it is stated that the final food is highly unlikely to contain novel protein or DNA due to the purification steps used in its production. This is verified by analytical testing and is reflected in the different GRAS notifications and in the EFSA evaluations.
In consequence, it can be stated with confidence that the safety of the products has been shown with all different strain subtypes used for fermentation and guaranteed independently of the strain subtype used.

Singapore:
2'-FL is authorized by the Agri-Food & Veterinary Authority Singapore (see also 1.3.2.4 CFS) for the use as an ingredient in infant formula inclusive follow up formula and growing up milk independent of the way of manufacture (by fermentation or synthetically).

It addition, it shall be noted that the HMO product is not produced in Australia or New Zealand and in particular, the fermentation production steps are also not performed in Australia or New Zealand. Only chemically defined purified compounds and their specified mixtures of mono- and oligosaccharides in which both GMMs and newly introduced genes have been removed are imported to Australia and New Zealand. The final product 2'-FL does not contain any GMM as verified by the test on residual proteins and of absence of DNA which is also confirmed in the assessment of FSANZ.

2. ***FSANZ's preliminary position is to set specifications for 2'-FL and LNnT using those provided by the applicant*** *(Point 2.2.6 of the CFS report).*

BASF proposes to base the purity data of the specification for 2'-FL and LNnT on the specification as currently in force in Europe (see Table 1) for the following reasons:
The specification as proposed by the applicant with regard to the specification parameters (see point 2.3.4 of SD1) are based on the product specific European permission (based on old European novel food

legislation) and the GRAS notification. We want to point out that the European generic Novel Food authorization for 2'-FL has been revised in the meantime in order to have one generic specification considering all notifications of the different companies which have been assessed by different European health authorities according to the old Novel Food legislation in Europe. This means, that any food business operator can place an authorised Novel Food on the European Union market, provided the authorised conditions of use, labelling requirements, and specifications are respected. Under this new European Novel Food Regulation, all authorizations (new and old) are generic as opposed to the applicant-specific, restricted novel food authorisations under the old Novel Food regime.
The European Commission therewith support a competitive food industry as well as provision of the market with important and beneficial human milk identical ingredients.
In addition, it shall be pointed out that the specifications as listed In SD1, Tables 2.3 and 2.4 which are proposed to be included in Schedule 3 also reference the Methods of Analysis (based on "In-House-Test-Methods"). We wish to draw attention that their inclusion in Schedule 3 would lead in an inconsistency with specifications currently included in Schedule 3 and moreover, would limit both manufacturers and enforcement agencies as well as for future analytical methods improvements and also, would provide indirectly for exclusivity in favor of the applicant as his "In-House Test Methods" are prescribed.

3. Point 2.4.3 of the CFS report: Desirability of an efficient and internationally competitive food industry

FSANZ has concluded that the "proposed permission would support an internationally competitive food industry for infant formula products and FSFYC". However, an efficient internationally competitive food industry is not supported by strain-specific authorizations, that are restricting market access for safe products. Instead, generic approvals for products meeting the specifications are supporting an efficient, internationally competitive food industry. As explained above the current manufacturers of 2'-FL have equivalent products and the developed strains are all suitable for the same purpose. There is no convincing reason to limit the permission to a specific proprietary production route. Furthermore, it would exclude other 2'-FL manufacturers to supply this important nutrient to the Australian infant food industry. Since 2'-FL is still a new ingredient in food supply the global capacities are still limited to cover the global needs. Excluding other suppliers from the Australian and New Zealand market by a company-specific authorization would limit the access of Australian and New Zealand companies to these new ingredients and therefore, limit the access of parents that need to also rely on infant formula to the best available infant formula ingredients.

Additionally, it has to be stated that listing of a specific subtype of the strain in the permission of 2'-FL would lead into numerous subtype specific novel food applications with FSANZ by individual as well as different companies without having any added value for the customer as the specification of the product are always the same. Limiting the point of entry for HMO producers to the infant food market in Australia and New Zealand.

4. Point 2.2.7 of the CFS report "exclusivity" of the assessment:

We understand the intention of the FSANZ NOVEL FOOD STANDARD for granting an exclusivity with regard to the importance of data protection and/or first to market advantage to ensure commercial advantage for the first applicant.
It shall be pointed out that in particular the product 2'-FL is manufactured by different companies with similar specification and is already available in different markets and cannot be considered a "novel"

ingredient anymore. The applied technologies for the production of the HMO products are common technological standard, as based on the same process principles and resulting in the same high-quality product which ensures all safety aspects as needed in particular when intended to be used in infant nutrition.

Moreover, please take note, that the safety of the products is shown and verified by various published toxicological studies[2] and also in several non-published studies. These studies could all demonstrate that the product is safe as the quality standard of the material provided by different companies is very high. In our point of view further animal studies are not required and should not be performed also in view of the animal welfare aspects.
If the current intention of FSANZ were followed to adopt the application of 2′-FL and LNnT as food produced using gene technology derived specifically from the applicant's GM production strains, and if FSANZ would adopt the specifications as proposed by the applicant naming the strain subtypes, FSANZ would provide exclusive permission to that applicant without the need for a specific brand name which means an unlimited exclusivity for the product. This would go far beyond the initially requested exclusivity period of 15 months. Scientifically and from the risk assessment point of view, the safety of the product is decisive for market approval, not the way of its production. If you have a safe product like 2'-FL, a safe production host like *E. coli* K12 and no GMM (and DNA) in the final product, then you have market approval, according to the novel food guiding principle in the EU.
The current FSANZ proposal for sub-strain specific product approval would result in numerous companies specific authorizations by naming their used strain subtype and in subsequently frequent applications by the same companies for updating the sub-type specific specification, i.e. each time when a strain optimization has been performed without any change in the product specifications.
This could be avoided if FSANZ would decide to base the Novel Food specification on a generic product authorization like e.g. in the EU.

In conclusion: In our point of view to set specifications for 2'-FL and LNnT on the specific strain subtypes used by the applicant is not scientifically justified and therefore should only be based on the name of the safe production host (*E.coli* K12) The proposed FSANZ permission of strain subtype specific approvals for HMOs is in full contrast to generic approvals in other jurisdictions. It would exclude competition in the Australian and New Zealand market with the risk of a possible insufficient supply by only one permission holder considering also the worldwide requirement for these important ingredients which narrow the nutritional gap between infant nutrition and human milk and which can provide benefits for many infants that cannot be breastfed. We are strongly in favor of keeping the concept of generic approvals for safe products (with or without the differentiation between produced by microbial fermentation or by chemical synthesis). For the sake of an efficient and internationally competitive food industry.

[2] M. **COULET**, P. Phothirath, L. Allais and B. Schilter. Pre-clinical safety evaluation of the synthetic human milk, nature-identical, oligosaccharide 20-O-Fucosyllactose (20FL). Regulatory Toxicology and Pharmacology 68 (2014) 59–69; doi: 10.1016/j.yrtph.2013.11.005
D. **VAN BERLO**, A. E. Wallinga, F. A. A. van Acker, D. J. Delsing. Safety assessment of biotechnologically produced 2′-Fucosyllactose, a novel food additive, Food and Chemical Toxicology (2018), doi: 10.1016/j.fct.2018.04.049
P. R. **HANLON** and B. A. Thorsrud. A 3-week pre-clinical study of 2′-fucosyllactose in farm piglets. Food and Chemical Toxicology 74 (2014) 343–348; doi: 10.1016/j.fct.2014.10.025