ORIGINAL SUBMISSION

Glycom A/S, Diplomvej 373 Ø, 1st fl., DK - 2800 Kongens Lyngby

GRN 000659

July 5, 2016

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RECEIVED
JUL 12 2016
OFFICE OF
FOOD ADDITIVE SAFETY

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835

Dear Dr. Gaynor:

Re: GRAS Exemption Claim for Lacto-*N*-neotetraose

In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the notifier [Glycom A/S, Diplomvej 373, DK-2800 Kgs. Lyngby, Denmark], a notice of the determination, on the basis of scientific procedures, that lacto-*N*-neotetraose (LNnT) produced by microbial fermentation by Glycom A/S, as defined in the enclosed documents, is GRAS under specific conditions of use in non-exempt term infant formula and in specified food products, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. This ingredient is chemically equivalent to the synthetic lacto-*N*-neotetraose notified to the FDA on September 16, 2014 (designated as GRN 547) and is intended for use as an alternative to the existing GRAS uses described therein. Information setting forth the basis for the GRAS determination, which includes detailed information on the notified substance and a summary of the basis for the GRAS determination, as well as a consensus opinion of an independent panel of experts in support of the safety of lacto-*N*-neotetraose under the intended conditions of use, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection Virus and Spyware Protection (Definition July-04-16 r1).

Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Christoph H. Röhrig, Ph.D.
Senior Scientist, Regulatory Affairs Manager
Glycom A/S

GRAS Exemption Claim for Lacto-*N*-neotetraose (LNnT) Produced by Fermentation

Submitted to:

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740-3835
USA

Submitted by:

Glycom A/S
Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark

July 5, 2016

GRAS Exemption Claim for Lacto-*N*-neotetraose (LNnT)

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I GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

Glycom A/S hereby claims that the use of lacto-*N*-neotetraose (LNnT) in non-exempt term infant formula and conventional food and beverage products, as described in Section I.D below, is Generally Recognized as Safe (GRAS) under its intended conditions of use and is therefore exempt from the requirement of premarket approval of the *Federal Food, Drug, and Cosmetic Act*. This determination is based on scientific procedures and unanimous consensus among experts qualified by scientific training and expertise.

Signed,

(b) (6)

06 July 2016

Christoph H. Röhrig, Ph.D.
Senior Scientist & Regulatory Affairs Manager
Glycom A/S
christoph.roehrig@glycom.com

Date

I.B Name and Address of Notifier

Glycom A/S
Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark
Tel: +45 4525 2247
Fax: +45 3841 1720

I.C Common Name of the Notified Substance

Lacto-*N*-neotetraose

I.D Conditions of Intended Use

Lacto-*N*-neotetraose is intended for use in term non-exempt infant formulas at a use level of up to 600 mg/L of the ready-to-drink or reconstituted formula. The maximum use level is proposed on the basis of providing a similar level of LNnT as that which occurs in mature human breast milk.

LNnT also is intended for use in various conventional food and beverage products across multiple categories as described in Table I.D-1.

Table I.D-1 Summary of the Individual Proposed Food-Uses and Use-Levels for Lacto-*N*-neotetraose (LNnT) in Conventional Food and Beverage Products and Infant Formula

Food Category	Proposed Food-Uses	RACC	Proposed Use Level (g/RACC)	Maximum Proposed Use Level (g/kg or g/L)
Beverages and Beverage Bases	Meal Replacement Drinks, for Weight Reduction	240 mL	0.6	2.5
	Sports, Isotonic, and Energy Drinks	240 mL	0.14	0.58
Dairy Product Analogs	Imitation Milks	240 mL	0.14	0.58
	Non-Dairy Yogurt	225 g	0.6	2.67
Infant and Toddler Foods	Term Infant Formulas	100 mL[a]	0.06	0.60
	Toddler Formulas	100 mL[a]	0.06	0.60
	Other Baby Foods for Infants and Young Children	7 to 170 g	0.02 to 0.68	3.0
	Other Drinks for Young Children	120 mL	0.07	0.58
Grain Products and Pastas	Meal Replacement Bars, for Weight Reduction	30 g	0.6	20.0
Milk, Whole and Skim	Unflavored Pasteurized and Sterilized milk[b]	240 mL	0.14	0.58
Milk Products	Buttermilk	240 mL	0.14	0.58
	Flavored Milk	240 mL	0.14	0.58
	Milk-Based Meal Replacement Drinks, for Weight Reduction	240 mL	0.6	2.5
	Yogurt	225 g	0.6	2.67
Processed Fruits and Fruit Juices	Fruit Juices and Nectars	240 mL	0.14	0.58

LNnT = lacto-*N*-neotetraose; RACC = Reference Amounts Customarily Consumed (21 CFR §101.12 – U.S. FDA, 2015a); U.S. = United States.
[a] RACC not available, 100 mL employed as an approximation.
[b] Milk is a standardized food in the United States. When the milk is fortified with LNnT it will then be classified as a milk product.

I.E Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30 of the *Code of Federal Regulations* (CFR) (U.S. FDA, 2015b), LNnT produced by microbial fermentation has been determined by Glycom A/S to be GRAS for uses in non-exempt term infant formula and specified conventional food and beverage products, as described herein, on the basis of scientific procedures.

I.F Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the United States (U.S.) Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Glycom A/S
Diplomvej 373
DK-2800 Kgs. Lyngby
Denmark

Should the FDA have any questions or additional information requests regarding this notification, Glycom will supply these data and information.

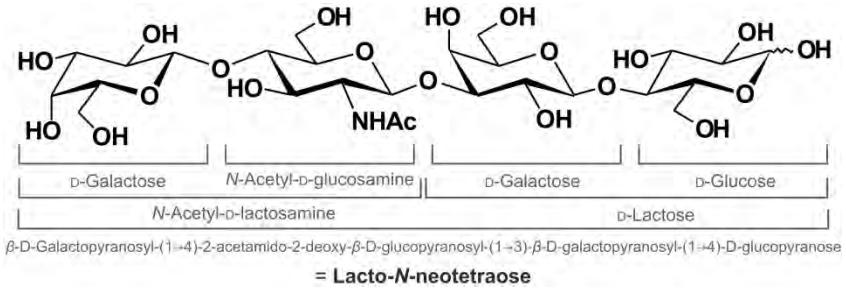
II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

II.A Identity

II.A.1 Chemical Identity

Common Name:	Lacto-*N*-neotetraose
Common Abbreviation:	LNnT
IUPAC Name:	β-D-Galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose
Alternative Denotations:	Gal-β-(1→4)-GlcNAc-β-(1→3)-Gal-β-(1→4)-Glc *N*-Acetyl-D-lactosamine-β-(1→3)-D-lactose
Chemical Abstracts Service (CAS) Registry Number:	13007-32-4
Chemical Formula:	$C_{26}H_{45}NO_{21}$
Molecular Weight:	707.63

Structural Formula:



II.A.2 Chemical and Physical Characteristics

LNnT is a naturally occurring tetrasaccharide detected in some mammalian milks with the highest concentrations present in human milk, and is therefore typically referred to as a human milk oligosaccharide (HMO). LNnT is a chemically defined linear tetrasaccharide consisting of D-galactose, *N*-acetyl-D-glucosamine, D-galactose and D-glucose, which occurs as one specific constitutional isomer.

The molecular structure of LNnT was elucidated by Richard Kuhn in 1962 and since then a number of publications reported detailed structure characterization by ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) techniques (Strecker *et al.*, 1989; Urashima *et al.*, 2002, 2005; Landersjö *et al*., 2005). Based on ^{1}H- and ^{13}C-NMR-, mass spectrometry (MS)-, and high performance liquid chromatography (HPLC) with corona charged aerosol detector data, it is confirmed that LNnT produced by microbial fermentation is chemically and structurally identical to LNnT produced by chemical synthesis described in GRN 547 and to LNnT present in human breast milk.

II.B Method of Manufacture

LNnT is manufactured in compliance with current Good Manufacturing Practices (cGMP) and the principles of Hazard Analysis Critical Control Point (HACCP). The raw materials from which LNnT is derived include D-lactose as a substrate, with D-glucose, D-glycerol and ammonium salts used as carbon and nitrogen sources for fermentation. The manufacturing process can be broadly divided into 2 stages: in Stage 1 [upstream processing (USP)], D-lactose is converted *via* the metabolic intermediate "lacto-*N*-triose II" to LNnT by the cellular enzymes of the LNnT production organism. In Stage 2, the downstream processing (DSP), a series of purification and isolation steps generate the final high-purity LNnT product.

II.B.1 Production Microorganism

II.B.1.1 Host

The genotypic characteristics of the host organism, *Escherichia coli* (*E. coli*) K-12 DH1, are presented in Table II.B.1.1-1 below. The host organism is well characterized at the phenotypic

and genotypic level, and the genome of *E. coli* K-12 has been sequenced and bioinformatic comparisons of the genome of *E. coli* K-12 with other safe laboratory strains and various pathogenic isolates have been conducted (Blattner *et al.*, 1997; Lukjancenko *et al.*, 2010). *E. coli* K-12 DH1 (*λ⁻ gyrA96 recA1 relA1 endA1 thi-1 hsdR17 supE44*) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) culture collection, and the construction of strain DH1 has been described in the literature (Hanahan, 1983; Luli and Strohl, 1990; Bachmann, 1996).

Table II.B.1.1-1	**Characteristics of the Host Organism *Escherichia coli* K-12 DH1**
Genotype	*F⁻*, *λ⁻*, *gyrA96, recA1*, *relA1*, *endA1*, *thi-1*, *hsdR17*, *supE44*.
Genus	*Escherichia*
Species	*Escherichia coli*
Subspecies	not applicable
Strain	*E. coli* strain K-12 DH1
Culture collection	The German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen)
Deposition number	DSM 4235 (ATCC33849)

The DH1 strain is resistant to nalidixic acid due to the gyrA96 mutation (Hanahan, 1983). The K-12-derived strains cannot colonize the human gastrointestinal system, and do not produce protein-type toxins (U.S. EPA, 1997). The strain can grow in minimal medium, provided that it is supplemented with thiamine due to the thi-1 mutation. Further, the recA1 mutation minimizes the recombination and increases the stability of plasmids and chromosomal DNA of the strain.

II.B.1.2 Host Modification

The host strain *E. coli* K-12 DH1 (DSMZ, 2015) was optimized for general oligosaccharide expression features (used as a “platform host strain”) by the introduction of 7 genetic modification events related to the metabolism of various sugars, thereby improving the efficiency of the strain. The modified strain is designated as strain MDO. The 7 genetic modifications that lead from DH1 to MDO are:

(1) Deletion of the β-galactosidase gene lacZ, which encodes an enzyme that degrades lactose into galactose and glucose. Deletion of this gene allows to use lactose as substrate for LNnT production.

(2) Insertion of the Plac promoter upstream of the intrinsic GDP-mannose-4,6-dehydratase gene gmd to increase GDP-fucose synthesis under isopropyl-β-D-1-thiogalactoside (IPTG) induction. This modification has no impact on the LNnT production capability of the strain.

(3) Deletion of the sialic acid metabolism-related gene cluster nanKETA. This modification is not relevant for LNnT synthesis but makes the derived strain suitable as a general platform host strain, including synthesis of sialylated oligosaccharides. The nanKETA cluster includes the genes nanK, nanE, nanA and nanT, encoding the enzymes *N*-acetylmannosamine kinase, *N*-acetylmannosamine-6-phosphate 2-epimerase, *N*-acetylneuraminate lyase and the sialic acid transporter nanT, respectively.

(4) Deletion of the galactoside *O*-acetyltransferase gene lacA, which encodes for an enzyme that acetylates the galactose residues of oligosaccharides and would thereby lead to increased carbohydrate-type impurities.

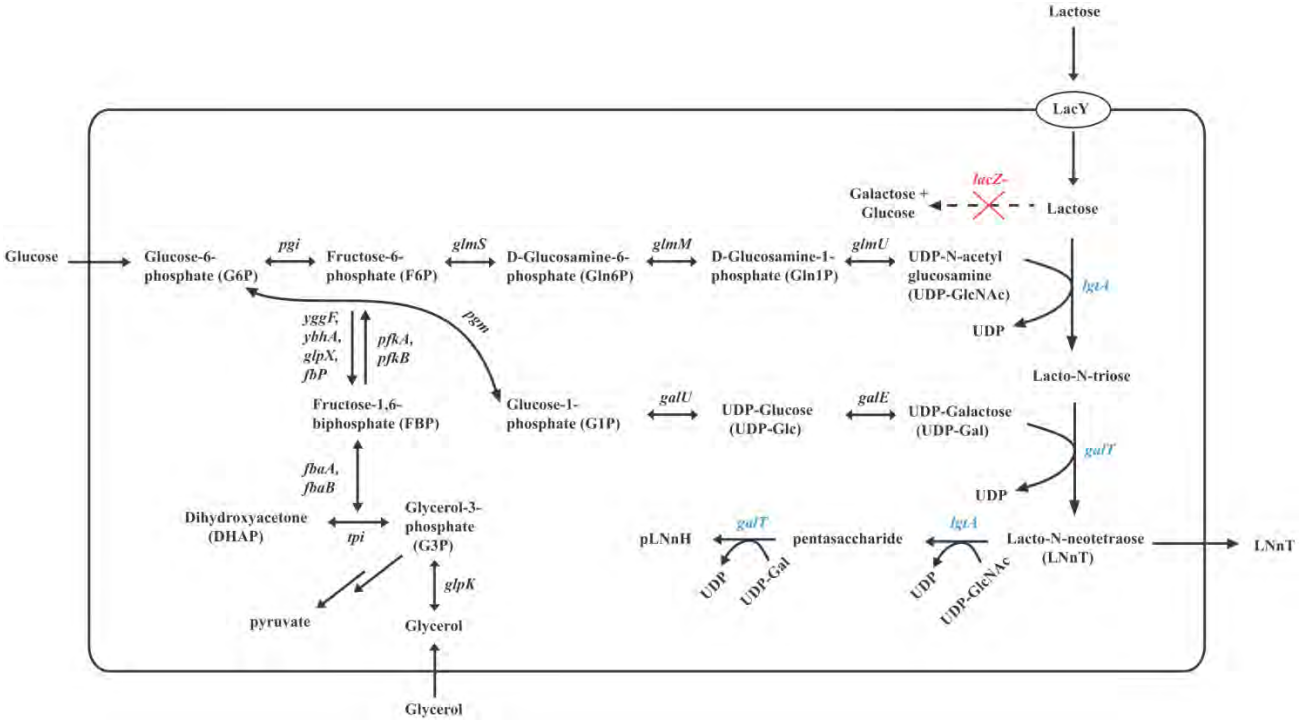
(5) Deletion of the α-galactosidase gene melA. This modification is not relevant for LNnT synthesis but makes the derived strain suitable as a general platform host strain, including synthesis of α-linked oligosaccharides. As a result of this modification the strain is not able to grow on melibiose (Gal-α-(1→6)-Glc).

(6) Deletion of the wcaJ gene that encodes the UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase, a lipid carier transferase involved in colonic acid biosynthesis. Colanic acid is an extracellular polysaccharide containing fucose and its overproduction increases dramatically the viscosity of the culture medium. The wcaJ knock-out prevents high culture medium viscosity.

(7) Deletion of the glucans biosynthesis glucosyltransferase H gene mdoH. The enzyme encoded by this gene is involved in the biosynthesis of periplasmic glucans, the presence of which would complicate the isolation and purification of other targeted oligosaccharides to be expressed by the strain.

The resulting strain, MDO, constitutes a general platform starting strain for the generation of specific strains for the fermentative synthesis of a diverse range of oligosaccharides. To enable LNnT production by the MDO platform strain, 2 heterologous genes from *Neisseria meningitides*, and *Helicobacter pylori* were introduced into the organism which encode for a β-1,3-*N*-acetylglucosaminyltransferase that converts lactose into lacto-*N*-triose II, and a β-1,4-galactosyltransferase that converts lacto-*N*-triose II to LNnT. The schematic pathway for LNnT biosynthesis from lactose, glucose and glycerol and involving the newly introduced enzymes, is presented in Figure II.B.2-1 below.

Figure II.B.2-1 Schematic Biochemical Pathway of Cell Fermentation to Produce LNnT



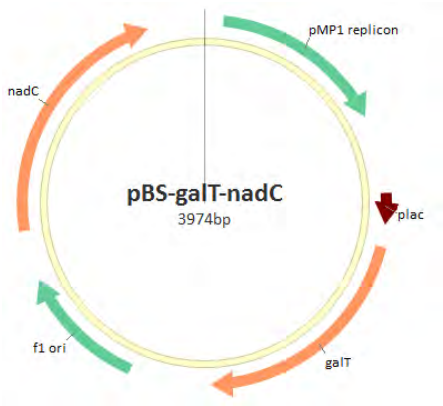
The LgtA enzyme can, in principle, also add another GlcNAc on the newly formed LNnT to result in a LNnT-derived pentasaccharide (albeit substrate recognition by the enzyme is diminished in case of LNnT as compared to lactose), which is then further converted to the LNnT-derived hexasaccharide, *para*-lacto-*N*-neohexaose (pLNnH), by the activity of the GalT enzyme (see Scheme above). However, the conditions of the fermentation process with strain MP572 have been optimized in order to reduce the formation of the penta- and hexasaccharide during the bioconversion process of lactose to LNnT. Nevertheless, these by-products, and the lacto-*N*-triose II intermediate, are principle impurities of the fermentative LNnT production process.

For optimization of LNnT biosynthesis multiple copies of a codon-optimized sequence of the β-1,3-N-acetylglucosaminyltransferase gene were introduced into the chromosomal DNA of the MDO platform strain at multiple targeted loci involved in sugar metabolism. To support an antibiotic resistance marker-free plasmid system, the *nadC* gene encoding the enzyme quinolinate phosphorribosyltransferase, was deleted from the genome of the platform strain. Finally, the gene encoding the repressor of the Lac operon, *lacI*, was deleted in order to enable the induction of gene expression from the introduced Plac promoters without the need of IPTG addition during the fermentation.

To enable LNnT production, the strain MP572 was transformed with a high-copy plasmid (pBlueScript, pBS) carrying the β-1,3-*N*-acetylglucosaminyltransferase (Figure II.B.2-2). To retain plasmid stability without the need for antibiotics, the ampicillin marker that was present in

the DNA sequence of the pBS plasmid was replaced by the native regulatory (promoter) and coding sequences (CDS) of the *nadC* gene from the host organism *E. coli* K12 DH1 (*i.e.*, the same gene deleted from host chromosomal DNA described above).

Figure II.B.2-2 Plasmid Carried by Strain MP572



The resulting strain was called MP572, and both strains (MDO and MP572) have been deposited at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) in Braunschweig, Germany. Donor genes introduced to the host genome originate from *Neisseria meningitidis*, *Helicobacter pylori* or are native to *E. coli* K12 DH1.

The final production strain, MP572, is highly stable, reliable, and yields high titers of LNnT, which is excreted into the fermentation broth. A discussion of the safety of the donor genes and corresponding expression products is presented in Section IV.G.

II.B.2 Raw Materials and Processing Aids

The D-lactose, D-glucose and D-glycerol raw materials used during fermentation meet the specifications established in the European Pharmacopeia or food grade equivalent standard. The raw materials are sterilized before use and monitored by HPLC for potential side products (*e.g.*, lactose can isomerize to lactulose through the well-known aldose-ketose isomerization). Fermentation is performed in a chemically well-defined, salt-based, minimal medium that excludes the use of antibiotics, IPTG induction, or nitrilotriacetic acid chelating agents. D-glucose, D-glycerol and ammonium salts are used as carbon and nitrogen sources and D-lactose serves as a substrate for LNnT synthesis.

Fermentation-aids and other processing-aids and filters and filter aids used in the production of LNnT are food grade quality[1] and are used in accordance with an applicable federal regulation,

[1]Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard.

previous GRAS determination, and/or have been the subject of an effective food contact notification (see Table II.B.2-1).

Table II.B.2-1 Raw Material and Processing Aids Used in the Manufacture of LNnT by Fermentation

Material	Function
Raw Material Substrates (Bioreagents)	
D-lactose	Substrate/source raw material
D-glucose	Energy/carbon source
D-glycerol	Energy/carbon source
Other Components of Fermentation Medium	
Ammonium hydroxide	Fermentation medium ingredient (nitrogen source)
Magnesium sulfate	Fermentation medium ingredient (essential element)
Ammonium dihydrogen phosphate	Fermentation medium ingredient (nitrogen and phosphate source)
Potassium dihydrogen phosphate	Fermentation medium ingredient (essential element and phosphate)
Potassium hydroxide	pH adjustment
Sodium hydroxide	pH adjustment
Citric acid	Fermentation medium ingredient (essential element)
Processing Aids	
Methanol	Crystallization
Ultrafiltration	Removal of cell matter and proteins
Nanofiltration	Removal of small molecules
Ion-exchange resin	Removal of small molecules
Electrodialysis	Removal of small charged molecules (*e.g.*, salts)
Charcoal filter	Decolorization and removal of impurities
Microfiltration	Filter sterilization

LNnT = lacto-*N*-neotetraose

II.B.3 Manufacturing Process

The manufacturing process for LNnT can be broadly divided into 2 stages, which are described in brief in Section II.B.3.1 and II.B.3.2, respectively (see Figure II.B.3-1 below).

Figure II.B.3-1 Schematic Overview of the Manufacturing Process for LNnT, Produced by Microbial Fermentation

Stage	Step No	PROCESS STEP	PURIFICATION
Upstream Processing (USP)	01	Media Preparation	
	02	Propagation	
	03	Seed Fermentation	
	04	FERMENTATION	*Production of LNnT*
	05	Ultrafiltration-Diafiltration (UF/DF)	*Removal of cells and large biomolecules (e.g. protein, nucleic acids and lipopolysaccharides)*
Downstream Processing (DSP)	06	Nanofiltration or Nanofiltration-Diafiltration (NF/DF)	*Concentration. Reduction water, minerals and very small biomolecules*
	06a	*Optional Microfiltration*	*Removal of potential microbiological contamination*
	07	Ion Removal (*e.g.*, ion-exchange resin or electrodialysis)	Removal of small charged molecules and salts (*e.g.*, trace metals)
	07a	*Optional Pre-concentration (e.g., evaporation and/or Nanofiltration)*	
	08	Decoloration (*e.g.*, active charcoal filtration)	*Removal of color and impurities by adsorbent*
	09	Microfiltration	*Removal of potential microbiological contamination*
	10	*Optional Pre-concentration* (*e.g.,* evaporation and/or Nanofiltration)	
	11	Chromatography	*Removal of lactose, para-LNnH and lacto-N-triose II*
	12	Pre-concentration (*e.g.,* evaporation and/or Nanofiltration)	
	13	Crystallization (from water with methanol)	*Highly efficient removal of micro-impurities (traces of protein and DNA, amino acids, carbohydrate-type impurities, trace elements, etc.)*
	14	Solid-Liquid-Separation (SLS)	
	15	Washing	
	16	Drying	*Removal of water and methanol*
	17	Milling	
	18	Sampling and Packaging	
	19	Quality Control	*Specifications are tested and CoA issued*
	20	Batch Release	

CoA = certificate of analysis; DSP = downstream processing; LNnH = lacto-*N*-neohexaose; LNnT = lacto-*N*-neotetraose; SLS = solid-liquid-separation; UF = ultrafiltration; USP = upstream processing.

II.B.3.1 Manufacturing Stage 1: Fermentation Procedure

In Stage 1 of the manufacturing process, D-lactose is converted by the production organism into LNnT by cell fermentation. The fermentation is maintained for several days until In-Process Controls indicate a favorable ratio LNnT to other carbohydrates and high consumption of D-lactose.

LNnT is excreted into the fermentation broth; therefore disruption of the cells is not required for isolation of LNnT from the culture broth. The microbial biomass containing the production organism is then removed from the culture supernatant containing LNnT by ultrafiltration/ diafiltration and the separated microbial biomass is deactivated by heat treatment. The quality of the clear ultrafiltration/diafiltration permeate is assessed by a range of In-Process Controls and then further purified by the second stage of the production process, the downstream processing.

II.B.3.2 Manufacturing Stage 2: Purification and Isolation

Stage 2 of the manufacturing process consists of a series of purification steps, most notably the final selective crystallization step, that generate the single, isolated, high-purity, crystalline tetrasaccharide LNnT ingredient.

The ultrafiltration permeate is concentrated through a nanofiltration aid to remove water, minerals, and very small molecules. Depending on processing time of the nanofiltration, an optional microfiltration step can be applied in order to minimize the risk of microbiological growth. Ion- and residual biomolecule removal is applied by treating the product stream with ion exchange resin. If required, the pH of the solution is adjusted to $4.0 < pH < 6.0$ to reduce the risk of LNnT fructose isomer formation *via* the pH-dependent Lobry de Bruyn-van Ekenstein aldose-keto isomerism. Subsequently, decoloration of the solution is achieved by treatment with an adsorbent (*e.g.*, activated charcoal), and an optional microfiltration step may be applied to minimize the risk of microbial growth. Again, the pH of the solution may be controlled and adjusted to $4.0< pH < 6.0$ if necessary. The product solution is pre-concentrated to a defined LNnT concentration either by vacuum distillation or nanofiltration (and an optional microfiltration step may also be applied thereafter). Purification by chromatography is then undertaken to remove any carbohydrate-type impurities, and the product fractions from chromatography are concentrated to a defined LNnT concentration either by vacuum distillation or nanofiltration. Defined portions of methanol and LNnT seeding crystals are then added in defined intervals of time and temperature, leading to highly controlled crystallization of LNnT. A solid-liquid-separation removes the LNnT crystals from the mother liquor[2], and the crystals then are washed with methanol to remove remaining traces of salts, biomolecules, and carbohydrate impurities, and then dried. Milling of the pre-dried product is then performed to yield a homogenized

[2] The solution that is removed by filtration from the crystallized product is called the “mother liquor”. It contains potential impurities and traces of product.

powder, and LNnT is further dried by exposure to a stream of wet nitrogen to reduce remaining traces of methanol. Alternatively, final methanol removal can be achieved by spray drying. Milling and sieving of the product is undertaken for optimal particle size distribution, and a final drying step can be taken to reduce the final water content in the finished product. Quality control measures are in place during the entire purification and isolation process to ensure that final batches of LNnT released conform to the product specifications.

The LNnT produced by fermentation is chemically equivalent to LNnT present in human milk from lactating women, and to LNnT produced by chemical synthesis as described in GRN 547 (Glycom A/S, 2014).

II.B.4 Quality Control

As previously indicated, the manufacture of LNnT by microbial fermentation is consistent with Good Manufacturing Practice and HACCP principles.

Due to the principal raw materials and the final product being single, well-characterized and pure compounds the whole production process can be followed in detail by a range of analytical techniques. These techniques are applied either as in-process controls or at batch release (by certificate of analysis) to allow full control of the production process.

Both manufacturing stages are controlled by a HACCP plan which includes specifications for the equipment, raw materials, product, and packaging materials used in the manufacturing process. Master operating instructions are followed, batch records are kept, a number of in-process controls are applied, and the final isolated LNnT product is controlled by certificates of analyses and batch release routines.

II.C Specifications for Food Grade Material and Product Analysis

II.C.1 Specifications for Food Grade Material

Food-grade specifications have been established for LNnT (Table II.C.1-1). The ingredient is specified as a white to off-white powder or agglomerate with a LNnT assay value of at least 92% based on HPLC with a corona charged aerosol detector (HPLC-cCAD) and a purity (defined as the sum of human-identical milk saccharides) of at least 95%. Upper limits have been established for the raw materials and processing aids used in the manufacturing (*e.g.,* D-lactose, methanol), the carbohydrates formed during the fermentation (*e.g.*, lacto-*N*-triose II, *para*-lacto-*N*-neohexaose, LNnT fructose isomer), heavy metals, and microbiological parameters, to ensure the purity of the final product.

Table II.C.1-1 Proposed Product Specifications for LNnT		
Parameter	**Specification**	**Method**
Appearance	Powder or agglomerates	MSZ ISO 6658:2007
Color	White to off white	MSZ ISO 6658:2007
Identification	RT of standard ± 3%	Glycom method HPLC-106-1C6-002
Assay (water free) Human-identical Milk Saccharides[a]	Not less than 95.0 w/w %	Glycom method HPLC-106-1C6-002
Assay (water free) Lacto-N-neotetraose	Not less than 92.0 w/w %	Glycom method HPLC-106-1C6-002
D-Lactose	Not more than 3.0 w/w %	Glycom method HPLC-106-1C6-002
Lacto-*N*-triose II	Not more than 3.0 w/w %	Glycom method HPLC-106-1C6-002
para-Lacto-*N*-neohexaose	Not more than 3.0 w/w %	Glycom method HPLC-106-1C6-002
LNnT fructose isomer	Not more than 1.0 w/w %	Glycom method HPLC-106-1C6-002
pH (20°C, 5 % solution)	4.0 – 7.0	Eur. Ph. 2.2.3
Water	Not more than 9.0 w/w %	Karl-Fischer (Ph. Eur. 2.5.12)
Ash, sulfated	Not more than 1.5 w/w %	Eur. Ph. 6.7 04/2010:20414
Methanol	Not more than 100 mg/kg	GC-HS (Ph. Eur. 2.4.24)
Isopropanol	Not more than 200 mg/kg	GC-HS (Ph. Eur. 2.4.24)
Residual proteins	Not more than 0.01 w/w %	Bradford Assay; Glycom method UV-001
Heavy metals		
Lead	Not more than 0.1 mg/kg	ICP-MS by EPA 6020A:2007
Microbiological Parameters		
Total plate count	Not more than 500 CFU/g	MSZ-EN-ISO 4833-1:2014
Yeasts	Not more than 10 CFU/g	MSZ-ISO 7954:1999
Molds	Not more than 10 CFU/g	MSZ-ISO 7954:1999
Enterobacteriaceae	Absent in 10 g	ISO 21528-1:2004, MSZ ISO 21528-2:2007
Salmonella	Absent in 25 g	MSZ-EN-ISO 6579:2006
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	ISO-TS 22964:2006
Listeria monocytogenes	Absent in 25 g	MSZ-EN-ISO 11290-1:1996/A1:2005, MSZ EN ISO 11290-1:1998
Bacillus cereus	Not more than 50 CFU/g	MSZ-EN-ISO 7932:2005
Residual endotoxins	Not more than 10 EU/mg	Eur. Ph. 2.6.14

CFU = colony forming units; Eur. Ph. = European Pharmacopeia; EU = endotoxin units; GC-HS = headspace gas chromatography; HPLC = high performance liquid chromatography; ISO = International Organization for Standardization; LNnT = lacto-*N*-neotetraose; RT = retention time.

[a] Human-identical milk oligosaccharides is defined as the sum of LNnT, lactose, lacto-*N*-triose II, and *para*-lacto-*N*-hexaose.

II.C.2 Product Analysis

Batch analyses for 4 independent commercial batches supporting the product specifications laid out in Section II.C.1 are presented in Table II.C.2-1.

Table II.C.2-1 Batch Analyses for LNnT

Parameter	Specification	Manufacturing Batch Number			
		5247750801	**2547750901**	**2547750902**	**2547750903**
Appearance	Powder or agglomerates	Powder with agglomerates	Slightly agglutinated powder	Slightly agglutinated powder	Powder
Color	White to off white	White	White	White	White
Identification	RT of standard ± 3%	Complies	Complies	Complies	Complies
Assay (water free) HiMS[a]	Not less than 95.0 w/w %	98.5 %	97.9 %	97.2 %	98.6 %
Assay (water free) Lacto-N-neotetraose	Not less than 92.0 w/w %	97.1 %	94.4 %	95.0 %	97.2 %
D-Lactose	Not more than 3.0 w/w %	0.25 %	0.66 %	0.38 %	0.21 %
Lacto-*N*-triose II	Not more than 3.0 w/w %	1.01 %	1.62 %	0.65 %	0.65 %
para-Lacto-*N*-neohexaose	Not more than 3.0 w/w %	0.13 %	0.95 %	1.02 %	0.45 %
LNnT fructose isomer	Not more than 1.0 w/w %	0.03 %	0.40 %	0.41 %	0.29 %
pH (20°C, 5 % solution)	4.0 – 7.0	5.3	5.8	5.4	6.0
Water	Not more than 9.0 w/w %	6.6 %	8.0 %	7.8 %	7.6 %
Ash, sulfated	Not more than 1.5 w/w %	< 0.03 %	< 0.01 %	0.03 %	<0.01 %
Methanol	Not more than 100 mg/kg	57 mg/kg	19 mg/kg	32 mg/kg	22 mg/kg
Isopropanol	Not more than 200 mg/kg	76 mg/kg	29 mg/kg	134 mg/kg	173 mg/kg
Residual proteins	Not more than 0.01 w/w %	<LOQ[b]	<LOQ[c]	<LOQ[b]	<LOQ[b]
Heavy metals					
Lead	Not more than 0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg	<0.1 mg/kg
Microbiological Parameters					
Total plate count	Not more than 500 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Yeasts	Not more than 10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Molds	Not more than 10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Enterobacteriaceae	Absent in 10 g	0.215 EU/mg	0.0015 EU/mg	0.035 EU/mg	0.0003 EU/mg

Table II.C.2-1 Batch Analyses for LNnT					
Parameter	**Specification**	**Manufacturing Batch Number**			
		5247750801	**2547750901**	**2547750902**	**2547750903**
Salmonella	Absent in 25 g	Complies	Complies	Complies	Complies
Cronobacter (Enterobacter) sakazakii	Absent in 10 g	Complies	Complies	Complies	Complies
Listeria monocytogenes	Absent in 25 g	Complies	Complies	Complies	Complies
Bacillus cereus	Not more than 50 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Residual endotoxins	Not more than 10 EU/mg	Complies	Complies	Complies	Complies

CFU = colony forming units; EU = endotoxin units; HiMS = human-identical milk saccharides; LNnT = lacto-*N*-neotetraose; LOQ = Limit of Quantitation; RT = retention time.
[a] Human-identical milk oligosaccharides is defined as the sum of LNnT, lactose, lacto-*N*-triose II, and para-lacto-*N*-hexaose.
[b] LOQ = 0.0017%.
[c] LOQ = 0.0005%.

II.C.3 Additional Quantitative and Qualitative Analyses

II.C.3.1 Chemical/Structural Confirmation – NMR and MS

LNnT produced by fermentation with *E. coli* K-12 MP572 is chemically identical to LNnT that is present in human breast milk and to LNnT produced by chemical synthesis described in GRN 547 (Glycom A/S, 2014). The chemical identity was confirmed using the same methods described previously in GRN 547 and included analyses by a combination of ^{1}H-, ^{13}C-nuclear magnetic resonance (NMR), and mass spectrometry techniques.

II.C.3.1 Carbohydrate Impurity Profile

Batch analyses from multiple lots of LNnT produced by fermentation have been analyzed using HPLC-cCAD and all batches exhibit comparable impurity profiles, thereby demonstrating consistency of the manufacturing process. Figure II.C.3-1 shows a representative impurity profile for LNnT, as measured by HPLC using a Corona Charged Aerosol Detector (HPLC-cCAD) and Figure II.C.3-2 presents the results of three batches analyzed for impurities. As shown in Figure II.C.3-1 small amounts of carbohydrate-type impurities may be detected in the LNnT ingredient; however, these compounds are human-identical milk oligosaccharides (HiMOs) themselves or fall into the general structural patterns observed in HMOs. Such carbohydrate-type compounds that may form during the fermentation process include lacto-*N*-triose II, *para*-lacto-*N*-neohexaose, and LNnT fructose isomer. Isomerization of carbohydrates is also known as the Lobry de Bruyn–van Ekenstein transformation (Angyal, 2001; Wang, 2010). This type of isomerization is pH and temperature dependent and has been commonly reported for the closely related conversion of D-lactose into D-lactulose during heat treatment [*i.e.*, ultra-high temperature (UHT) processing and pasteurization] of milk, including human

donor milk (Beach and Menzies, 1983; Schuster-Wolff-Bühring *et al.*, 2010; Gómez de Segura *et al.*, 2012). Although the presence of the isomerization product of LNnT has not been specifically evaluated in heat-treated human donor milk, D-lactulose has been detected at significant proportions of D-lactose (Gómez de Segura *et al.*, 2012). Therefore, it can be reasonably assumed that the LNnT fructose isomer is present at comparable ratios to that of heat-treated human milk and can therefore be equally regarded to have a history of safe consumption.

Specifications for these carbohydrate-type compounds have been established and in-process controls (*i.e.*, for temperature and pH) and purification steps (*i.e.*, chromatography) have been included in the manufacturing process in order to minimize the formation of these substances. The results of batch analyses (Table II.C.3-2) demonstrate that concentrations of each compound do not exceed 2% of the total ingredient, and at such low levels of concentration, they are not anticipated to have any significant effect on the safety or nutritive value of the LNnT ingredient produced by Glycom.

Figure II.C.3-1 Representative Impurity Profile for LNnT, as Measured by High Performance Liquid Chromatography with a Corona Charged Aerosol Detector

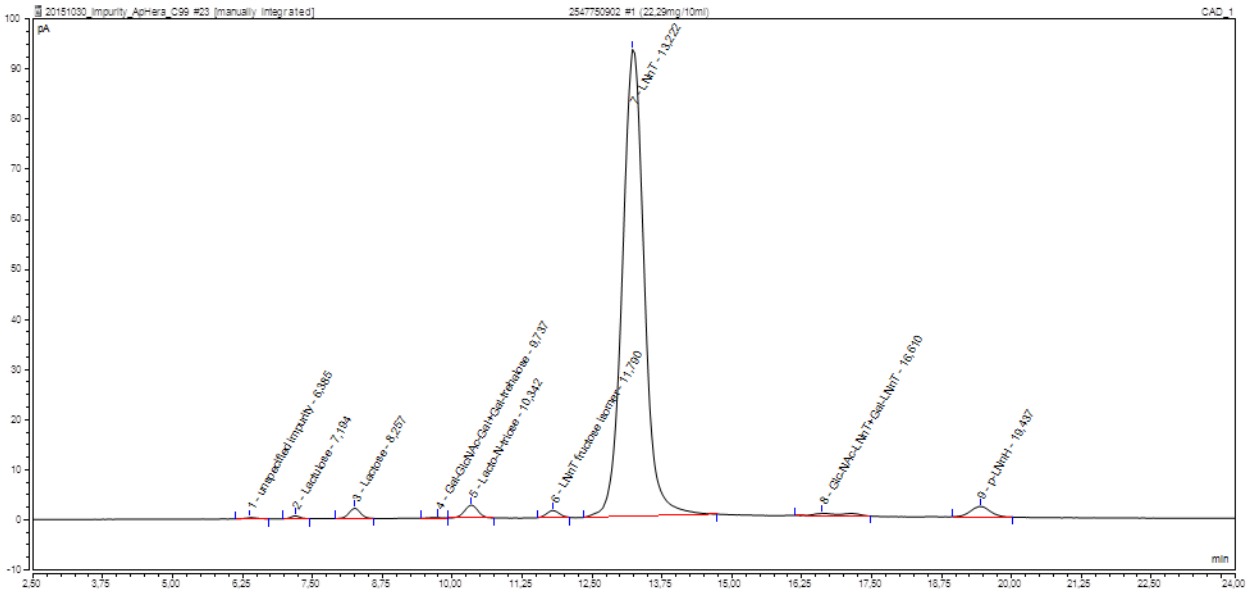


Figure II.C.3-2 Impurity Profile for Three Batches of LNnT, as Measured by High Performance Liquid Chromatography with a Corona Charged Aerosol Detector

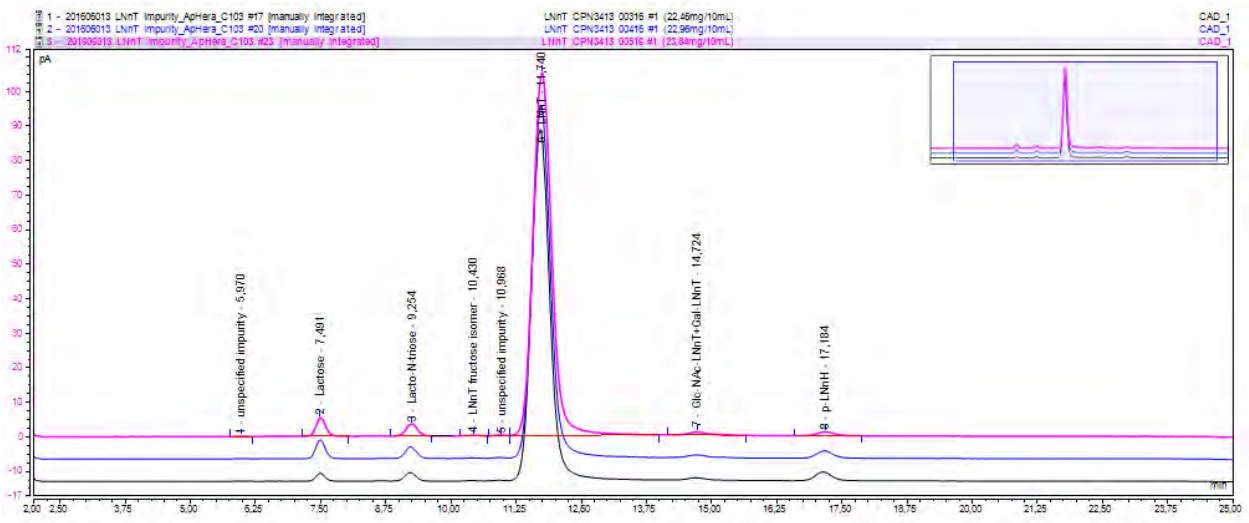


Table II.C.3-2 Carbohydrate-Type Impurities in the Lacto-*N*-neotetraose (LNnT) Ingredient

Parameter	Specification	Fermented LNnT			
		Batch Results			
		2547750801	2547750901	2547750902	2547750903
D-Lactose (water-free)	Max. 3.0 % w/w	0.25 %	0.66 %	0.38 %	0.21 %
Lacto-*N*-triose II (water free)	Max. 3.0 % w/w	1.01 %	1.62 %	0.65 %	0.65 %
Para-LNnH	Max. 3.0 % w/w	0.13 %	0.95 %	1.02 %	0.45 %
LNnT fructose isomer (water free)	Max. 1.0 % w/w	0.03 %	0.40 %	0.41 %	0.29 %
Lactulose	Max. 0.3 % w/w	<0.03 %	0.23 %	0.11 %	0.06 %
Gal-LNnT + GlcNAc-LNnT	Max. 0.6% w/w	0.08 %	0.21 %	0.41 %	0.15 %
Gal-GlcNAc-Gal + 3-Gal-trehalose	Max. 0.6% w/w	0.04 %	0.03 %	0.04 %	0.03 %
p-LNnH fructose isomer	Max. 0.3% w/w	<0.03 %	<0.03 %	<0.03 %	<0.03 %
Total unspecified impurities by HPLC	Max. 1.5 % w/w	na	0.06 %	0.06 %	<0.03 %

HPLC = high performance liquid chromatography; LNnT = lacto-*N*-neotetraose; Max. = maximum; na = not available.

II.C.4 Manufacturing Impurities and Contaminants

II.C.4.1 Amino Acids and Biogenic Amines

LNnT is secreted into the fermentation broth and no disruption of the production microorganism is required during manufacture. Although LNnT is harvested without a specific lysis step, production batches have been analyzed for secondary metabolites and cellular components that

may originate from the fermentation medium. Various theoretical cell metabolism impurities produced during fermentation (*e.g.*, glutamic acid, GABA, histamine, tyramine, spermidine, cadaverine and putrescine) were not present at detectable levels in the final ingredient using sensitive HPLC based analyses methods (data not shown).

II.C.4.2 Microbial Endotoxins

The host strain, *E. coli* K12, is a gram-negative bacterium and these bacteria possess complex glycolipids of high molecular weight, called lipopolysaccharides (LPS), in their cell membrane. Internal specifications for LPS have been established as an additional quality control point to ensure that any microbial endotoxins are efficiently removed and/or not introduced during the production process. The endotoxin specification established for LNnT is assayed using the *Limulus* amebocyte lysate kinetic chromogenic assay described in the European Pharmaceopoeia. Batch analyses of LNnT demonstrate compliance to the endotoxins specifications.

II.C.4.3 Residual Protein

The transfer of residual protein from the fermentation process into the finished LNnT ingredient is significantly reduced by the use of ultrafiltration, adsorption (*e.g.*, activated carbon treatment) and multiple crystallization/wash steps employed during the manufacturing process. LNnT has been evaluated for protein content using a modified Bradford method with a quantification limit of 0.0017% and a residual protein specification of no more than 0.01% has been established. Based on the results of batch analyses, no detectable levels of protein are observed in the finished LNnT ingredient.

II.C.4.4 Absence of Production Organism

The production microorganism is efficiently removed by the ultrafiltration step during USP, which is applied directly after fermentation. Additionally, during DSP, various sequential purification processes are applied to ensure purity. The overall purification process comprises of the following steps:

1. Ultrafiltration/diafiltration
2. Nanofiltration
3. Optional microfiltration
4. Ion removal
5. Decoloration (*e.g.*, active charcoal filtration)
6. Microfiltration
7. Optional pre-concentration
8. Chromatography
9. Pre-concentration

10. Crystallization
11. Solid-liquid-separation (*e.g.*, filtration)
12. Washing; and
13. Drying and Milling.

The absence of the microorganisms in the ingredient is demonstrated by microbial testing for *Enterobacteriaceae* during batch analyses according to internationally-recognized methods (ISO 21528-1:2004, MSZ ISO 21528-2:2007). The ISO 21528-1:2004 method includes a pre-enrichment step to allow for resuscitation of the microorganism before enrichment. Additionally, the ISO 7251:2005 method for analysis of *E. coli* also has been applied to production batches of the ingredient during QC testing to further corroborate the absence of *Enterobacteriaceae*.

Finally, the absence of the production organism in the finished ingredient is also supported by analyses for residual DNA in final production batches. As demonstrated in Table II.C.4.4-1, the absence of residual DNA from the production organism is confirmed by 3 different validated quantitative PCR (qPCR) methods. These qPCR methods target short subsequences of the inserted genes as well as a short subsequence of the multicopy operon encoding the 23S ribosomal subunit of *E. coli*. Analysis of 4 batches of LNnT demonstrate no detectable levels of residual DNA (limit of detection of 0.004 mg/kg) present in the final ingredient.

Table II.C.4.4-1 Levels of Residual DNA in 4 Batches of LNnT Produced by Fermentation

Parameter	Specification	Average Batch Result	2547750801	2547750901	2547750902	2547750903
Residual DNA by qPCR (*gene 1* assay)	<LOQ[a]	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Residual DNA by qPCR (*gene 2* assay)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Residual DNA by qPCR (EC23S assay)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

LNnT = lacto-*N*-neotetraose; LOQ = limit of quantitation; qPCR = quantitative polymerase chain reaction.
[a] LOQ = 0.004 mg/kg

II.C.4.5 Additional Analyses

LNnT is produce by fermentation and therefore may result in the concentration of trace elements and minerals as carry-over from the fermentation medium. However, the use of ion-exchange filtration, or alternatively, electrodialysis, are sufficient to reduce any appreciable carry-over of minerals from fermentation into the final ingredient. The results of trace element analyses are presented in Table II.C.4.5-1 below.

Table II.C.4.5-1 Presence of Trace Elements in Lacto-*N*-Neotetraose (LNnT) Produced by Fermentation

Trace Element	LNnT Internal Specification	Batch Results			
		2547750801	2547750901	2547750902	2547750903
Phosphorus (as orthophosphate) (%)	Max. 0.5	<0.0010	<0.0010	<0.0010	<0.0010
Chloride (Cl) (%)	Max. 0.1	<0.0010	<0.0010	<0.0010	<0.0010
Sodium (Na) (mg/kg)	Max. 5,000	<10	<10	<10	<10
Potassium (K) (mg/kg)	Max. 5,000	<10	<10	<10	<10
Magnesium (Mg) (mg/kg)	Max. 3,000	<10	<10	<10	<10
Calcium (Ca) (mg/kg)	Max. 1,000	<10	<10	<10	<10
Iron (Fe) (mg/kg)	Max. 20	3	<1	<1	<1
Zinc (Zn) (mg/kg)	Max. 20	0.3	0.2	0.2	0.4
Copper (Cu) (mg/kg)	Max. 20	<0.1	<0.1	<0.1	<0.1
Manganese (Mn) (mg/kg)	Max. 10	<0.1	<0.1	<0.1	<0.1
Aluminum (Al) (mg/kg)	Max. 10	<2	<2	<2	<2
Chromium (Cr) (mg/kg)	Max. 3.0	0.2	<0.1	<0.1	<0.1
Molybdenum (Mo) (mg/kg)	Max. 1.0	0.1	<0.1	<0.1	<0.1
Cobalt (Co) (mg/kg)	Max. 1.0	<0.1	<0.1	<0.1	<0.1
Arsenic (As) (mg/kg)	Max. 0.4	<0.1	<0.1	<0.1	<0.1
Cadmium (Cd) (mg/kg)	Max. 0.05	<0.01	<0.01	<0.01	<0.01
Mercury (Hg) (mg/kg)	Max. 0.2	<0.01	<0.01	<0.01	<0.01

LNnT = lacto-*N*-Neotetraose; Max. = maximum

II.D Stability

II.D.1 Bulk Stability

LNnT produced by fermentation is chemically identical to LNnT produced by chemical synthesis (previously notified under GRN 547), and crystalline material from both processes exhibit identical crystal forms (crystal morphology and crystalline space group). No differences in the stability profile between the ingredient described herein and the chemically synthesized equivalent is anticipated. Bulk stability data from the chemically synthesized ingredient is incorporated by reference to GRN 547 (Glycom A/S, 2014). The results of the 5-year real time stability study, two 2-year accelerated stability studies, and the interim results of a 5-year real time stability study indicate that there are no changes in organoleptic properties of LNnT, no appreciable degradation of LNnT, no changes in impurity profile, and no alterations in the microbiological quality of the ingredient following storage for up to 5 years under ambient storage conditions and for up to 2 years under accelerated conditions.

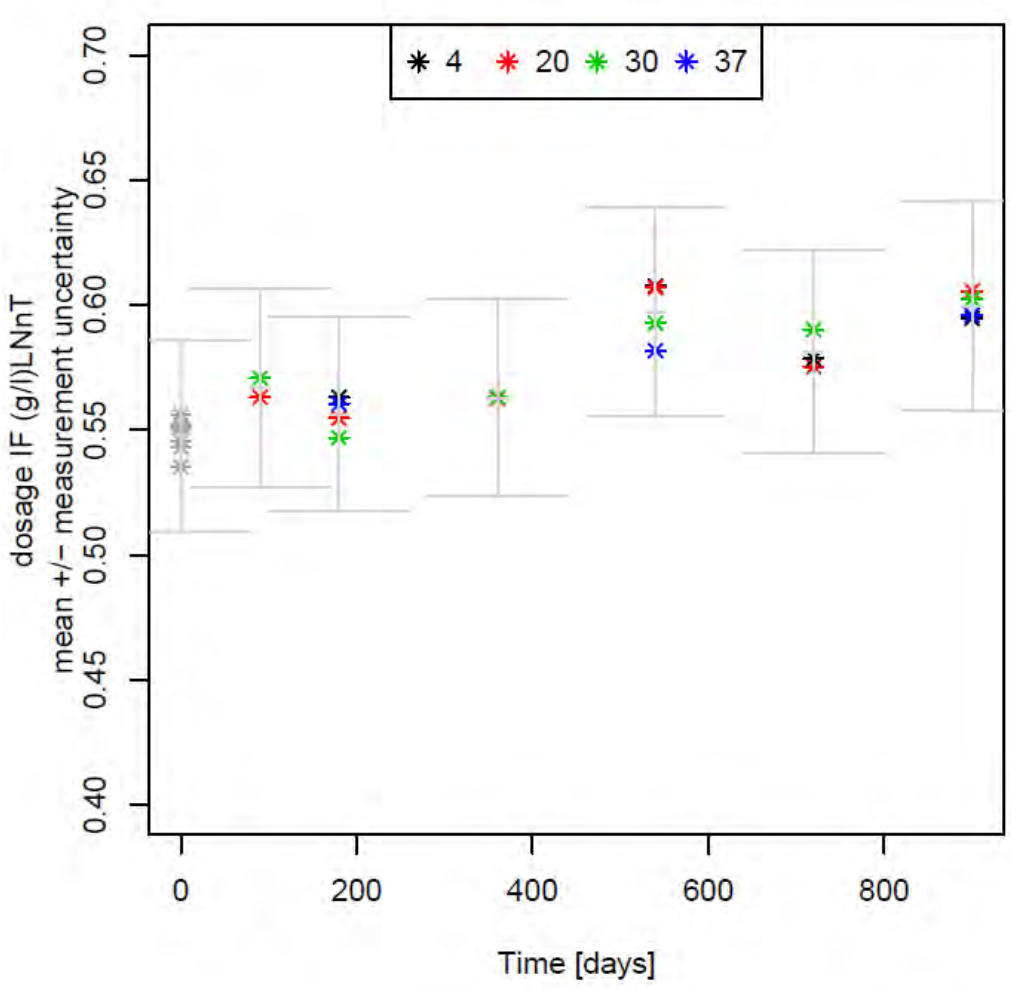
II.D.2 Stability Under the Intended Conditions of Use

II.D.2.1 Stability in Powdered Infant Formula

The stability of LNnT in combination with 2'-*O*-fucosyllactose (2'-FL) in infant formula has been previously investigated in long-term studies. Briefly, 3 independently formulated, commercially representative infant formula powders containing a target concentration of 0.45 g LNnT and 0.90 g 2'-FL per 100 g (dry matter) of infant formula[3] respectively were subjected to typical production processing steps and stored in gassed (N_2/CO_2) tin cans (1 can per time and temperature point) at temperatures of 4, 20, 30, or 37°C. LNnT content was measured at regular time intervals for up to 900 days of storage. No significant loss of LNnT was detected when added to infant formula powder prior to processing and subject to the different storage conditions. The results from one production batch are presented in Figure II.D.2.1-1 below.

[3] For details regarding the composition of the infant formula, please refer to GRN 547.

Figure II.D.2.1-1 Concentration of LNnT in a Commercially Representative Infant Formula Following Storage for Up to 900 Days at Increasing Temperatures



II.D.2.3 Stability in Other Food Matrices

The stability of LNnT has been evaluated in representative food products, including yogurts, ready-to-drink flavored milk, and citrus fruit beverages. LNnT content was measured by HPLC with fluorescent detection in the foods at pre-processing, and following food processing[4] for up to 28 days of storage at 4°C. The results of the tests are incorporated by reference to Section II.D.2 of GRN 547 and demonstrate that there was no loss of LNnT in any of the food matrices examined (Glycom A/S, 2014).

[4] Processes included pasteurization and ultra-high temperature processing.

III. SELF-LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with Glycom's LNnT ingredient.

IV. BASIS FOR GRAS DETERMINATION

Glycom's determination that LNnT, as described herein, is GRAS for its intended uses in non-exempt term infant formula and conventional food and beverage products, is based on scientific procedures. The safety of LNnT is principally established through a history of human consumption (Section IV.C) on the basis that LNnT manufactured by Glycom is chemically equivalent to LNnT in human milk, and as a nutritive component of human milk can be considered GRAS for addition to infant formula at quantitatively similar concentrations. The safety of LNnT is corroborated by published and unpublished studies characterizing the toxicity and mutagenicity/genotoxicity of LNnT (as manufactured by Glycom using chemical synthesis and fermentation) in weanling rats (Section IV.E) and findings from safety and tolerance studies in infants administered infant formula supplemented with LNnT (Section IV.F). The aforementioned data and information also has been the subject of comprehensive critical and independent evaluation by a Panel of qualified experts (GRN 547) and by authoritative bodies including the FDA (2015c) and the European Food Safety Authority (EFSA, 2015a); these independent experts have similarly concluded that LNnT manufactured by Glycom is GRAS for addition to infant formula at a use level of up to 600 mg/L and in specified conventional food and beverage products at use levels providing between 0.02 to 0.68 g/serving.

For the purposes of identifying any new data relevant to the safety of LNnT published since Glycom's previous GRAS determination, a comprehensive search of the published scientific literature was conducted in March 2016. The search was conducted using the electronic search tool, ProQuest, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS reviews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. A discussion of all newly available published and unpublished studies, both favorable and unfavorable, is provided throughout Section IV. Furthermore, information on the safety of the production strain and associated biotechnological modifications are discussed in Section IV.G.

IV.A Probable Consumption

Dietary Intake of LNnT Among Infants and Toddlers

LNnT manufactured by Glycom using fermentation is intended for use in non-exempt term infant formula, and baby foods, including toddler formula (Table I.D-1). Potential dietary exposures to LNnT among infant and toddler consumers of term infant formula and baby foods to which LNnT

may be added have been evaluated previously, and the reader is therefore directed to Sections IV.A.1 and IV.A.2 of GRN 547 for detailed summaries of the stratified assessment of LNnT intake by this population group (Glycom A/S, 2014). Considering that LNnT produced by fermentation will serve as an alternative to LNnT described in GRN 547, the proposed uses of LNnT produced by fermentation will not increase dietary exposures in this population group.

The estimated mean and 90th percentile intake of LNnT, based on intended uses in conjunction with infant formula consumption data included in the U.S. National Center for Health Statistics' (NCHS) 2009-2010 National Health and Nutrition Examination Surveys (NHANES), was determined to be 0.51 and 0.73 g/person/day in infants aged 0 to 6 months (USDA, 2012). On a body weight basis, these intakes were determined to be 83.2 and 133.9 mg/kg body weight/day, respectively. In infants 7 to 12 months of age, the estimated mean and 90th percentile all-user intakes of LNnT from infant formulas were determined to be 0.42 g/person/day (48.5 mg/kg body weight/day) and 0.66 g/person/day (79.5 mg/kg body weight/day), respectively (Sections IV.A.1 and IV.A.2 of GRN 547, Glycom A/S, 2014).

Dietary Intake in General U.S. Population from all Proposed Food Uses

Stratified assessments of dietary intake of LNnT among U.S. consumers of various conventional food and beverage products to which LNnT may be added have been conducted previously (*i.e.*, GRN 547). As described in Table I.D-1, the food uses of LNnT have been revised and no longer include applications in baked goods and baking mixes, carbonated beverages, flavored and enhanced waters, coffee and tea, beverage whiteners, fruit flavored drinks and ades, vegetable juices and nectars, and table top sweeteners. Furthermore, the use levels of LNnT in non-dairy yogurt and yogurt have been lowered from 1.2 g/serving to a maximum proposed use level of 0.6 g/serving (2.67 g/kg). Estimates for the daily intake of LNnT were therefore updated to incorporate the lowered uses of LNnT in certain food categories as well as employ the more recently published 2011-2012 NHANES food consumption data. The following sections summarize the estimated mean and 90th percentile daily intake of LNnT among the U.S. population.

Estimates for the intake of LNnT were calculated based on the individual food uses and maximum use levels presented in Table I.D-1 in conjunction with the food consumption data included in the most recent release of NHANES available (USDA, 2014; CDC, 2015). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (U.S. FDA, 2015d). Product-specific adjustment factors were developed based on data provided in the standard recipe file for the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-1996, 1998 survey (USDA, 2000). Estimates for the total daily intake of LNnT from all intended food uses are summarized in Table IV.A-1 on a per person basis by population group. Table IV.A-2 presents these data on a per kilogram body weight basis.

Approximately 85.2% of the U.S. population was identified as potential consumers of foods containing LNnT (designated as “users”). As a result of the high percentage of users identified within all population groups, the intake estimates for the all-person (*i.e.*, all individuals surveyed) and all-user (*i.e.* consumers only) categories were similar; therefore, only the all-user results are discussed in detail.

The mean and 90th percentile intake of LNnT by the all-user population from all intended food uses was estimated to be 304 and 646 mg/person/day, respectively. On a body weight basis, the estimated mean and 90th percentile of intakes were determined to be 8.1 and 16.8 mg/kg body weight/day, respectively.

Among the individual population groups, the highest mean and 90th percentile intakes of LNnT on both an absolute and per body weight basis were identified in toddlers. The mean and 90th percentile of intakes of LNnT in this population group were determined to be 514 mg/person/day (equivalent to 38.4 mg/kg body weight/day), and 901 mg/person/day (equivalent to 67.7 mg/kg body weight/day), respectively.

Elderly adults had the lowest mean all-user intakes on an absolute and per body weight basis of 243 mg/person/day (3.4 mg/kg body weight/day) and also had the lowest 90th percentile intake on a body weight basis of 7.5 mg/kg body weight/day. On an absolute basis, females of childbearing age had the lowest 90th percentile intakes of 526 mg/person/day.

As expected, the deletion of the intended uses of LNnT in select food categories and the reduction of the use levels of LNnT in non-dairy and dairy yogurts resulted in a reduction in overall intake of LNnT among U.S. consumers.

Table IV.A-1 Summary of the Estimated Daily Intake of LNnT from All Proposed Food and Beverage Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Toddlers	1 to 3	510	901	99.3	561	514	901
Children	4 to10	344	648	99.1	1,161	347	648
Female Teenagers	11 to 18	222	529	90.0	513	247	546
Male Teenagers	11 to 18	288	627	90.3	476	319	632
Female Adults of child bearing age	19 to 40	212	500	85.0	702	249	526
Female Adults	19 to 64	202	493	81.6	1,448	248	541
Male Adults	19 to 64	253	634	79.9	1,318	317	680
Elderly Adults	65 and up	214	513	88.2	818	243	550
Total Population	All Ages	259	609	85.2	6,595	304	646

LNnT = lacto-*N*-neotetraose; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Table IV.A-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of LNnT from All Proposed Food and Beverage Uses in the U.S. by Population Group (2011-2012 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Toddlers	1 to 3	38.1	67.7	99.3	558	38.4	67.7
Children	4 to10	13.6	27.1	99.1	1,161	13.7	27.1
Female Teenagers	11 to 18	4.1	9.3	89.9	506	4.6	10.2
Male Teenagers	11 to 18	4.9	10.8	90.2	473	5.4	11.1
Female Adults of child bearing age	19 to 40	3.1	7.4	84.9	692	3.6	8.0
Female Adults	19 to 64	2.9	7.1	81.7	1,429	3.5	7.6
Male Adults	19 to 64	3.0	7.3	79.8	1,309	3.8	8.1
Elderly Adults	65 and up	3.0	6.9	88.1	806	3.4	7.5
Total Population	All Ages	6.9	14.6	85.2	6,542	8.1	16.8

bw = body weight; LNnT = lacto-*N*-neotetraose; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

IV.B History of Safe Consumption

HMOs, such as LNnT, are biosynthesized exclusively in the lactating mammary gland and secreted into breast milk. LNnT is an important member of the complex oligosaccharide mixture that is present in human milk; it is among the most abundant HMOs, and is the second-most

abundant core-structure, with a high number of HMOs derived by addition of L-fucose and/or sialic acid to the LNnT core-structure (Urashima *et al*., 2012).

As discussed previously in GRN 547, the LNnT content of human milk has been reported in several publications from independent research groups, whereby extensive data have been provided according to Secretor and Lewis-blood group status (Thurl *et al.*, 1997, 2010; Galeotti *et al.*, 2012), ethnicity (Erney *et al.*, 2000), lactation period (Coppa *et al.*, 1999; Erney *et al.*, 2000; Sumiyoshi *et al.*, 2003; Asakuma *et al.*, 2008; Leo *et al.*, 2010; Gabrielli *et al.*, 2011; Bao *et al.*, 2013), term/preterm birth (Nakhla *et al.*, 1999; Gabrielli *et al.*, 2011), and the average content of mature milk (Chaturvedi *et al.*, 1997, 2001a; Asakuma *et al.*, 2011). Based on all available data, the range of average concentrations of LNnT in pooled[5] mature breast milk (from full-term birth mothers at approximately lactation days 5 to 100) is reported as being between 110 and 630 mg/L (reviewed in Section IV.B.1 of GRN 547).

Based on the concentrations of LNnT in mature human breast milk as detailed above and based on a 6.5-kg infant consuming approximately 1 L of breast milk per day (Davies *et al.*, 1994; Hester *et al.*, 2012), the intake of LNnT from mature breast milk is calculated to be approximately 20 to 100 mg/kg body weight/day. Considering that Glycom's LNnT is chemically and structurally identical to the LNnT present in human breast milk, the background dietary range of LNnT intakes from mature human breast milk in infants serves as the safe range of Glycom's LNnT intakes for infants. In addition, considering that infants are a susceptible population group from a safety perspective [Scientific Committee on Food (SCF, 1998)], it may be concluded that this background dietary range of LNnT intakes in infants also would be safe for older population groups. This background exposure to LNnT therefore serves as the safe reference range of Glycom's LNnT intakes for all population groups.

IV.C Case-of-Need for Infant Formula

The presence of HMOs, including LNnT, in breast milk has been associated with a variety of nutritional effects including the following: establishment and maintenance of healthy intestinal bacterial microflora that is rich in *Bifidobacteria* (Bode, 2009, 2012; Chichlowski *et al.*, 2011, 2012; Jantscher-Krenn and Bode, 2012; Wang *et al.*, 2015); reducing the adhesion of pathogens to the intestinal wall (Andersson *et al.*, 1986; Zopf and Roth, 1996; Idänpään-Heikkilä *et al.*, 1997; Newburg, 2000; Newburg *et al.*, 2005; Bode *et al.*, 2012; Hester *et al.*, 2013; Li *et al.*, 2014); modulating the maturation of intestinal enterocytes and epithelial cells (Kuntz *et al*., 2008, 2009; Holscher *et al*., 2014); providing nutritional support to the neonatal immune system (Newburg and Walker, 2007; Newburg, 2009), and potentially supporting the maintenance of normal cognitive, learning and memory functions of the brain (Matthies *et al.*, 1996; Murrey and Hsieh-Wilson, 2008; Mountford *et al.*, 2015; Vázquez *et al.*, 2015). For more in-depth reviews,

[5] Data from "pooled milk" refers to data obtained from actual pooled milk samples from different women with varying milk types or to an average value calculated from individual values obtained from different women with varying milk types.

the reader is directed to the review/proceedings of the first "*International Conference on Glycobiology of Human Milk Oligosaccharides",* which was held in Copenhagen in May 2011 (Kunz *et al.*, 2012), or to a more recent review by Smilowitz *et al*. (2014).

LNnT is an important member of the complex oligosaccharide mixture that is present in human milk; it is among the most abundant HMOs, and is the second-most abundant core-structure, with a high number of HMOs derived by addition of L-fucose and/or sialic acid to the LNnT core-structure (Urashima *et al.*, 2012). The addition of LNnT to term infant formulas is therefore supported on a teleological basis and is consistent with efforts to produce infant formula that closely match the nutrient composition of human milk.

IV.D Absorption, Distribution, Metabolism, and Excretion

HMOs, including LNnT, do not undergo any significant digestion in the upper gastrointestinal tract (see GRN 547 for a comprehensive review of the metabolic fate of LNnT). The results of several *in vitro* experiments using simulated gastrointestinal tract conditions indicate that a large proportion of an ingested amount of HMOs goes undigested in the intestinal tract (see Engfer *et al.*, 2000; Gnoth *et al.*, 2000), and therefore, only small amounts of monosaccharides derived from HMOs would be available for absorption (absorption of saccharides occurs at the monosaccharide level).

Studies in infants suggest that some HMOs are absorbed intact to a certain extent, and a small portion of the ingested amount is excreted unchanged in the urine (approximately 1 to 2% of the total administered amount) (Rudloff *et al*., 1996, 2006, 2012; Obermeier *et al.*, 1999; Chaturvedi *et al.*, 2001b; Rudloff and Kunz, 2012; Dotz *et al*., 2014). The mechanism by which HMOs are absorbed intact has not yet been fully elucidated; however, data from *in vitro* studies using the Caco-2 human intestinal epithelial cell model suggest that neutral HMOs, such as LNnT, are transported transcellularly across the intestinal epithelium by receptor-mediated transcytosis, as well as by paracellular means (Gnoth *et al*., 2001). Acidic HMOs were determined to be absorbed by paracellular transport (Gnoth *et al*., 2001). LNnT, specifically, has been detected unchanged in the plasma and urine of breast-fed infants; furthermore, plasma levels of LNnT are positively correlated with concentrations in breast milk, indicating that LNnT is among the HMOs absorbed intact by breastfed infants (Goehring *et al.*, 2014).

While the precise proportion of ingested HMO that is orally absorbed has not been quantified, the "relative concentrations" of HMOs in plasma and urine (*i.e.,* concentrations relative to breast milk) indicate that intact HMOs are absorbed to a certain extent and detected at relative fractions of approximately 4% in the urine and 0.1% in the plasma (Goehring *et al.*, 2014). Furthermore, the results of infant studies analyzing HMO digestion by intestinal bacterial microflora and HMO fecal excretion indicate that the proportion absorbed would be relatively small. The data indicate that a large proportion of an ingested physiological amount of HMOs by infants undergoes fermentation by the intestinal bacterial microflora as assessed by the

lactulose hydrogen breath test (Brand-Miller *et al.*, 1995, 1998). Specifically, the proportion subjected to fermentation was estimated to be on average 100% following a loading test in infants with the purified HMO fraction isolated from their respective mother's milk (Brand-Miller *et al.*, 1998). In other studies, HMOs, including LNnT, were detected unchanged (by HPLC) in fecal samples of infants at total levels amounting to 40 to 50% of the ingested amount following consumption of breast milk (Chaturvedi *et al.*, 2001b; Coppa *et al.*, 2001). While some discrepancy exists among these studies regarding the proportion of ingested HMOs that is excreted in the feces *versus* the proportion that is fermented by the intestinal microflora, the available data indicate that the majority of an ingested amount of an HMO, including LNnT, reaches the large intestine where the HMO serves as a substrate for bacterial (intestinal bacterial microflora) metabolism or is excreted unchanged in the feces.

IV.E Toxicological Studies

The general recognition of safety of LNnT under the specified conditions of use in term infant formula and conventional food and beverage products is largely based on published studies characterizing the concentrations of LNnT in human milk (see Section IV.B), the corresponding history of safe consumption of LNnT by breast-feeding infants, and upon data demonstrating that LNnT produced by fermentation is of high purity and is chemically equivalent to human LNnT.

The results of published and unpublished toxicological studies in neonatal and mature rats further corroborate the safety of the ingredient. Comprehensive discussions of the published toxicity studies as they apply to the safety of LNnT for use in infant formula and foods are incorporated by reference to Section IV.B.5 of GRN 547, and are briefly summarized below in Section IV.E.1.

New studies relevant to the safety of LNnT include a 90-day oral toxicity study, an *in vitro* bacterial reverse mutation assay, and an *in vitro* micronucleus assay conducted using LNnT produced by fermentation by Glycom as described herein. Findings from these studies are consistent with available published data characterizing the toxicity of LNnT and therefore provide additional evidence to corroborate safety of this ingredient for use in infant formula and food. No new studies were identified from a recent literature search that would suggest that LNnT may be unsafe for consumption.

IV.E.1 Repeated Dose Toxicity

Studies Described Previously in GRN 547

The potential toxicity of LNnT manufactured by Glycom using chemical synthesis has been investigated in a 14-day dose range finding study, a subacute (28-day) oral toxicity study, and a subchronic (90-day) oral toxicity study in neonatal rats administered LNnT by gavage (Coulet *et*

al., 2013) (see Section IV.B.5 of GRN 547 for comprehensive discussions on the toxicological data). The subacute and subchronic studies were conducted consistent with the Organization for Economic Co-operation and Development (OECD) Test Guidelines 407 and 408, respectively, adapted to include the use of juvenile Wistar [Crl:WI(Han)] rats to better reflect the use of the ingredient for consumption by infants. In the 14-day dose range finding study, the subacute oral toxicity study, and the subchronic oral toxicity study, rats were administered LNnT by gavage at doses of 0 (water vehicle control), 1,000, 2,000, or 5,000 mg/kg body weight/day. A reference control group was administered 5,000 mg/kg body weight/day of oligofructose. In both the 14-day and subacute study, LNnT was well-tolerated at doses of up to 5,000 mg/kg body weight/day, with the study investigators noting only transient soft, colored/liquid feces in the high-dose group during the first few days of the administration period in the subacute study.

Similarly in the subchronic study, LNnT was well-tolerated with soft, liquid, yellow-colored feces observed in both the reference control group and the high-dose group (Coulet *et al.*, 2013). Several statistically significant changes in hematological and clinical chemistry parameters were observed between the LNnT groups compared with the controls; however, these were deemed by the study investigators to be slight in nature and not toxicologically significant, and/or did not exhibit a dose response or lacked histopathological correlative findings. The study investigators established a no observed adverse effect level (NOAEL) of 5,000 mg/kg body weight, the highest dose tested, based on the results of the study.

These studies were submitted in support of a novel food application submitted to the Irish Food Safety Authority and therefore have been recently reviewed by EFSA when drafting their Scientific Opinion for the use of LNnT in infant formula and conventional food products (EFSA, 2015a). EFSA's Panel on Dietetic Products, Nutrition and Allergies stated that "*Based on the observations on reticulocytes, platelet counts, Hb levels and PCV in the high-dose LNnT group (5 000 mg/kg body weight per day) and the decrease in the zymogen content in acinar cells in three animals in the high-dose LNnT group, the Panel considers that the no observed adverse effect level (NOAEL) is 2 500 mg/kg body weight per day*." (EFSA, 2015a). Glycom notes that the reduction in hemoglobin levels considered adverse by EFSA are based on hemoglobin values being 3.2% lower in the high-dose males on day 91 *vs*. control animals. Significant differences in hemoglobin levels were not reported in the female LNnT groups and were not reported in male or female treatment groups on day 28. Platelet count was 9.6% lower ($P<0.05$) in the high-dose males relative to controls; however, no significant differences were reported for females and no differences or trends towards reduced platelet counts were reported in any group at day 28. Therefore, Glycom considered differences in various hematological parameters to be a spurious finding of no biological significance. With respect to reduced zymogen content in pancreatic acinar cells of 3 animals in the high-dose group, the findings were of low severity (slight to minimal), within the normal background variation and not reported in the recovery animals. Glycom considers these findings to be incidental and unrelated to LNnT. Glycom also noted that similar changes in pancreatic acinar cells were not reported in

subsequent subchronic toxicity studies conducted using LNnT produced by fermentation (see following section). Therefore, based on the reasons described above, and in consideration of new toxicity information not previously available to EFSA, it is the opinion of Glycom that the NOAEL of 5,000 mg/kg body weight/day as originally established by Coulet *et al.* (2013) was appropriate.

Lastly, Prieto (2005) described the results of a 28-day and 4-month repeat dose oral toxicity study for LNnT manufactured by a yeast fermentation process. The author reported a NOAEL of 400 mg/kg body weight/day for gavage administration of LNnT (highest dose tested *via* gavage) to 15-day-old Cri:CD®BR rats for 28 days. A NOAEL of 5% LNnT in the diet, the highest dietary concentration tested, was determined in 31- to 37-day-old rats (strain and number not reported) administered LNnT for 4 months (Prieto, 2005). However, details of the study methodology and tabulated results are not reported by Prieto (2005), and this information was previously presented as corroborating information in support of the GRAS determination of LNnT described in GRN 547.

LNnT Produced by Fermentation

The following study was conducted using LNnT (94%; Glycom A/S) produced by fermentation from *E. coli* K12 MP572 and meeting specifications set forth in Table II.C.1-1.

LNnT was evaluated in a subchronic (90-day) oral toxicity study in 7-day-old weanling Wistar [Crl:WI(Han)] rats (Penard, 2016; select results presented in Appendix A). The study was conducted using the same methodology described by Coulet *et al*. (2013) and was performed in accordance with the OECD Principles of Good Laboratory Practices (GLP) and OECD Test Guideline 408 (OECD, 1998a,b). Fructo-oligosaccharide (FOS) was used as the reference control. Rats (10/sex/group) were administered test articles by gavage at doses providing 0 (water vehicle control), 1,000, 2,500, or 5,000 mg/kg body weight/day of LNnT (94.4% LNnT by assay) or the reference compound, FOS (5,000 mg/kg body weight/day) for 90 or 91 days. Recovery groups containing 5 males and 5 females were terminated after a 28-day recovery period. Individual dams with reconstituted litters of at least 5 male and 5 female pups were housed in plastic cages until weaning on post-natal day (PND) 21. After weaning rats were housed separately in plastic cages according to sex and dose group. A standard diet (A04C-10) and water were provided *ad libitum*. Animals were observed twice daily for mortality and morbidity, and clinical observations were performed daily. A detailed clinical examination was performed weekly. Body weights were assessed at time of randomization, prior to dosing, twice weekly during the first 8 weeks of the administration period, and then once weekly thereafter. Food intake also was measured twice weekly after weaning and for the first 6 weeks post-weaning, and then once weekly thereafter. Ophthalmological examinations were performed on all animals from the control, high-dose LNnT, and FOS groups during the last week of administration (day 90). Fasting blood and urine samples were collected from all animals of all groups for clinical pathology analysis (*i.e.*, hematology, coagulation, clinical chemistry, and

urinalysis) at the end of the administration period. Physical development endpoints included pinna unfolding (evaluated daily from PND 2 until confirmation), eye opening (evaluated daily from PND 12 until confirmation), incisor eruption (evaluated daily from PND 7 until confirmation), and left tibia length (evaluated weekly from PND 7 onward) for all pups. For females, day of vaginal opening and corresponding body weight on the day of the occurrence were recorded. In males, detection of the day of balano preputial skinfold cleavage and body weight on the day of the occurrence was evaluated daily from PND 38 until detection. Reflex tests were conducted on all pups and assessments of neurological development were evaluated by water maze (on weeks 8 or 9) and open field test (on week 10). Complete necropsy was performed and selected organs were removed and weighed for all animals at the end of the treatment period or at the end of the 4-week recovery period. Histopathological examinations of select organs and tissues were performed for all early decedents and animals administered the vehicle control or high-dose LNnT.

No test article-related mortalities occurred during the study. Isolated occurrences of hypersalivation were noted in 1 high-dose male and 3 high-dose females receiving LNnT but were considered by the study investigators to be unrelated to LNnT administration. No test article-related ophthalmological findings were observed. No remarkable effects in body weight, body weight gain, or food consumption were observed. No toxicologically relevant effects in tibia length, reflex and physical development, time to sexual maturation, learning capacity, memory, motor activity (as evaluated in the Morris water maze), exploratory behavior, or general movement (as evaluated in the open-field test) were observed at any dose level.

Statistically significant differences in hematology parameters were noted[6], however lacked a dose-response relationship, were minimal in magnitude (*i.e.*, ~10% or less), and were considered unrelated to LNnT administration (see Appendix A for a summary of hematological results). Similarly, no test article-related effects in serum clinical chemistry parameters were observed[7] and any statistically significant differences in serum clinical chemistry parameters were considered to be unrelated to LNnT administration.

A statistically significant increase in urine volume in high-dose animals and a statistically significant decrease in specific gravity were noted in high-dose animals compared with controls. However, these were deemed by the study investigators to be incidental and of no toxicological relevance due to the lack of dose-response or histopathological changes in the kidney. No treatment-related differences in organ weights, macroscopic observations, or histological observations were observed between rats receiving LNnT and the control and reference groups.

[6] These changes included a decrease activated partial thromboplastin time in high-dose males and females (≤10.1%), decreases in fibrinogen in low-, mid-, and high-dose males and high-dose females (≤12.6%), decrease in red blood cell count in low-dose females (≤6.0%), and decreases in absolute (but not relative) reticulocytes in females receiving LNnT and FOS (≤10.3%).

[7] These changes included decreases in mean total bilirubin concentration in low-, mid-, and high-dose males and low-dose females, decreases in calcium in low-dose males, decrease in glucose in low-dose females, and an increase in creatinine in high-dose females. See Appendix A for a summary of results.

A NOAEL of 5,000 mg/kg body weight/day, the highest dose tested, was determined based on the results of this study.

IV.E.2 Genotoxicity

Studies Described Previously in GRN 547

The potential mutagenicity of LNnT produced by chemical synthesis was evaluated in the bacterial reverse mutation assay in *Salmonella Typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 in the presence or absence of metabolic activation (S9) using the plate-incorporation and pre-incubation methods (Coulet *et al.*, 2013; previously summarized in Section IV.B.5.3 of GRN 547). Based on the results of the study, LNnT was determined to be non-mutagenic in the bacterial reverse mutation assay up to the limit concentration of 5,000 µg/plate.

LNnT also was non-mutagenic in an *in vitro* mammalian cell gene mutation test in L5178Y tk+/- mouse lymphoma cells (Coulet *et al.*, 2013; previously summarized in Section IV.B.5.3 of GRN 547). In the study, no statistically or biologically significant increases in the frequency of mutations were observed in cells treated with LNnT, with or without S9, at concentrations of up to 4,250 µg/mL.

LNnT Produced by Fermentation

The following studies were conducted using LNnT (94%; Glycom A/S) produced by fermentation from *E. coli* K12 MP572 and meeting specifications set forth in Table II.C.1-1.

LNnT was evaluated in the bacterial reverse mutation assay using *S. Typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *E. coli* WP2uvrA in the presence or absence of metabolic activation (S9), using the plate incorporation and pre-incubation methods (Verspeek-Rip, 2016). The study was conducted in accordance with the OECD principles of GLP and OECD Test Guideline No. 471 (OECD, 1997, 1998a). The water vehicle served as a negative control for all strains. Positive controls assays conducted in the absence of S9 included 2-nitrofluorene (TA98, TA1537, pre-incubation assay), methylmethanesulfonate (TA100), sodium azide (TA1535), ICR-191 (TA1537, direct plate assay), 9-aminoacridine (TA1537), or 4-nitroquinoline n-oxide (WP2uvrA). For assays conducted in the presence of S9, 2-aminoathracene was used.

Using the plate incorporation method, bacterial strains were treated with LNnT at concentrations of 52, 164, 512, 1,600, or 5,000 µg/plate in the presence or absence of S9. For the pre-incubation method, bacterial strains also were incubated with LNnT at concentrations of 52, 164, 512, 1,600, or 5,000 µg/plate in the presence or absence of S9. No cytotoxicity or precipitation was observed. Treatment with LNnT did not result in a biologically significant

increase in the number of revertant colonies compared with the negative control at any concentration in both experiments either in the presence or absence of S9. Positive control agents induced a significant increase in the number of revertant colonies compared to the negative control. LNnT was determined to be non-mutagenic under the conditions of the bacterial reverse mutation assay in the presence or absence of exogenous metabolic activation at concentrations up to 5,000 µg/plate.

The genotoxicity of LNnT also was investigated in an *in vitro* micronucleus assay conducted in cultured peripheral human lymphocytes (Verbaan, 2016). The study was conducted in compliance with the OECD principles of GLP and consistent with OECD Test Guideline No. 487 (OECD, 1998a, 2014). Mitomycin C and colchicine were used as the positive controls in the absence of metabolic activation (S9 mix) and cyclophosphamide was used as the positive control in the presence of S9 mix. Water was used as the negative control. In the short-term exposure experiment, lymphocytes were incubated with LNnT at concentrations of 512, 1,600, or 2,000 µg/mL for 3 hours in the presence or absence of S9, following which the cells were rinsed and incubated for another 24 hours prior to scoring. In the long-term exposure experiment, cells were treated with LNnT at concentrations of 512, 1,600, or 2,000 µg/mL for 24 hours in the absence of S9. At least 1,000 binucleated cells and 1,000 mononucleated were scored for micronuclei under each treatment condition.

In both experiments, there were no signs of precipitation or cytotoxicity (as determined by the cytokinesis block proliferation index) observed in cells treated with LNnT at any concentration. No statistically or biologically significant increases in the frequency of mono- or bi-nucleated cells with micronuclei were observed in cells treated with LNnT. The positive controls produced the expected responses. Thus, LNnT was determined to be non-clastogenic and non-aneugenic in human lymphocytes under the conditions of the assay.

IV.F Human Studies

IV.F.1 Infants

IV.F.1.1 Previous Studies Described in GRN 547

As summarized in Section IV.B.6 of GRN 547, the tolerability of LNnT produced using a yeast fermentation process was reported by Prieto (2005). The study was conducted using 228 healthy male and female infants and toddlers between the ages of 6 to 24 months attending day care centers of the Junta Nacional De Jardines Infantiles of Santiago De Chile (Chile) who were provided infant formula supplemented with LNnT at a use level of 220 mg/L. Infants in the control group received the same formula without LNnT. Formulas were provided for 112 days and study endpoints included measures of oropharyngeal colonization rate of *S. pneumonia*, otitis media and related adverse ear pathologies, formula intake, and body weight and length. Average daily consumption of the control formula ranged from 511 to 602 mL/infant/day and

average daily consumption of the LNnT supplemented formula ranged from 506 to 559 mL/ infant/day (no statistically significant difference in overall formula intake between groups). Based on the results of the study, LNnT produced using a yeast fermentation process was well-tolerated in infants and was without adverse effects on growth and ear health at a concentration of 220 mg/L.

IV.F.1.2 LNnT in Combination with 2'-FL in Infant Formula

The safety of LNnT (Glycom A/S, Denmark) manufactured by chemical synthesis as described in GRN 547 was evaluated in a randomized, blinded, controlled, multi-center, parallel-design study in healthy full-term infants (0 to 6 months of age) provided a standard term infant formula supplemented with LNnT (at a target concentration of 0.5 g LNnT/L reconstituted formula) in combination with 2'-FL (at a target concentration of 1.0 g/L reconstituted formula) (Puccio *et al.*, 2016). A comparator group receiving a standard intact protein infant formula without HMOs also was included. Infants received the test formulas on a daily basis from enrolment at 0 to 6 months of age until the age of 12 months. All infants were converted to test formula's containing intact cow's milk protein at 6 to 12 months of age.

Weight gain through 4 months was evaluated as the primary endpoint, with secondary endpoints of anthropometry (including body length, head circumference), digestive tolerance, formula compliance, and morbidity including adverse event (AE) reporting.

A total of 175 infants were enrolled in the study. The mean weight gain in the test group was non-inferior[8] to the mean weight gain in the control group. Infants receiving the test formula did not differ from control with regard to weight, length, head circumference, body mass index (BMI) or corresponding z-scores or digestive tolerance. Differences in reports of some AEs were noted in infants receiving the test formula (compared to the control); infants receiving the test formula were less likely to report bronchitis through 4 months (p=0.010), 6 months (p=0.005) and 12 months (p=0.004), and less likely to report AE clusters for lower respiratory tract infections through 12 months (p=0.027). Infants in the test group were less likely to report receiving antipyretics through 4 months (p=0.032) and antibiotics through 6 months (p=0.047) and 12 months (p=0.016). The study investigators concluded that "*Infant formula with 2'-FL and LNnT is safe, well-tolerated, and supports age-appropriate growth; it reduced the likelihood of reporting morbidity, particularly bronchitis and medication use vs control*".

Additional outcomes from this trial evaluating effects on early intestinal microbiota have been reported by Steenhout *et al*. (2016). The authors used 16S rRNA gene sequencing and NMR metabolic profiling to evaluate stool samples for effects of LNnT and 2'-FL on fecal microbiota composition. The global average microbial composition for the sub-group of infants with stool

[8] Weight gain in the test group was considered "non-inferior" if the lower bound of the one-sided 97.5% confidence interval on the difference between the test and control groups was greater than the non-inferiority margin of 3 grams/day, based on the recommendations from the American Academy of Pediatrics.

samples that followed the study protocol showed similar pattern between control (n=65) and test (n=58) at the genus level, although samples obtained from infants receiving the test formula were closer to breastfed (n=34) than control samples. Calculations of microbial *alpha* diversity and comparison of the global microbiota composition confirmed that test was different from control at the genus level ($p<0.001$) and closer to the breastfed reference. Statistical analysis (corrected for false discovery rate) identified several taxa differentially present in control and test including *Bifidobacterium* (p=0.01), *Escherichia* (p=0.008) and unclassified *Coprobacillaceae* (p=0.01). Multivariate analysis identified several influential metabolites that discriminated between test, control and breastfed groups including phenyalanine, isoleucine, tyrosine, fecal organic acids and fucosylated compounds. The values observed for test were more similar to those observed in the breast fed group compared with control, a finding that suggests reduced protein fermentation.

The authors concluded the following: "*Together these findings indicate that the addition of 2'-FL and LNnT to a starter infant formula shifts the stool microbiota and metabolic signature towards those observed in breastfed infants*". Glycom notes that findings from this study corroborate the safety of LNnT and secondary measures reported in the study demonstrate that the addition of LNnT to infant formula is associated with nutritional outcomes that are more similar to those found in breast-fed infants.

IV.F.2 Adults

LNnT Alone and in Combination with 2'-FL

The safety and tolerability of LNnT, produced by chemical synthesis as described in GRN 547, was investigated in a randomized, placebo-controlled, double-blind, parallel-design study in which healthy adult volunteers (51 men and 49 women; mean age of 36.0 years) were provided LNnT and 2'-FL alone or in combination at different doses for 2 weeks (Salomonsson *et al.,* 2016, and summarized in EFSA, 2015a). A comparator control group receiving glucose as a placebo was also included. The intervention groups used in the study are summarized in Table IV.F.2-1 below. All interventions were provided as single daily bolus doses. Test articles were provided in powder form and participants were instructed to dissolve the powder in approximately 250 mL of water prior to intake in the morning with breakfast. Compliance was evaluated using a subject diary in which subjects were instructed to record the intake of the test article, which was confirmed by the collection of empty and un-opened bottles at the end of the intervention period.

Table IV.F.2-1 Interventions Used in the Two-Week Healthy Adult Study		
Group No.	**Daily Dose of LNnT (grams)**	**Daily Dose of 2'-FL (grams)**
1	0	20
2	0	10
3	0	5
4	20	0
5	10	0
6	5	0
7	6.67	13.33
8	3.33	6.67
9	1.67	3.33
Control	2 grams Dextropure (glucose)	

2'-FL = 2'-*O*-fucosyllactose; LNnT = lacto-*N*-neotetraose.

Adverse events were monitored during the study. Blood samples were collected at baseline and at the end of the intervention period (2 weeks) and evaluated for standard hematological and blood biochemistry parameters. Feces were collected at baseline and at the end of the intervention period (2 weeks) and evaluated for biomarkers of gastrointestinal inflammation, gut mucosal immunity, malabsorption, and dietary fiber fermentation products (including calprotectin, secretory IgA, glucose, galactose, lactose, and short-chain fatty acids). Fecal samples were also collected to assess microbiota composition at baseline, during the first week of supplementation, and at week 2. Gastrointestinal symptoms were evaluated using the Gastrointestinal Symptom Rating Scale (GSRS) and changes in bowel habits were assessed using the Bristol Stool Form Scale (BSFS).

Three subjects from each intervention group were randomly selected to participate in a bioavailability and kinetic study in which blood and urine samples were obtained. Blood was collected at pre-dose, 3, 6, and 9 hours following the intake of the study product. Urine was collected at pre-dose and once more during the day.

All adverse events reported during the study were judged to be "mild" and there were no cases of premature discontinuation from the trial due to adverse events. Most adverse events were judged to be "possibly" related to the test article; however, many symptoms were noted by the study investigators to be common and difficult to ascertain whether they were related to the test article, to normal day-to-day variation, or to increased awareness of gastrointestinal symptoms during the trial period.

Hematological and blood biochemistry analyses obtained at the 2-week time-point remained within the normal range for all subjects and any minor changes over the course of the study compared to baseline values were not considered clinically relevant. The GSRS scores indicated that both LNnT and 2'-FL were well-tolerated. At the end of the intervention, a significantly higher incidence of passing gas was reported by participants who consumed either

10 or 20 g of LNnT compared with the placebo group. The BSFS scores indicated there was no statistical difference between the treatment groups and the placebo controls.

Overall, the results support that the consumption of LNnT, either alone or in combination with 2'-FL, at the doses tested, was safe and well-tolerated in healthy adult men and women. Acute intake of a bolus dose of 20 g of LNnT (or 2'-FL) may represent a gastro-intestinal tolerability threshold for some individuals; however, bolus exposures of 20 g are highly unlikely to be experienced by the consumer given the proposed use-levels and consequently required food intakes that would lead to such intakes.

IV.G Safety of the Production Strain

IV.G.1 Taxonomic Identity and History of Food Use

Table IV.G.1-1	**Characteristics of the Host Organism *Escherichia coli* K-12 DH1**
Genus	*Escherichia*
Species	*Escherichia coli*
Subspecies	not applicable
Strain	*E. coli* strain K-12 DH1
Culture collection	The German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen)
Deposition number	DSM 4235 (ATCC33849)

The taxonomic identity of the host organism *E. coli* K-12 DH1 is presented in the table above. As mentioned, *E. coli* K-12 and its derivatives (*e.g.*, DH1) are known as laboratory "safety-strains". These strains cluster together in the pan-genome comparison, and possess a smaller genome, fewer genes, and the absence of gene families that would allow them to colonize the human gut and/or produce protein-type toxins (Manning *et al.*, 1977; Smith, 1978; Bachmann, 1996; Lukjancenko *et al.*, 2010). The genomes of *E. coli* K-12 and closely related strains (*i.e.*, K and B strains) have been sequenced and compared to other strains of *E. coli*, including pathogenic strains (Blattner *et al.*, 1997; Lukjancenko *et al.*, 2010).

The construction of *E. coli* K-12 strain DH1 has been described in the literature (Hanahan, 1983; Luli and Strohl, 1990; Bachmann, 1996). The *E. coli* K-12 strain DH1 (*λ^- gyrA96 recA1 relA1 endA1 thi-1 hsdR17 supE44*), was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, 2015). The DH1 strain is resistant to nalidixic acid due to the gyrA96 mutation (Hanahan, 1983). The strain can grow in minimal medium provided that it is supplemented with thiamine due to the thi-1 mutation. The recA1 mutation minimizes the recombination and increases the stability of plasmids and chromosomal DNA of the strain.

The host strain *E. coli* K-12 and its derivatives are generally recognized as safe and suitable for use as a host organism for use in the construction of modified strains used for the production of

food ingredients. For example, in the European Union (EU), *E. coli* K-12 has been recommended as a safe host organism by the EU Commission and has been repeatedly assessed by EFSA as safe for the production of food and feed ingredients, additives, and food enzymes [*e.g.,* chymosin (JECFA, 2006), gamma cyclodextrin (ACNFP, 2012), L-methionine (EFSA, 2013), L-valine (EFSA, 2008), L-threonine (EFSA, 2014a), L-lysine (EFSA, 2014b), L-isoleucine (EFSA, 2010), L-tryptophan (EFSA, 2015b)]. Additionally, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has assessed *E. coli* K-12 as a safe host for food enzyme preparations [*e.g*., chymosin (JECFA, 1991, 2006)] and the French government explicitly lists chymosin derived from *E. coli* K-12 fermentation as an approved processing aid (see www.legifrance.gouv.fr) (Legifrance, 2006). In the U.S., *E. coli* K-12 and B-strain derivatives are used in the production of a range of GRAS ingredients and food enzymes [*e.g.,* alpha cyclodextrin (U.S. FDA, 2004), chymosin (U.S. FDA, 2015e), L-leucine (U.S. FDA, 2010a,b), and β-galactosidase (U.S. FDA, 2014)]. Comprehensive safety assessments of *E. coli* K-12 *and its derivatives* have been conducted by the U.S. Environmental Protection Agency (U.S. EPA, 1997).

Other food ingredients and/or food additives produced with *E. coli* K-12-derived strains include xylitol (Khankal *et al.*, 2008; von Rymon Lipinski, 2014), thaumatin (Daniell *et al.*, 2000; von Rymon Lipinski, 2014), tagatose (Roh *et al.*, 2000; von Rymon Lipinski, 2014), formic acid (Murarka *et al.*, 2008; Shams Yazdani and Gonzalez, 2008), L-phenylalanine (Karau and Grayson, 2014), L-tyrosine (Karau and Grayson, 2014), and L-valine (Karau and Grayson, 2014).

The production strain is removed by ultrafiltration after fermentation and before the down-stream purification sequence. Absence of the production organism from the finished ingredient (including the production strain) is verified by a number of microbiological purity criteria, including *E. coli* and *Enterobacteriaceae* and the absence of residual proteins, residual DNA, and residual bacterial endotoxins (see Section II.C.4).

IV.G.2 Safety of Modified Organism and Introduced Genes

IV.G.2.1 Introduction of Undesirable Traits

The use of *E. coli* K-12 and its derivatives has a long history of safe use in the production of food ingredients and drugs. The annotated genome and metabolic properties of the strain have been the subject of considerable scientific evaluation and it is well-established that *E. coli* K-12 DH1 does not exhibit inherent capacity to produce protein toxins or toxic secondary metabolites. The potential of the introduced genetic elements and/or corresponding expression proteins to alter the phenotypic properties of the source organism was considered. The genes encoding the 2 enzymes used in LNnT biosynthesis originate from pathogenic donor organisms (*i.e*., *Neisseria meningitides*, *Helicobacter pylori*); however, the cloned gene fragments were either synthesized from verified published gene sequences using automated solid-phase

oligonucleotide synthesis, or were obtained using targeted PCR amplification and purification. All DNA inserts were fully sequenced to verify the identity of the genes, ensuring that introduction of extraneous genetic material did not occur.

All introduced genes are well characterized with respect to their function, do not have homology to known protein toxins, and as enzymes involved in LNnT biosynthesis, are not reasonably expected to introduce toxicogenic/pathogenic attributes to the host. As such, safety concerns related to the introduced genes and corresponding gene expression products are low. In addition, successful integration of all introduced gene fragments were verified using PCR based analyses and Southern blotting techniques. Finished batches of the LNnT ingredient manufactured using the production strain are free from viable counts of *E. coli* and *Enterobacteriaceae* and also have been demonstrated to be absent of detectable quantities of DNA (corresponding to the introduced genes) and total protein. No antibiotic resistance markers are present in the production strain and no antibiotics are used during manufacture of LNnT.

IV.G.2.2 Potential Allergenicity of Expressed Proteins

The allergenic potential of the introduced enzymes in the production strain have been evaluated using the database and search algorithms by the Allergen Online tool (version 16) of the University of Nebraska. The database was updated last on 27 January 2016 and contains sequences of 1,956 putative and known allergens (University of Nebraska, 2016).

Allergen online searches were conducted using default settings and searches were conducted for matches to the full length sequences, matches to 80 amino acid sequence segments (sliding window) and 8-mer sequence alignments. Full length FASTA matches with *E* values of (<1e-7) and/or sequence identity greater than 50% were considered potentially cross-reactive with the aligned sequence. In accordance with Codex guidelines, FASTA also was used to search for 80 amino acid sliding window segments aligning with a match ≥35% identity to a protein in the allergen database (Codex Alimentarius Commission, 2003).

No full length sequence alignments ≥50% were identified for any inserted protein. No 80-amino acid segments matched with >35% identity to an allergen, and no 8-mer sequence alignment was identified. Therefore, based on the information provided above, no evidence exists that might indicate that the introduced proteins would cross-react with known or putative allergens.

IV.H Expert Panel Evaluation

Glycom A/S has determined that LNnT produced by fermentation, manufactured consistent with cGMP and meeting appropriate food grade specifications, is GRAS for use in infant formula and in food as described in Section I.D on the basis of scientific procedures. This GRAS determination is based on data generally available in the public domain pertaining to the safety

of LNnT and on a unanimous opinion among a panel of experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of infant formula ingredients and food ingredients. The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Ronald Kleinman (Mass General Hospital for Children), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), and Dr. John A. Thomas (Indiana University School of Medicine).

The Expert Panel, convened by Glycom A/S, independently and critically evaluated all data and information presented herein, and concluded that LNnT produced by fermentation was GRAS for use in non-exempt term infant formula and in food as described in Section I.D based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of LNnT is presented in Appendix B.

IV.I Conclusion

Based on a critical evaluation of the data and information presented herein, Glycom A/S has concluded that the intended uses of LNnT in non-exempt term infant formula and specified conventional food and beverage products, as described in Section I.D, is GRAS based on scientific procedures. General recognition of Glycom A/S's GRAS determination is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of LNnT in infant formula and in food, who similarly concluded that the intended use of LNnT in infant formula and in food as described herein is GRAS.

LNnT therefore may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

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Appendix A

LNnT 13-Week Oral (Gavage) Juvenile Toxicity Study in the Rat

Summary of Body Weights, Hematology, Clinical Chemistry, Histopathology Data

14. Tables and Figures

Day 1: First day of treatment.

Abbreviations:
Mean: Arithmetic mean
S.D.: Standard deviation
N: Number of animals or cages
m: Male
f: Female

Statistical significances arise from automatic comparisons with the principal control.
Principal Control Group: 1.

*: 5 % significance level
**: 1 % significance level
t: excluded from the trend analysis

Table 1 Group mean body weights

Due to technical error on Day 54, value from group 1 male no. 15 was excluded from the mean on this day.

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Group mean body weights

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Body Weight (g)

Group	Sex		Day numbers relative to Start Date 1	5	8	12	15	19
1	m	Mean	17.39	25.65	31.55	39.67	51.15	67.81
		S.D.	1.07	1.26	1.38	1.73	2.11	4.03
		N	15	15	15	15	15	15
2	m	t Mean	15.77*(1)	20.93**(1)	26.59**(1)	33.93**(1)	46.35	60.11**(1)
		S.D.	2.35	4.18	3.94	5.00	6.12	6.88
		N	15	15	15	15	15	15
3	m	Mean	15.12**	22.19*	27.17**	35.20*	44.92*	59.36*
		S.D.	1.91	2.22	2.56	3.64	5.43	7.23
		N	15	15	15	15	15	15
4	m	Mean	15.30**	23.22*	29.28	37.35	47.66	63.02*
		S.D.	2.33	2.82	3.30	4.00	5.79	6.50
		N	15	15	15	15	15	15
5	m	Mean	16.15**	24.89*,*(2)	31.75**(2)	40.05**(2)	52.03*(2)	66.21*,**(2)
		S.D.	1.32	1.99	2.28	2.71	3.37	4.80
		N	15	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex		Day numbers relative to Start Date 22	26	29	33	37	40
1	m	Mean	85.03	112.35	131.13	157.57	182.03	200.81
		S.D.	5.15	6.26	6.89	7.43	8.54	9.68
		N	15	15	15	15	15	15
2	m	t Mean	76.83**(1)	102.97**(1)	120.49**(1)	148.21*(1)	172.62	190.73
		S.D.	8.96	11.00	11.80	14.75	16.79	17.77
		N	15	15	15	15	15	15
3	m	Mean	74.99*	99.34*	117.50*	141.99*	165.64*	182.65*
		S.D.	9.43	11.24	12.41	15.28	17.45	17.56
		N	15	15	15	15	15	15
4	m	Mean	78.63*	103.01*	121.65*	143.85*	165.29*	183.04*
		S.D.	7.26	9.54	11.05	11.95	13.15	13.53
		N	15	15	15	15	15	15
5	m	Mean	82.55*,*(2)	108.32*	128.10*	153.62*	178.78*	197.24*
		S.D.	6.78	8.65	9.93	10.26	12.56	14.59
		N	15	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex			Day numbers relative to Start Date 43	47	50	54	57
1	m		Mean	220.61	247.21	267.15	286.41	297.19
			S.D.	10.34	14.12	15.73	14.77	17.39
			N	15	15	15	14	15
2	m	t	Mean	208.86	234.53	254.04	271.38*(1)	286.43
			S.D.	19.54	19.93	21.87	22.63	23.64
			N	15	15	15	15	15
3	m		Mean	201.31*	228.97*	246.87*	261.26*	279.29
			S.D.	18.34	20.34	20.29	20.49	21.95
			N	15	15	15	15	15
4	m		Mean	200.50*	227.07*	247.36*	262.78*	282.42
			S.D.	14.85	15.72	17.23	16.51	18.13
			N	15	15	15	15	15
5	m		Mean	216.67*	243.06*	262.08*	278.10*	294.35
			S.D.	16.05	18.60	20.11	22.58	24.71
			N	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex			Day numbers relative to Start Date 64	71	78	85	91
1	m		Mean	323.53	350.85	367.97	384.69	396.85
			S.D.	20.93	22.76	24.41	26.24	24.19
			N	15	15	15	15	15
2	m	t	Mean	311.38	336.60	353.83	368.24	381.15
			S.D.	25.77	27.56	29.86	31.26	32.73
			N	15	15	15	15	15
3	m		Mean	306.03	329.45	347.37	361.59	373.37
			S.D.	23.44	27.20	29.45	33.83	34.30
			N	15	15	15	15	15
4	m		Mean	309.05	332.99	352.07	368.07	381.01
			S.D.	19.06	21.90	23.77	24.64	26.75
			N	15	15	15	15	15
5	m		Mean	321.33	343.79	361.98	381.44	388.92
			S.D.	28.58	32.04	37.39	40.32	41.27
			N	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex		Day numbers relative to Start Date 1	5	8	12	15	19
1	f	Mean	16.82	25.03	30.74	38.81	50.28	66.56
		S.D.	1.06	1.29	1.54	1.91	2.69	3.86
		N	15	15	15	15	15	15
2	f	t Mean	15.18*(1)	20.58**(1)	25.70**(1)	33.55**(1)	45.28**(1)	58.73**(1)
		S.D.	2.52	3.36	4.10	4.75	6.41	7.09
		N	15	15	15	15	15	15
3	f	Mean	14.93*	21.99*	26.91**	34.61**	44.40**	59.39**
		S.D.	1.83	2.34	2.55	3.14	4.31	5.33
		N	15	15	15	15	15	15
4	f	Mean	14.55*	22.49*,*(2)	28.67*(2)	36.27*(2)	46.71*	60.49**
		S.D.	1.70	2.19	2.64	2.97	4.11	4.39
		N	15	15	15	15	15	15
5	f	Mean	16.05*	24.63*,**(2)	31.23**(2)	39.85**(2)	51.15**(2)	63.81**,**(2)
		S.D.	1.72	1.92	2.16	2.66	3.17	4.45
		N	15	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex		Day numbers relative to Start Date 22	26	29	33	37	40
1	f	Mean	83.82	104.89	118.81	132.65	144.91	153.80
		S.D.	4.55	5.76	6.30	6.83	7.62	8.59
		N	14	14	14	14	14	14
2	f	t Mean	74.03**(1)	96.02**(1)	109.62**(1)	124.55*(1)	137.75	149.16
		S.D.	8.56	10.93	13.11	14.71	16.87	17.44
		N	15	15	14	14	14	14
3	f	Mean	73.89**	96.23*	109.63*	126.22	138.69	145.24*
		S.D.	6.18	7.28	8.00	9.32	9.75	10.39
		N	15	15	15	15	15	15
4	f	Mean	73.96**	95.26*	108.30*	123.01*	136.86	144.28*
		S.D.	4.66	5.52	5.78	6.27	7.68	5.99
		N	15	15	15	15	15	15
5	f	Mean	78.53**,*(2)	101.70*,*(2)	116.81*,*(2)	132.87*(2)	146.28*(2)	153.64
		S.D.	4.82	6.22	7.21	8.58	8.74	9.69
		N	15	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex		Day numbers relative to Start Date 43	47	50	54	57
1	f	Mean	162.29	174.29	182.04	187.36	196.10
		S.D.	10.43	12.25	13.16	14.58	14.43
		N	14	14	14	14	14
2	f	t Mean	156.91	168.13	176.79	181.61	191.94
		S.D.	18.50	19.38	20.40	21.52	22.08
		N	14	14	14	14	14
3	f	Mean	153.29	165.41	175.22	178.19	186.33
		S.D.	10.66	10.50	10.42	11.80	11.62
		N	15	15	15	15	15
4	f	Mean	152.41	165.33	173.75	177.27	187.03
		S.D.	7.34	8.63	9.04	9.20	10.39
		N	15	15	15	15	15
5	f	Mean	162.81	175.56	181.61	185.22	195.96
		S.D.	9.74	10.94	11.44	13.75	13.01
		N	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights

AB20758

Body Weight (g)

Group	Sex		Day numbers relative to Start Date 64	71	78	85	91
1	f	Mean	207.81	221.72	228.39	235.64	240.54
		S.D.	14.31	17.11	18.98	19.95	19.11
		N	14	14	14	14	14
2	f	t Mean	204.68	214.44	223.02	233.80	236.26
		S.D.	21.72	23.13	26.38	24.99	26.06
		N	14	14	14	14	14
3	f	Mean	198.19	210.25	217.47	226.65	229.23
		S.D.	13.58	13.61	13.97	16.33	13.78
		N	15	15	15	15	15
4	f	Mean	198.99	210.69	218.67	225.27	230.18
		S.D.	11.08	12.63	14.00	13.15	14.60
		N	15	15	15	15	15
5	f	Mean	208.21	219.33	225.52	237.27	237.77
		S.D.	14.68	14.62	15.90	15.61	16.81
		N	15	15	15	15	15

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (3) v (2), (4) v (2), (2) v (1), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

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Group mean body weights - Treatment-free period (1)

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Body Weight (g)

Group	Sex			Day numbers relative to Start Date 91	99	106	113	119
1	m		Mean	393.82	403.28	412.36	424.74	434.34
			S.D.	21.06	23.04	23.38	23.22	24.43
			N	5	5	5	5	5
2	m	t	Mean	395.96	404.16	419.92	429.24	439.48
			S.D.	30.54	32.27	36.61	39.69	41.03
			N	5	5	5	5	5
3	m		Mean	350.78	358.76	366.72	373.72	378.74*(2)
			S.D.	27.64	28.78	27.32	24.51	21.75
			N	5	5	5	5	5
4	m		Mean	366.60	377.66	390.00	401.68	406.96
			S.D.	35.48	38.20	40.00	44.52	43.07
			N	5	5	5	5	5
5	m		Mean	365.96	372.94	387.36	396.50	404.86
			S.D.	62.70	62.62	66.19	66.15	70.51
			N	5	5	5	5	5

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean body weights - Treatment-free period (1)

AB20758

Body Weight (g)

Group	Sex			Day numbers relative to Start Date 91	99	106	113	119
1	f		Mean	246.36	249.56	252.36	260.44	259.82
			S.D.	13.38	14.85	15.26	16.09	19.48
			N	5	5	5	5	5
2	f	t	Mean	250.73	254.35	257.13	266.55	268.40
			S.D.	28.02	30.63	25.51	29.81	30.54
			N	4	4	4	4	4
3	f		Mean	228.40	233.42	237.18	245.58	242.44
			S.D.	13.48	13.29	14.21	13.11	13.18
			N	5	5	5	5	5
4	f		Mean	235.02	240.68	245.38	250.92	250.56
			S.D.	8.87	6.67	6.00	6.10	11.43
			N	5	5	5	5	5
5	f		Mean	247.66	252.76	255.22	262.66	265.48
			S.D.	19.55	18.70	17.32	19.07	18.61
			N	5	5	5	5	5

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Figures 1 and 2 Mean body weights

Figure 1
Mean body weights - Males

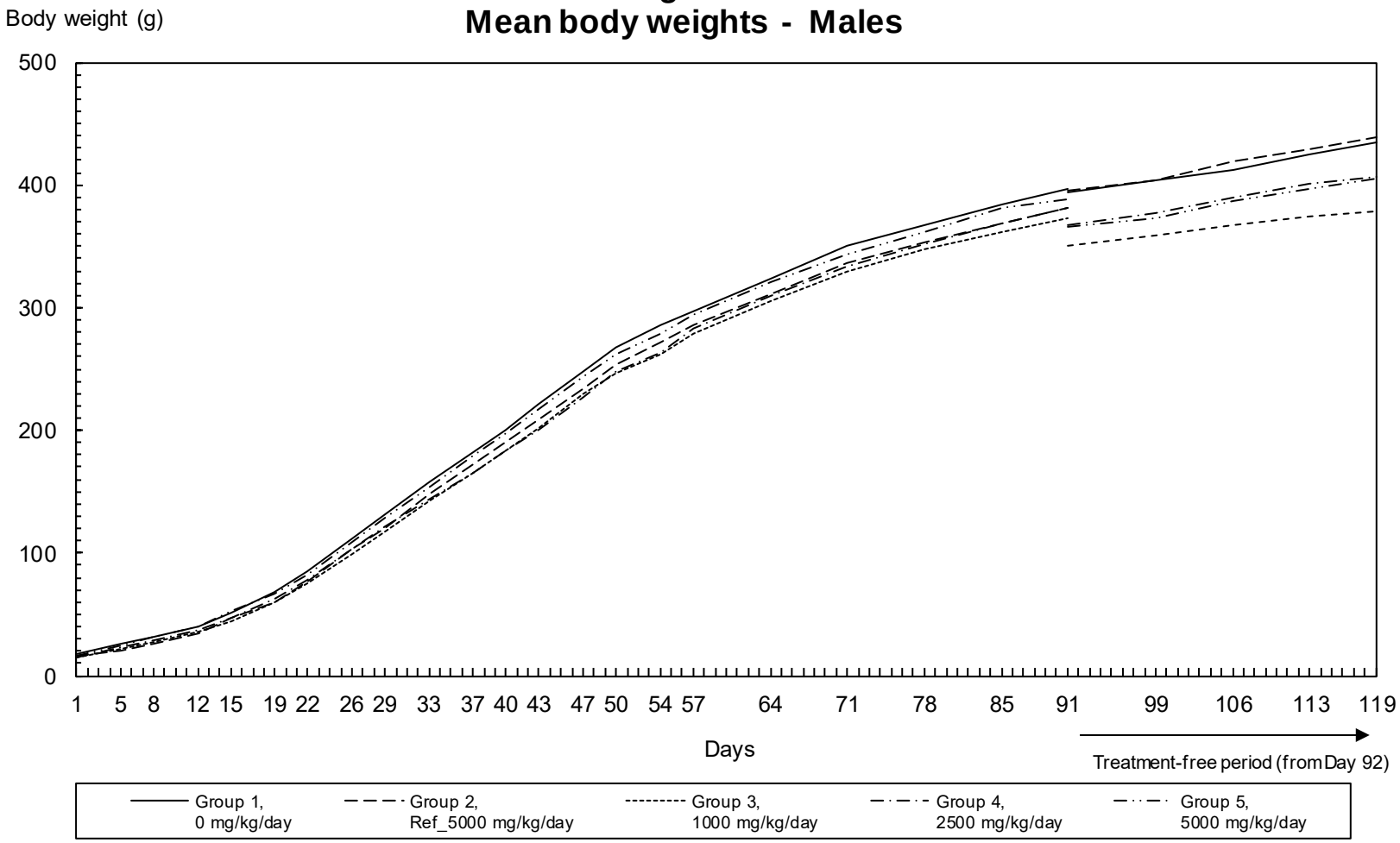

Figure 2
Mean body weights - Females

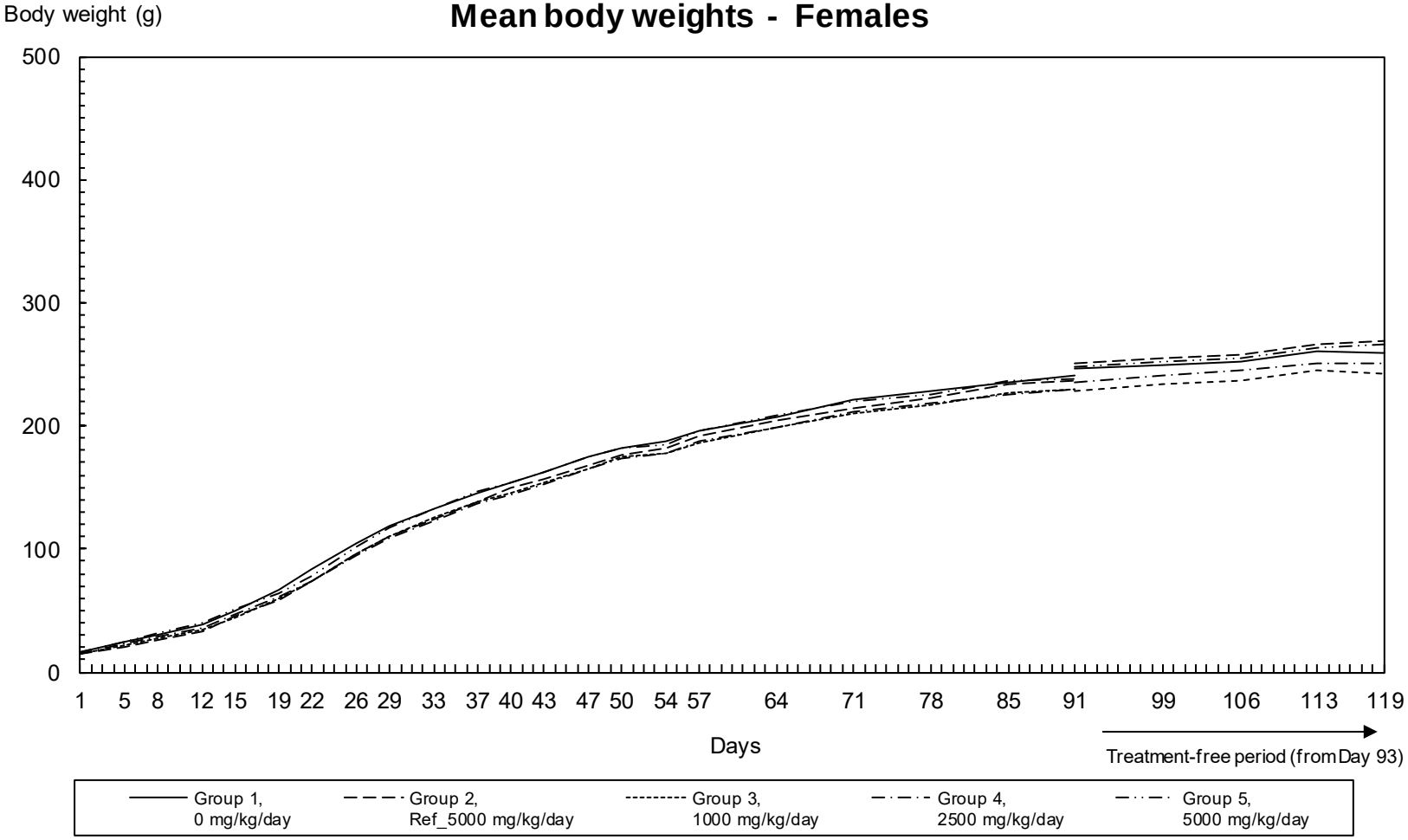

Table 9 Group mean hematology parameters

Abbreviations:

RBC: Red blood cell count

Hb: Hemoglobin

PCV: Packed cell volume

MCV: Mean corpuscular volume

MCH: Mean corpuscular hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

Reti.: Reticulocyte count

Plat.: Platelet count

APTT: Activated partial thromboplastin time

Prothrom: Prothrombin time

Fibrinog Conc.: Fibrinogen concentration

WBC: Total white blood cell count

N: Polymorphonuclear neutrophils

L: Lymphocytes

M: Monocytes

E: Polymorphonuclear eosinophils

B: Polymorphonuclear basophils

LUC: Large unstained cells

Abs.: Absolute count

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Group mean hematology parameters

AB20758

Day: 92 relative to Start Date

Group	Sex		RBC T/L	Hb g/L	PCV %	MCV fL	MCH pg
1	m	Mean	8.459	156.5	46.46	55.06	18.61
		S.D.	0.599	7.3	2.81	3.47	1.48
		N	10	10	10	10	10
2	m	t Mean	8.670	156.8	47.30	54.58	18.11
		S.D.	0.389	5.2	1.92	0.71	0.53
		N	9	9	9	9	9
3	m	Mean	8.462	155.4	46.22	54.69	18.36
		S.D.	0.478	6.0	2.22	2.14	0.88
		N	10	10	10	10	10
4	m	Mean	8.553	154.6	46.12	53.97	18.10
		S.D.	0.398	3.3	1.15	1.96	0.93
		N	10	10	10	10	10
5	m	Mean	8.459	155.9	45.92	54.33	18.43
		S.D.	0.273	2.7	1.11	1.44	0.65
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

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Group mean hematology parameters

AB20758

Day: 92 relative to Start Date

Group	Sex		MCHC g/L	Reti.(%) %	Reti.abs Giga/L	Plat. Giga/L	APTT s
1	m	Mean	337.5	2.73	230.10	824.7	17.03
		S.D.	11.0	0.22	23.12	89.2	0.73
		N	10	10	10	10	10
2	m	t Mean	331.6	2.57	223.57	789.4	16.76
		S.D.	5.8	0.34	30.72	84.2	0.66
		N	9	9	9	9	9
3	m	Mean	336.1	2.67	225.39	842.6	17.00
		S.D.	6.6	0.33	21.13	111.1	0.62
		N	10	10	10	10	10
4	m	Mean	335.3	2.47	211.89	813.4	16.99
		S.D.	7.8	0.35	31.43	96.0	1.96
		N	10	10	10	10	10
5	m	Mean	339.4*(2)	2.52	213.75	824.2	15.96**,*(2)
		S.D.	6.7	0.34	29.37	105.7	0.63
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

AB20758

Day: 92 relative to Start Date

Group	Sex		Prothrom s	Fibrinog Conc. g/L	WBC Giga/L	N.Abs. Giga/L	N (%) %
1	m	Mean	22.35	3.3507	7.032	1.360	19.90
		S.D.	0.54	0.2567	1.265	0.161	4.17
		N	10	10	10	10	10
2	m	t Mean	23.36*(1)	2.9841*(1)	5.688*(1)	1.129	19.61
		S.D.	1.15	0.4100	0.911	0.416	4.91
		N	9	9	9	9	9
3	m	Mean	21.92**(2)	3.0039**	7.004	1.299	18.81
		S.D.	0.55	0.2208	2.177	0.423	3.55
		N	10	10	10	10	10
4	m	Mean	22.00**(2)	2.9276*	7.120*(2)	1.288	18.15
		S.D.	1.19	0.2805	1.873	0.396	3.66
		N	10	10	10	10	10
5	m	Mean	22.01**(2)	3.0343*	6.843	1.394	20.35
		S.D.	0.98	0.3210	0.859	0.284	3.08
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

AB20758

Day: 92 relative to Start Date

Group	Sex		L.Abs. Giga/L	L (%) %	M.Abs. Giga/L	M (%) %	E.Abs. Giga/L
1	m	Mean	5.335	75.27	0.183	2.64	0.107
		S.D.	1.184	4.43	0.037	0.60	0.036
		N	10	10	10	10	10
2	m	t Mean	4.306	75.93	0.136*(1)	2.40	0.080
		S.D.	0.656	4.95	0.042	0.64	0.035
		N	9	9	9	9	9
3	m	Mean	5.407	76.83	0.168	2.37	0.083
		S.D.	1.796	3.75	0.063	0.37	0.027
		N	10	10	10	10	10
4	m	Mean	5.488*(2)	76.99	0.172	2.45	0.126*(2)
		S.D.	1.517	3.90	0.043	0.55	0.049
		N	10	10	10	10	10
5	m	Mean	5.136	75.08	0.176	2.55	0.093
		S.D.	0.666	3.45	0.042	0.45	0.037
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

AB20758

Day: 92 relative to Start Date

Group	Sex		E (%) %	B.Abs. Giga/L	B (%) %	LUC.Abs. Giga/L	LUC (%) %
1	m	Mean	1.52	0.013	0.17	0.035	0.48
		S.D.	0.41	0.007	0.07	0.014	0.10
		N	10	10	10	10	10
2	m t	Mean	1.42	0.007*(1)	0.12	0.029	0.51
		S.D.	0.53	0.005	0.04	0.021	0.30
		N	9	9	9	9	9
3	m	Mean	1.30	0.011	0.16	0.033	0.49
		S.D.	0.54	0.009	0.10	0.020	0.31
		N	10	10	10	10	10
4	m	Mean	1.78	0.011	0.15	0.037	0.48
		S.D.	0.59	0.007	0.07	0.023	0.19
		N	10	10	10	10	10
5	m	Mean	1.39	0.013*(2)	0.16	0.031	0.46
		S.D.	0.67	0.005	0.05	0.010	0.11
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

AB20758

Day: 93 relative to Start Date

Group	Sex		RBC T/L	Hb g/L	PCV %	MCV fL	MCH pg
1	f	Mean	8.484	156.2	47.13	55.64	18.47
		S.D.	0.458	5.0	2.31	2.99	1.09
		N	9	9	9	9	9
2	f	t Mean	8.279	150.6**(1)	45.76	55.28	18.20
		S.D.	0.413	5.3	2.21	1.25	0.74
		N	9	9	9	9	9
3	f	Mean	7.973*	152.7	44.98	56.47	19.18**(2)
		S.D.	0.419	4.3	1.77	1.69	0.71
		N	10	10	10	10	10
4	f	Mean	8.167	155.0*(2)	46.29	56.67	18.97*(2)
		S.D.	0.274	3.5	1.54	1.36	0.62
		N	10	10	10	10	10
5	f	Mean	8.243	154.3	45.83	55.63	18.75
		S.D.	0.350	3.8	1.56	1.48	0.67
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

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Group mean hematology parameters

AB20758

Day: 93 relative to Start Date

Group	Sex			MCHC g/L	Reti.(%) %	Reti.abs Giga/L	Plat. Giga/L	APTT s
1	f		Mean	331.4	2.89	243.92	890.1	17.20
			S.D.	7.8	0.35	25.70	80.6	0.97
			N	9	9	9	9	9
2	f	t	Mean	329.2	2.54*(1)	210.90*(1)	894.4	16.82
			S.D.	11.3	0.36	30.93	122.2	0.98
			N	9	9	9	9	10
3	f		Mean	339.9**(2)	2.43	193.22*	824.8	15.87*
			S.D.	5.3	0.42	28.96	84.7	1.38
			N	10	10	10	8	9
4	f		Mean	334.7	2.70	220.72*	830.9	16.17*
			S.D.	7.0	0.19	19.16	96.9	1.02
			N	10	10	10	9	10
5	f		Mean	337.3*(2)	2.64	218.88*	815.4	15.47**,**(2)
			S.D.	6.9	0.42	34.88	58.9	1.03
			N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

AB20758

Day: 93 relative to Start Date

Group	Sex			Prothrom s	Fibrinog Conc. g/L	WBC Giga/L	N.Abs. Giga/L	N (%) %
1	f		Mean	23.78	2.3568	3.574	0.613	17.77
			S.D.	0.66	0.2918	0.959	0.163	5.40
			N	9	9	9	9	9
2	f	t	Mean	24.17	2.1580	3.998	0.673	17.19
			S.D.	0.43	0.2453	1.016	0.267	5.78
			N	10	10	9	9	9
3	f		Mean	22.89*,**(2)	2.4419	3.927	0.901	22.99
			S.D.	0.96	0.2969	0.837	0.354	8.21
			N	9	9	10	10	10
4	f		Mean	22.38*,**(2)	2.3488	4.461	0.748	20.00
			S.D.	1.40	0.4297	1.743	0.248	12.85
			N	10	10	10	10	10
5	f		Mean	22.85*,**(2)	2.5131*(2)	4.601	0.864	18.61
			S.D.	0.89	0.3273	0.880	0.272	4.05
			N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

AB20758

Day: 93 relative to Start Date

Group	Sex			L.Abs. Giga/L	L (%) %	M.Abs. Giga/L	M (%) %	E.Abs. Giga/L
1	f		Mean	2.794	77.44	0.081	2.27	0.062
			S.D.	0.865	5.84	0.030	0.90	0.022
			N	9	9	9	9	9
2	f	t	Mean	3.104	77.34	0.102	2.58	0.092
			S.D.	0.882	5.99	0.036	0.55	0.036
			N	9	9	9	9	9
3	f		Mean	2.830	72.07	0.090	2.29	0.089
			S.D.	0.751	9.65	0.025	0.63	0.060
			N	10	10	10	10	10
4	f		Mean	3.490	74.90	0.113	2.60	0.086
			S.D.	1.478	13.35	0.039	0.51	0.061
			N	10	10	10	10	10
5	f		Mean	3.531	76.95	0.088	1.85*(2)	0.096
			S.D.	0.646	4.29	0.042	0.81	0.049
			N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean hematology parameters

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Group	Sex		E (%) %	B.Abs. Giga/L	B (%) %	LUC.Abs. Giga/L	LUC (%) %
1	f	Mean	1.87	0.004	0.12	0.018	0.51
		S.D.	0.80	0.005	0.07	0.008	0.18
		N	9	9	9	9	9
2	f	t Mean	2.32	0.003	0.11	0.020	0.46
		S.D.	0.81	0.005	0.06	0.010	0.19
		N	9	9	9	9	9
3	f	Mean	2.19	0.002	0.06	0.018	0.47
		S.D.	1.50	0.004	0.07	0.007	0.21
		N	10	10	10	9	9
4	f	Mean	2.02	0.006	0.12	0.027	0.54
		S.D.	1.14	0.005	0.11	0.020	0.29
		N	10	10	10	7	7
5	f	Mean	2.07	0.003	0.09	0.021	0.47
		S.D.	0.86	0.005	0.06	0.012	0.25
		N	10	10	10	9	9

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Table 10 Group mean serum clinical chemistry parameters

Abbreviations:
Na: Sodium
K: Potassium
Ca: Calcium
Gluc.: Glucose
Creat.: Creatinine
Chol.: Total cholesterol
Trigs.: Triglycerides
T.Bili: Total bilirubin
Prot.: Total protein
Alb.: Albumin
Glob.: Globulin
A.G ratio: Albumin/globulin ratio
ALP: Alkaline phosphatase
ASAT: Aspartate aminotransferase
ALAT: Alanine aminotransferase
mcmol: µmol

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Group mean serum clinical chemistry parameters

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Group	Sex		Na mmol/L	K mmol/L	Ca mmol/L	Gluc. mmol/L	Urea mmol/L	Creat. mcmol/L
1	m	Mean	143.6	4.37	2.646	7.399	5.054	40.0
		S.D.	0.7	0.35	0.082	0.831	0.584	2.9
		N	10	10	10	10	10	10
2	m	t Mean	143.4	4.53	2.556**(1)	7.574	4.763	38.0
		S.D.	1.3	0.19	0.051	0.836	0.817	2.7
		N	10	10	10	10	10	10
3	m	Mean	144.4*(2)	4.18**(2)	2.562**	7.178	5.133	39.4
		S.D.	1.3	0.21	0.031	0.622	0.423	1.6
		N	10	10	10	10	10	10
4	m	Mean	143.1	4.52	2.597	7.172	5.293	40.0
		S.D.	0.7	0.29	0.055	0.531	0.629	3.5
		N	10	10	10	10	10	10
5	m	Mean	143.0	4.47	2.621*(2)	7.791	5.379*(2)	38.8
		S.D.	1.2	0.24	0.049	0.738	0.398	1.8
		N	10	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean serum clinical chemistry parameters

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Day: 92 relative to Start Date

Group	Sex		Chol. mmol/L	Trigs. mmol/L	T.Bili mcmol/L	Prot. g/L	Alb. g/L
1	m	Mean	1.791	1.008	2.85	63.99	33.36
		S.D.	0.425	0.332	0.85	1.31	1.11
		N	10	10	10	10	10
2	m	t Mean	1.268**(1)	0.944	2.44	60.49**(1)	32.24*(1)
		S.D.	0.154	0.260	0.38	2.48	0.77
		N	10	10	10	10	10
3	m	Mean	1.662**(2)	0.949	2.31*	63.04**(2)	33.16*(2)
		S.D.	0.338	0.346	0.22	2.23	1.05
		N	10	10	10	10	10
4	m	Mean	1.690**(2)	1.379*(2)	2.56*	64.01**(2)	33.26*(2)
		S.D.	0.382	0.561	0.27	1.80	0.80
		N	10	10	10	10	10
5	m	Mean	1.490	1.246	2.28**	63.63**(2)	33.04
		S.D.	0.258	0.428	0.30	1.27	1.14
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean serum clinical chemistry parameters

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Day: 92 relative to Start Date

Group	Sex			Glob. g/L	A.G ratio	ALP IU/L	ASAT IU/L	ALAT IU/L
1	m		Mean	30.63	1.09	267.9	78.3	25.5
			S.D.	0.76	0.03	70.5	9.3	6.4
			N	10	10	10	10	10
2	m	t	Mean	28.25**(1)	1.14	263.3	75.5	20.7*(1)
			S.D.	2.07	0.08	57.0	7.4	3.9
			N	10	10	10	10	10
3	m		Mean	29.88*(2)	1.12	249.1	77.9	25.0
			S.D.	1.49	0.06	44.8	15.7	4.7
			N	10	10	10	10	10
4	m		Mean	30.75**(2)	1.07*(2)	329.0*(2)	85.5	25.5*(2)
			S.D.	1.32	0.05	78.7	8.7	5.0
			N	10	10	10	10	10
5	m		Mean	30.59**(2)	1.09	292.6	78.5	23.2
			S.D.	1.00	0.06	58.7	13.9	5.1
			N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean serum clinical chemistry parameters

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Day: 93 relative to Start Date

Group	Sex			Na	K	Ca	Gluc.	Urea	Creat.
				mmol/L	mmol/L	mmol/L	mmol/L	mmol/L	mcmol/L
1	f		Mean	142.9	3.93	2.612	5.812	6.170	40.3
			S.D.	0.6	0.21	0.047	0.537	1.150	4.8
			N	9	9	9	9	9	9
2	f	t	Mean	141.9*(1)	4.04	2.622	5.948	6.888	41.2
			S.D.	1.3	0.25	0.087	0.339	0.755	2.2
			N	10	10	10	10	10	10
3	f		Mean	143.2**(2)	3.95	2.591	5.000*,**(2)	6.672	41.5
			S.D.	1.1	0.28	0.050	0.629	0.707	2.6
			N	10	10	10	10	10	10
4	f		Mean	143.2**(2)	4.06	2.652	5.571	6.479	41.2
			S.D.	0.8	0.36	0.067	1.012	1.002	2.1
			N	10	10	10	10	10	10
5	f		Mean	143.3**(2)	4.01	2.626	5.664	6.921	43.7*
			S.D.	1.2	0.18	0.057	0.755	0.685	4.1
			N	10	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean serum clinical chemistry parameters

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Day: 93 relative to Start Date

Group	Sex		Chol. mmol/L	Trigs. mmol/L	T.Bili mcmol/L	Prot. g/L	Alb. g/L
1	f	Mean	1.620	0.528	2.47	64.01	35.44
		S.D.	0.305	0.135	0.47	2.96	2.73
		N	9	9	9	9	9
2	f	t Mean	1.556	0.705	2.56	63.90	35.92
		S.D.	0.296	0.326	0.26	2.17	0.97
		N	10	10	10	10	10
3	f	Mean	1.615	0.460*(2)	2.28*(2)	66.60*(2)	36.57
		S.D.	0.393	0.172	0.18	2.96	1.53
		N	10	10	10	10	10
4	f	Mean	1.726	0.448*(2)	2.61	66.46*(2)	37.13
		S.D.	0.416	0.077	0.39	2.25	1.69
		N	10	10	10	10	10
5	f	Mean	1.577	0.614	2.38	64.56	34.93
		S.D.	0.351	0.345	0.23	1.69	1.54
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Group mean serum clinical chemistry parameters

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Day: 93 relative to Start Date

Group	Sex		Glob. g/L	A.G ratio	ALP IU/L	ASAT IU/L	ALAT IU/L
1	f	Mean	28.57	1.24	146.2	68.9	14.1
		S.D.	0.95	0.10	39.8	7.2	2.8
		N	9	9	9	9	9
2	f	t Mean	27.98	1.28	101.7**(1)	69.2	13.3
		S.D.	1.45	0.06	13.8	10.0	2.1
		N	10	10	10	10	10
3	f	Mean	30.03**(2)	1.21	145.8**(2)	81.5*(2)	16.0*(2)
		S.D.	1.99	0.07	21.9	16.4	2.1
		N	10	10	10	10	10
4	f	Mean	29.33*(2)	1.26	153.6**(2)	72.1	14.8
		S.D.	1.33	0.08	37.9	10.1	3.7
		N	10	10	10	10	10
5	f	Mean	29.63*(2)	1.18*(2)	139.0**(2)	70.9	14.0
		S.D.	1.46	0.09	28.0	13.6	3.2
		N	10	10	10	10	10

Statistical analysis performed - Arithmetic mean values presented.
Pairwise comparisons requested: (2) v (1), (3) v (2), (4) v (2), (5) v (2).
Statistical significances marked with a number in brackets arise from the requested pairwise comparison with that group. Others arise from automatic comparisons with the principal control.
Principal Control Group: 1

Group 1 - 0 mg/kg/day Group 2 - Ref_5000 mg/kg/day Group 3 - 1000 mg/kg/day
Group 4 - 2500 mg/kg/day Group 5 - 5000 mg/kg/day

Table 3 Summary of microscopic findings

Number of animals with microscopic findings by organ/group/sex (with grades)

In the following table, the organ “no correlate” should not be taken into account. It cannot be deleted due to constraints of the data capture system.

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Summary of microscopic findings

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Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
EYES;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	2	0	0	0	2
Haemorrhage; retrobulbar	(6)	(0)	(0)	(0)	(8)
minimal	3	0	0	0	6
slight	3	0	0	0	2
Inflammation; retrobulbar	(6)	(0)	(0)	(0)	(4)
minimal	6	0	0	0	4
OPTIC NERVES;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
BRAIN;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SPINAL CORD (CERVICAL SEGMENT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SPINAL CORD (LUMBAR SEGMENT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SPINAL CORD (THORACIC SEGMENT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
THYROID GLANDS;					
Examined	(10)	(0)	(0)	(0)	(10)

Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
THYROID GLANDS; (continued)					
Within Normal Limits	10	0	0	0	10
PARATHYROID GLANDS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
Not Examined: NOT PRESENT	0	0	0	0	0
PANCREAS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
THYMUS;					
Examined	(10)	(0)	(1)	(0)	(10)
Within Normal Limits	10	0	0	0	10
Cyst/embryonic remnants	(0)	(0)	(1)	(0)	(0)
present	0	0	1	0	0
Haemorrhage	(0)	(0)	(0)	(0)	(0)
slight	0	0	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
MANDIBULAR GLAND (LEFT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SUBLINGUAL GLAND (LEFT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10

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Summary of microscopic findings

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Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
PAROTID GLAND (LEFT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
PITUITARY GLAND;					
Examined	(10)	(0)	(0)	(1)	(10)
Within Normal Limits	10	0	0	1	10
ADRENAL GLANDS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SKELETAL MUSCLE (LEFT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SKIN STUDY PLAN SAMPLE;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
MAMMARY GLAND;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
Not Examined: NOT PRESENT	1	0	0	0	0
STOMACH;					
Examined	(10)	(1)	(0)	(0)	(10)
Within Normal Limits	10	1	0	0	9
Erosion; glandular part	(0)	(0)	(0)	(0)	(1)
slight	0	0	0	0	1

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
DUODENUM;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
AORTA;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
ESOPHAGUS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
Degeneration; muscle layer	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
TRACHEA;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
JEJUNUM;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
COLON;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
ILEUM;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
CECUM;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
Lymphoid cell infiltration; mucosal	(1)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0
MESENTERIC LYMPH NODE;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	8	0	0	0	8
Hyperplasia; lymphoid	(2)	(0)	(0)	(0)	(2)
minimal	1	0	0	0	2
slight	1	0	0	0	0
HEART;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	9
Inflammation	(1)	(0)	(0)	(0)	(1)
minimal	1	0	0	0	1
LIVER;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
Inflammation	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
SPLEEN;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	4	0	0	0	4
Extramedullary hematopoiesis, increased	(5)	(0)	(0)	(0)	(6)
minimal	5	0	0	0	6
Pigment deposits, increased	(1)	(0)	(0)	(0)	(0)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
SPLEEN; (continued)					
minimal	1	0	0	0	0
LUNGS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	6	0	0	0	9
Inflammatory cell infiltration; eosinophilic; perivascular	(4)	(0)	(0)	(0)	(0)
minimal	4	0	0	0	0
Metaplasia; osseous	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	1
BRONCHUS/BRONCHI;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
KIDNEYS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	7	0	0	0	6
Chronic nephropathy	(1)	(0)	(0)	(0)	(3)
minimal	1	0	0	0	3
Lymphoid cell infiltration	(1)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0
Cyst(s)	(1)	(0)	(0)	(0)	(1)
present	1	0	0	0	1
TESTES;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	9
Tubular hypoplasia/atrophy	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	1

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
OVARIES;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
OVIDUCTS;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Cyst(s)	(-)	(-)	(-)	(-)	(-)
moderate	-	-	-	-	-
PROSTATE GLAND;					
Examined	(10)	(0)	(1)	(0)	(10)
Within Normal Limits	7	0	1	0	4
Lymphoid cell infiltration	(3)	(0)	(0)	(0)	(6)
minimal	3	0	0	0	4
slight	0	0	0	0	2
SEMINAL VESICLES;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
URINARY BLADDER;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
UTERUS;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Dilatation due to oestrus cycle	(-)	(-)	(-)	(-)	(-)
slight	-	-	-	-	-
moderate	-	-	-	-	-

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
UTERUS; (continued)					
Cyst(s)	(-)	(-)	(-)	(-)	(-)
present	-	-	-	-	-
CERVIX;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
VAGINA;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Proestrus phase of the cycle	-	-	-	-	-
Oestrus phase of the cycle	-	-	-	-	-
Dioestrus phase of the cycle	-	-	-	-	-
EPIDIDYMIDES;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
STERNUM;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
BONE MARROW (STERNUM);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
FEMUR;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	10	10	10	10	10
Number of Animals Completed:	(10)	(10)	(10)	(10)	(10)
STIFLE JOINT, FEMORO-TIBIAL, LEFT;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
HARDERIAN GLANDS;					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10
SKIN/SUBCUTIS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Acanthosis	(1)	(0)	(0)	(0)	(0)
moderate	1	0	0	0	0
Serocellular crust	(1)	(0)	(0)	(0)	(0)
moderate	1	0	0	0	0
NO CORRELATE;					
Examined	(1)	(1)	(1)	(1)	(2)
Within Normal Limits	0	0	0	0	0
No correlate	1	1	1	1	2
SCIATIC NERVE (LEFT);					
Examined	(10)	(0)	(0)	(0)	(10)
Within Normal Limits	10	0	0	0	10

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
EYES;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	4	0	0	0	3
Haemorrhage; retrobulbar	(5)	(0)	(0)	(0)	(6)
minimal	4	0	0	0	3
slight	1	0	0	0	3
Inflammation; retrobulbar	(1)	(0)	(0)	(0)	(2)
minimal	1	0	0	0	2
OPTIC NERVES;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
BRAIN;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SPINAL CORD (CERVICAL SEGMENT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SPINAL CORD (LUMBAR SEGMENT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SPINAL CORD (THORACIC SEGMENT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
THYROID GLANDS;					
Examined	(9)	(0)	(0)	(0)	(10)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
THYROID GLANDS; (continued)					
Within Normal Limits	9	0	0	0	10
PARATHYROID GLANDS;					
Examined	(8)	(0)	(0)	(0)	(10)
Within Normal Limits	8	0	0	0	10
Not Examined: NOT PRESENT	1	0	0	0	0
PANCREAS;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
THYMUS;					
Examined	(9)	(0)	(0)	(1)	(10)
Within Normal Limits	9	0	0	0	10
Cyst/embryonic remnants	(0)	(0)	(0)	(0)	(0)
present	0	0	0	0	0
Haemorrhage	(0)	(0)	(0)	(1)	(0)
slight	0	0	0	1	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(9)	(1)	(0)	(0)	(10)
Within Normal Limits	9	1	0	0	10
MANDIBULAR GLAND (LEFT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SUBLINGUAL GLAND (LEFT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10

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Summary of microscopic findings

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Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
PAROTID GLAND (LEFT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
PITUITARY GLAND;					
Examined	(9)	(1)	(0)	(0)	(10)
Within Normal Limits	9	1	0	0	10
ADRENAL GLANDS;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SKELETAL MUSCLE (LEFT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SKIN STUDY PLAN SAMPLE;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
MAMMARY GLAND;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
Not Examined: NOT PRESENT	0	0	0	0	0
STOMACH;					
Examined	(9)	(0)	(1)	(0)	(10)
Within Normal Limits	9	0	1	0	10
Erosion; glandular part	(0)	(0)	(0)	(0)	(0)
slight	0	0	0	0	0

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Summary of microscopic findings

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Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
DUODENUM;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
AORTA;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
ESOPHAGUS;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	8	0	0	0	10
Degeneration; muscle layer	(1)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0
TRACHEA;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
JEJUNUM;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
COLON;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
ILEUM;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10

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Summary of microscopic findings

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Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
CECUM;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
Lymphoid cell infiltration; mucosal	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
MESENTERIC LYMPH NODE;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	8	0	0	0	8
Hyperplasia; lymphoid	(1)	(0)	(0)	(0)	(2)
minimal	1	0	0	0	1
slight	0	0	0	0	1
HEART;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
Inflammation	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
LIVER;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	9
Inflammation	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	1
SPLEEN;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	7	0	0	0	9
Extramedullary hematopoiesis, increased	(2)	(0)	(0)	(0)	(0)
minimal	2	0	0	0	0
Pigment deposits, increased	(0)	(0)	(0)	(0)	(1)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
SPLEEN; (continued)					
minimal	0	0	0	0	1
LUNGS;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	8	0	0	0	9
Inflammatory cell infiltration; eosinophilic; perivascular	(1)	(0)	(0)	(0)	(1)
minimal	1	0	0	0	1
Metaplasia; osseous	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
BRONCHUS/BRONCHI;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
KIDNEYS;					
Examined	(9)	(1)	(0)	(0)	(10)
Within Normal Limits	9	1	0	0	10
Chronic nephropathy	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
Lymphoid cell infiltration	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
Cyst(s)	(0)	(0)	(0)	(0)	(0)
present	0	0	0	0	0
TESTES;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Tubular hypoplasia/atrophy	(-)	(-)	(-)	(-)	(-)
minimal	-	-	-	-	-

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
OVARIES;					
Examined	(9)	(1)	(0)	(0)	(10)
Within Normal Limits	9	1	0	0	10
OVIDUCTS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Cyst(s)	(0)	(1)	(0)	(0)	(0)
moderate	0	1	0	0	0
PROSTATE GLAND;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Lymphoid cell infiltration	(-)	(-)	(-)	(-)	(-)
minimal	-	-	-	-	-
slight	-	-	-	-	-
SEMINAL VESICLES;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
URINARY BLADDER;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
UTERUS;					
Examined	(9)	(2)	(1)	(2)	(10)
Within Normal Limits	5	0	0	0	7
Dilatation due to oestrus cycle	(3)	(2)	(1)	(2)	(3)
slight	0	0	0	1	0
moderate	3	2	1	1	3

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
UTERUS; (continued)					
Cyst(s)	(1)	(0)	(0)	(0)	(0)
present	1	0	0	0	0
CERVIX;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
VAGINA;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	3	0	0	0	2
Proestrus phase of the cycle	2	0	0	0	3
Oestrus phase of the cycle	2	0	0	0	1
Dioestrus phase of the cycle	2	0	0	0	4
EPIDIDYMIDES;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
STERNUM;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
BONE MARROW (STERNUM);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
FEMUR;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: TERMINAL SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	9	10	10	10	10
Number of Animals Completed:	(9)	(10)	(10)	(10)	(10)
STIFLE JOINT, FEMORO-TIBIAL, LEFT;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
HARDERIAN GLANDS;					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10
SKIN/SUBCUTIS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Acanthosis	(0)	(0)	(0)	(0)	(0)
moderate	0	0	0	0	0
Serocellular crust	(0)	(0)	(0)	(0)	(0)
moderate	0	0	0	0	0
NO CORRELATE;					
Examined	(2)	(3)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0
No correlate	2	3	1	0	0
SCIATIC NERVE (LEFT);					
Examined	(9)	(0)	(0)	(0)	(10)
Within Normal Limits	9	0	0	0	10

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Summary of microscopic findings

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Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: RECOVERY SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	5	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)
THYROID GLANDS;					
Examined	(0)	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	1
THYMUS;					
Examined	(1)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Haemorrhage	(1)	(1)	(0)	(0)	(0)
minimal	0	1	0	0	0
slight	1	0	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(0)	(1)	(0)	(0)	(1)
Within Normal Limits	0	1	0	0	0
Plasmacytosis, increased	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	1
Haemorrhage	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	1
STOMACH;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
LIVER;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
UTERUS;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Dilatation due to oestrus cycle	(-)	(-)	(-)	(-)	(-)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: RECOVERY SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	5	5	5	5	5
Number of Animals Completed:	(5)	(5)	(5)	(5)	(5)
UTERUS; (continued)					
slight	-	-	-	-	-
moderate	-	-	-	-	-
SKIN/SUBCUTIS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Acanthosis	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
Reduced hair follicles	(0)	(0)	(0)	(0)	(0)
present	0	0	0	0	0
NO CORRELATE;					
Examined	(0)	(1)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0
No correlate	0	1	0	0	1
MANDIBULAR LYMPH NODE (RIGHT);					
Examined	(0)	(1)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0
Plasmacytosis, increased	(0)	(1)	(0)	(0)	(0)
minimal	0	1	0	0	0
Haemorrhage	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	1
slight	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: RECOVERY SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	5	4	5	5	5
Number of Animals Completed:	(5)	(4)	(5)	(5)	(5)
THYROID GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
THYMUS;					
Examined	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Haemorrhage	(0)	(0)	(1)	(0)	(0)
minimal	0	0	1	0	0
slight	0	0	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Plasmacytosis, increased	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
Haemorrhage	(0)	(0)	(1)	(0)	(0)
minimal	0	0	1	0	0
STOMACH;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
LIVER;					
Examined	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	1	0	0
UTERUS;					
Examined	(0)	(0)	(0)	(1)	(2)
Within Normal Limits	0	0	0	0	0
Dilatation due to oestrus cycle	(0)	(0)	(0)	(1)	(2)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: RECOVERY SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	5	4	5	5	5
Number of Animals Completed:	(5)	(4)	(5)	(5)	(5)
UTERUS; (continued)					
slight	0	0	0	0	1
moderate	0	0	0	1	1
SKIN/SUBCUTIS;					
Examined	(1)	(4)	(0)	(0)	(0)
Within Normal Limits	0	2	0	0	0
Acanthosis	(1)	(1)	(0)	(0)	(0)
minimal	1	1	0	0	0
Reduced hair follicles	(0)	(2)	(0)	(0)	(0)
present	0	2	0	0	0
NO CORRELATE;					
Examined	(1)	(2)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0
No correlate	1	2	1	0	0
MANDIBULAR LYMPH NODE (RIGHT);					
Examined	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Plasmacytosis, increased	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
Haemorrhage	(0)	(0)	(1)	(0)	(0)
minimal	0	0	0	0	0
slight	0	0	1	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
EYES;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Fibroplasia; retrobulbar	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
OPTIC NERVES;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
BRAIN;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPINAL CORD (CERVICAL SEGMENT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPINAL CORD (LUMBAR SEGMENT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPINAL CORD (THORACIC SEGMENT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
THYROID GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
PARATHYROID GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
PANCREAS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
THYMUS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MANDIBULAR GLAND (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Immature	(0)	(0)	(0)	(0)	(0)
present	0	0	0	0	0
SUBLINGUAL GLAND (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
PAROTID GLAND (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
PITUITARY GLAND;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
ADRENAL GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SKELETAL MUSCLE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SKIN STUDY PLAN SAMPLE;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MAMMARY GLAND;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
STOMACH;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
DUODENUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
AORTA;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
ESOPHAGUS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
TRACHEA;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
JEJUNUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
COLON;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
ILEUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
CECUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MESENTERIC LYMPH NODE;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
HEART;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
LIVER;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPLEEN;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
LUNGS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
BRONCHUS/BRONCHI;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
KIDNEYS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Cyst(s)	(0)	(0)	(0)	(0)	(0)
present	0	0	0	0	0
OVARIES;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Immature	(-)	(-)	(-)	(-)	(-)
present	-	-	-	-	-
URINARY BLADDER;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
UTERUS;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Immature	(-)	(-)	(-)	(-)	(-)
present	-	-	-	-	-
CERVIX;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
VAGINA;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Dioestrus phase of the cycle	-	-	-	-	-
STERNUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
BONE MARROW (STERNUM);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
FEMUR;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Epiphyseal growth plate(s) open	0	0	0	0	0
STIFLE JOINT, FEMORO-TIBIAL, LEFT;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
HARDERIAN GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SCIATIC NERVE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
EYES;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Fibroplasia; retrobulbar	(1)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0
OPTIC NERVES;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
BRAIN;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SPINAL CORD (CERVICAL SEGMENT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SPINAL CORD (LUMBAR SEGMENT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SPINAL CORD (THORACIC SEGMENT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
THYROID GLANDS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
PARATHYROID GLANDS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
PANCREAS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
THYMUS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
MANDIBULAR GLAND (LEFT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Immature	(1)	(0)	(0)	(0)	(0)
present	1	0	0	0	0
SUBLINGUAL GLAND (LEFT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
PAROTID GLAND (LEFT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
PITUITARY GLAND;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
ADRENAL GLANDS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SKELETAL MUSCLE (LEFT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SKIN STUDY PLAN SAMPLE;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
MAMMARY GLAND;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
STOMACH;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
DUODENUM;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
AORTA;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
ESOPHAGUS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
TRACHEA;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
JEJUNUM;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
COLON;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
ILEUM;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
CECUM;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
MESENTERIC LYMPH NODE;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
HEART;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
LIVER;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SPLEEN;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
LUNGS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
BRONCHUS/BRONCHI;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
KIDNEYS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Cyst(s)	(1)	(0)	(0)	(0)	(0)
present	1	0	0	0	0
OVARIES;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Immature	(1)	(0)	(0)	(0)	(0)
present	1	0	0	0	0
URINARY BLADDER;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
UTERUS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Immature	(1)	(0)	(0)	(0)	(0)
present	1	0	0	0	0
CERVIX;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
VAGINA;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Dioestrus phase of the cycle	1	0	0	0	0
STERNUM;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
BONE MARROW (STERNUM);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
FEMUR;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Epiphyseal growth plate(s) open	1	0	0	0	0
STIFLE JOINT, FEMORO-TIBIAL, LEFT;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: MORIBUND SACRIFICE	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	1	0	0	0	0
Number of Animals Completed:	(1)	(0)	(0)	(0)	(0)
HARDERIAN GLANDS;					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0
SCIATIC NERVE (LEFT);					
Examined	(1)	(0)	(0)	(0)	(0)
Within Normal Limits	1	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
EYES;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
OPTIC NERVES;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
BRAIN;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPINAL CORD (CERVICAL SEGMENT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPINAL CORD (LUMBAR SEGMENT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SPINAL CORD (THORACIC SEGMENT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
THYROID GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
PARATHYROID GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
PANCREAS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
THYMUS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MANDIBULAR GLAND (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SUBLINGUAL GLAND (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
PAROTID GLAND (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
PITUITARY GLAND;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
ADRENAL GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
SKELETAL MUSCLE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
SKIN STUDY PLAN SAMPLE;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MAMMARY GLAND;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
STOMACH;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
DUODENUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
AORTA;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
ESOPHAGUS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
TRACHEA;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
JEJUNUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
COLON;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
ILEUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
CECUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
MESENTERIC LYMPH NODE;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
HEART;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
LIVER;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Vacuolation; hepatocellular	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
SPLEEN;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Extramedullary hematopoiesis, increased	(0)	(0)	(0)	(0)	(0)
slight	0	0	0	0	0
LUNGS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Haemorrhage	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
BRONCHUS/BRONCHI;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
KIDNEYS;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Degeneration; tubular cell	(0)	(0)	(0)	(0)	(0)
minimal	0	0	0	0	0
OVARIES;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
URINARY BLADDER;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: ACCIDENTAL DEATH

	MALES				
	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
UTERUS;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
CERVIX;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
VAGINA;					
Examined	(-)	(-)	(-)	(-)	(-)
Within Normal Limits	-	-	-	-	-
Dioestrus phase of the cycle	-	-	-	-	-
STERNUM;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
BONE MARROW (STERNUM);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
FEMUR;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
STIFLE JOINT, FEMORO-TIBIAL, LEFT;					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
HARDERIAN GLANDS;					
Examined	(0)	(0)	(0)	(0)	(0)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	MALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	0	0	0	0
Number of Animals Completed:	(0)	(0)	(0)	(0)	(0)
HARDERIAN GLANDS; (continued)					
Within Normal Limits	0	0	0	0	0
SCIATIC NERVE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Not Examined: NOT PRESENT	0	0	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
EYES;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
OPTIC NERVES;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
BRAIN;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
SPINAL CORD (CERVICAL SEGMENT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
SPINAL CORD (LUMBAR SEGMENT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
SPINAL CORD (THORACIC SEGMENT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
THYROID GLANDS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
PARATHYROID GLANDS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: ACCIDENTAL DEATH

	FEMALES				
	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
PANCREAS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
THYMUS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
MANDIBULAR LYMPH NODE (LEFT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
MANDIBULAR GLAND (LEFT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
SUBLINGUAL GLAND (LEFT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
PAROTID GLAND (LEFT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
PITUITARY GLAND;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
ADRENAL GLANDS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
SKELETAL MUSCLE (LEFT);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
SKIN STUDY PLAN SAMPLE;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
MAMMARY GLAND;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
STOMACH;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
DUODENUM;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
AORTA;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
ESOPHAGUS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
TRACHEA;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
JEJUNUM;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
COLON;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
ILEUM;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
CECUM;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
MESENTERIC LYMPH NODE;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
HEART;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
LIVER;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Vacuolation; hepatocellular	(0)	(1)	(0)	(0)	(0)
minimal	0	1	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
SPLEEN;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Extramedullary hematopoiesis, increased	(0)	(1)	(0)	(0)	(0)
slight	0	1	0	0	0
LUNGS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Haemorrhage	(0)	(1)	(0)	(0)	(0)
minimal	0	1	0	0	0
BRONCHUS/BRONCHI;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
KIDNEYS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Degeneration; tubular cell	(0)	(1)	(0)	(0)	(0)
minimal	0	1	0	0	0
OVARIES;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
URINARY BLADDER;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
UTERUS;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
CERVIX;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
VAGINA;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Dioestrus phase of the cycle	0	1	0	0	0
STERNUM;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
BONE MARROW (STERNUM);					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
FEMUR;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
STIFLE JOINT, FEMORO-TIBIAL, LEFT;					
Examined	(0)	(1)	(0)	(0)	(0)
Within Normal Limits	0	1	0	0	0
HARDERIAN GLANDS;					
Examined	(0)	(1)	(0)	(0)	(0)

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Summary of microscopic findings

AB20758

Observations: Neo-Plastic and Non Neo-Plastic	FEMALES				
Removal Reason: ACCIDENTAL DEATH	0 mg/kg/day	Ref_5000 mg/kg/day	1000 mg/kg/day	2500 mg/kg/day	5000 mg/kg/day
Number of Animals on Study :	0	1	0	0	0
Number of Animals Completed:	(0)	(1)	(0)	(0)	(0)
HARDERIAN GLANDS; (continued)					
Within Normal Limits	0	1	0	0	0
SCIATIC NERVE (LEFT);					
Examined	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0
Not Examined: NOT PRESENT	0	1	0	0	0

Appendix B

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Infant Formula and Food Uses of Human-identical Milk Oligosaccharide Lacto-*N*-neotetraose (LNnT)

Expert Panel Consensus Statement Concerning the Determination that Lacto-*N*-neotetraose (LNnT) is Generally Recognized as Safe (GRAS) for Uses in Infant Formula and Conventional Food Products

June 2, 2016

INTRODUCTION

Glycom A/S (Glycom) convened a panel of independent scientists (the "Expert Panel"), qualified by their scientific training and relevant national and international experience in the safety evaluation of food ingredients, to conduct a critical and comprehensive assessment of the available pertinent data and information on the human-identical milk oligosaccharide, lacto-*N*-neotetraose (LNnT), produced by fermentation using a modified strain of *Escherichia coli* K-12 DH1, and to determine whether the intended uses of LNnT in term infant formula and conventional food and beverage products (as described in Table A-1), would be Generally Recognized as Safe (GRAS) based on scientific procedures. The Expert Panel consisted of the below-signed qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University School of Medicine), Dr. Ronald Kleinman (Mass General Hospital for Children), Dr. Robert J. Nicolosi (University of Massachusetts Lowell), and Dr. John A. Thomas (Indiana University School of Medicine).

The Expert Panel, independently and collectively, critically evaluated a comprehensive package of scientific information and data compiled from the literature. This information was presented in a dossier provided by Glycom [Documentation Supporting the Determination that Lacto-*N*-neotetraose, Produced by Fermentation is Generally Recognized as Safe (GRAS) for Use in Infant Formula and Food], which included an evaluation of all available scientific data and information, both favorable and unfavorable, relevant to the safety of the intended food uses of Glycom's ingredient. This information was prepared in part from a comprehensive search of the scientific literature and also included information characterizing the identity and purity of the ingredient, manufacture of the ingredient, product specifications, supporting analytical data, intended conditions of use, estimated exposure under the intended uses, information on the history of consumption from human breast milk, and studies investigating the safety of LNnT. In addition, the Expert Panel evaluated other information deemed appropriate or necessary.

Following its independent critical evaluation, and on the basis of scientific procedures, the Expert Panel unanimously concluded that LNnT, produced by fermentation using a modified strain of *E. coli* K-12 DH1, meeting food-grade specifications and manufactured consistent with current Good Manufacturing Practice (cGMP), is GRAS for use in term infant formula and

conventional food products as described in Table A-1. A summary and discussion of the information critically evaluated by the Experts are presented below.

SUMMARY AND BASIS FOR GRAS

On September 25, 2014, Glycom A/S (Glycom) submitted a notice to the United States (U.S.) Food and Drug Administration (FDA), informing the Agency of Glycom's determination that a human-identical milk oligosaccharide (HiMO), LNnT, was Generally Recognized as Safe (GRAS) for use as an ingredient in non-exempt term infant formula at a maximum use level of 600 mg per liter, and in various conventional food and beverage products at use levels ranging from 0.02 to 1.2 g per serving. The notice was filed on October 10, 2014, and designated as GRN No. 547 (Glycom A/S, 2014). Following critical review of Glycom's notice, the agency concluded the following: "*Based on the information provided by Glycom, as well as other information available to FDA, the agency has no questions at this time regarding Glycom's conclusion that LNnT is GRAS under the intended conditions of use*." (U.S. FDA, 2015a)

Glycom has since revised the production process to also include the use of microbial fermentation for the synthesis of LNnT. LNnT is a linear tetrasaccharide consisting of D-galactose, *N*-acetyl-D-glucosamine, D-galactose and D-glucose. The LNnT ingredient, manufactured using microbial fermentation, is chemically equivalent to both the ingredient produced by chemical synthesis, and to naturally occurring LNnT from human milk. The structural identity has been confirmed by ^{1}H- and 2D-NMR-spectroscopy confirming its qualitative chemical equivalence to LNnT in human milk.

The Expert Panel critically reviewed the details of the manufacturing process for LNnT. The ingredient is produced consistent with current Good Manufacturing Practice (cGMP) and principles of Hazard Analysis and Critical Control Points (HACCP). The manufacturing process can be broadly divided into two stages: Stage 1 [upstream processing (USP)], and Stage 2 [downstream processing (DSP)].

In the first stage, USP, D-lactose is converted *via* the metabolic intermediate "lacto-*N*-triose II" to LNnT by the cellular enzymes of the LNnT production organism. In the second stage, the DSP, a series of purification and isolation steps generate the final high-purity LNnT product. The production microorganism is a derivative of *Escherichia coli* K-12 DH1, which is a safe laboratory strain with a well-characterized genetic history (Hanahan, 1983; Luli and Strohl, 1990; Bachmann, 1996). *E. coli* K-12 DH1 was optimized for general oligosaccharide expression features by the introduction of several modification events related to the metabolism of various sugars, then transformed with a high-copy plasmid carrying 2 enzymes necessary for LNnT synthesis. The resulting strain was designated MP572 and has been deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Germany.

LNnT is excreted into the fermentation broth and the microbial biomass containing the production organism is effectively removed from the culture supernatant by ultrafiltration/ diafiltration. The microbial biomass is then separately deactivated by heat treatment. During Stage 2 (DSP), a series of purification steps are employed, which include nanofiltration (for removal of minerals, salts and other small molecule impurities from fermentation), ion removal (for removal of charged molecules), decoloration (for removal of colored and lipophilic impurities), followed by chromatographic isolation (for removal of other carbohydrates) and a final and highly selective crystallization step (for removal of final traces of virtually any kind of impurities), which results in the production of a high-purity, crystalline tetrasaccharide LNnT.

Both Manufacturing Stages (USP and DSP) are controlled by a HACCP plan which includes specifications for equipment, raw materials, product, and packaging materials. Master operating instructions are followed, batch records kept, a number of in-process controls are applied, and the isolated product is controlled by certificates of analysis and batch release routines.

Glycom has established food-grade specifications for LNnT. Specifications include limits related to purity, physical properties, water content, ash content, heavy metal content, and microbial contaminants. The purity of LNnT is set at a minimum assay value of 92.0% on a dry weight basis and a total human milk saccharide content of at least 95.0%. Specifications have been set for carbohydrate-type impurities (including D-Lactose, Lacto-*N*-triose II, *para*-Lacto-*N*-neohexaose, and LNnT fructose isomer) as well as residual solvents and proteins. All analytical methods are nationally or internationally recognized standard methods or have been validated using in-house procedures by Glycom. The Expert Panel reviewed the batch results from 4 independent production batches of LNnT and confirms that the data demonstrate that the manufacturing process yields a consistent material in conformance with the product specifications.

The results of impurity and contaminant analyses on the same batches were made available to the Expert Panel. Upon review of the test results for the potential presence of amino acids and biogenic amines, microbial endotoxins, residual proteins, *Enterobacteriaceae* (production organism), DNA, trace elements and minerals, the Expert Panel confirmed that the ingredient does not contain undesirable levels of those substances. In addition, the results of bulk stability tests on LNnT has been comprehensively evaluated under several long-term studies conducted in ambient (25°C, 60% relative humidity) and accelerated conditions (40°C, 75% relative humidity), the results of which demonstrate that LNnT is stable for up to 5 years under ambient storage conditions and for up to 2 years under accelerated conditions. The results of stability studies conducted in representative food matrices, including infant formula, yogurts, ready-to-drink flavored milk, and citrus fruit beverages, confirm that the ingredient is expected to be stable in its intended food and beverage products.

LNnT, produced using microbial fermentation, is intended to serve as an alternative to LNnT produced by chemically synthesis described in GRN No. 547 (Glycom A/S, 2014). As detailed

in GRN 547, LNnT is intended for use in non-exempt term infant formula and follow-on formula at a use level of 600 mg/L of the reconstituted or ready-to-drink product. The Expert Panel noted that the uses of LNnT in conventional food and beverage products described in GRN 547 have been revised. Previous food uses of LNnT in baked goods and baking mixes, carbonated beverages, flavored and enhanced waters, coffee and tea, beverage whiteners, fruit flavored drinks and ades, vegetable juices and nectars, and table top sweeteners have been deleted. Furthermore, the intended inclusion levels of LNnT in non-dairy and dairy yogurt have been lowered from a maximum of 1.2 grams per serving to 0.6 grams per serving (Table A-1). As infants (<1 year of age) are not frequent consumers of conventional food products to which LNnT may be added, the aforementioned revision of the food uses and use levels for various conventional food products will not change estimated exposures to infants previously described in GRN 547, and the introduction of LNnT, produced by fermentation, to the U.S. marketplace will not change dietary intakes in this population group. Mean and 90th percentile intakes of LNnT were 0.51 and 0.73 g/person/day (83.2 and 133.9 mg/kg body weight respectively) in infants aged 0 to 6 months of age. In infants 7 to 12 months of age dietary intakes of LNnT were estimated to be 0.42 and 0.66 g/person/day (48.5 and 79.5 mg/kg body weight respectively).

Revised intake estimates for LNnT among toddlers aged 1 to 3 and older population groups were generated using the most recently published food consumption data from the U.S. National Center for Health Statistics' (NCHS) 2011-2012 National Health and Nutrition Examination Surveys (NHANES). Among the individual population groups, the highest mean and 90th percentile intakes of LNnT on both an absolute and per body weight basis were identified in toddlers ages 1 to 3 years old. The mean and 90th percentile of intakes of LNnT in this population group were determined to be 514 mg/person/day (38.4 mg/kg body weight/day), and 901 mg/person/day (67.7 mg/kg body weight/day), respectively. Elderly adults had the lowest mean all-user intakes on an absolute and per body weight basis of 243 mg/person/day (3.4 mg/kg body weight/day) and also had the lowest 90th percentile intake on a body weight basis of 7.5 mg/kg body weight/day. On an absolute basis, females of childbearing age had the lowest 90th percentile intakes of 526 mg/person/day. As expected, the deletion of the intended uses of LNnT in select food categories and the reduction of the use levels of LNnT in non-dairy and dairy yogurts resulted in a reduction in overall intake of LNnT among U.S. consumers.

The Expert Panel critically evaluated published data and information characterizing the safety of LNnT. LNnT is an important member of the complex oligosaccharide mixture that is present in human milk. It is among the most abundant oligosaccharides of human milk, and is the second-most abundant core-structure, with a high number of HMOs derived by addition of L-fucose and/or sialic acid to the LNnT core-structure (Urashima *et al*., 2012). Glycom reviewed published studies examining the concentration of LNnT in human milk samples. Levels of LNnT in pooled milk samples that have been reported are highly variable across studies: concentrations as low as 39.2 mg/L in milk samples from mothers 1-month post-partum were

reported in Alderete *et al.* (2015), and concentrations as high as 2,230 were reported in milk samples obtained from Se+/Le+ type mothers during first month of lactation (Galeotti *et al.*, 2012). The mean concentration of LNnT in pooled milk samples across all studies was 636 mg/L human milk; at this concentration an infant consuming 51 mg/kg body weight/day of breast milk per day (U.S. EPA, 2011) would theoretically be consuming approximately 32.4 mg LNnT/kg body weight/day. Among infants in the 90th percentile of human milk intake (108 mg/kg body weight/day), the corresponding consumption of LNnT would be 68.7 mg LNnT/kg body weight/day. The Expert Panel noted that these dietary intakes of LNnT among breast fed infants were quantitatively similar to those estimated for LNnT from intended uses in infant formula. The safety of LNnT for addition to term infant formula is therefore principally established through its history of human consumption, on the basis that LNnT manufactured by Glycom is chemically equivalent to LNnT in human milk, and, as a nutritive component of human milk from lactating women, can be considered GRAS for addition to infant formula at similar levels.

Published data and information characterizing the metabolic fate of LNnT was comprehensively reviewed in Section IV.B.4 of GRN No. 547 (U.S. FDA, 2015a). HMOs, including LNnT, do not undergo any appreciable level of digestion in the upper gastrointestinal tract and only small amounts of monosaccharides derived from HMOs would be available for absorption (see GRN No. 547, Engfer *et al.*, 2000; Gnoth *et al.*, 2000). The available data in infants indicate that the majority of an ingested amount of an HMO, including LNnT, reaches the large intestine where the HMO serves as a substrate for bacterial (intestinal bacterial microflora) metabolism or is excreted unchanged in the feces (Brand-Miller *et al.*, 1995, 1998; Chaturvedi *et al*., 2001; Coppa *et al.*, 2001).

Comprehensive discussions of the published toxicity studies, as they apply to the safety of LNnT for use in infant formula, were incorporated by reference to GRN 547. These studies include findings from short-term and subchronic toxicity studies in neonatal rats and *in vitro* evaluation of mutagenicity/genotoxicity using the Ames assay and mouse lymphoma assay (Coulet *et al*., 2013). In three separate studies neonatal (post-natal day 7) Wistar [Crl:WI(Han)] rat pups[1] were administered LNnT by gavage at doses of 0 (water vehicle control), 1,000 (low-dose), 2,000 (mid-dose), or 5,000 (high-dose) mg/kg body weight/day from post-natal day 7 to up to days 14, 28 or 91. A reference control group (15 rats/sex/group) was administered 5,000 mg/kg body weight/day of oligofructose (OF). LNnT was well-tolerated at doses of up to 5,000 mg/kg body weight/day in all studies, with the only notable observations reported by the authors being colored/liquid feces during the first few days of the administration period. A NOAEL of 5,000 mg/kg body weight, the highest dose tested, was established by the authors for the subchronic study. This study has been subsequently reviewed within a recent novel food opinion published by the European Food Safety Authority (EFSA, 2015) for the use of LNnT in

[1] The control and high-dose groups each consisted of 15 males and 15 females, while the low- and mid-dose groups each consisted of 10 males and 10 females.

infant formula and conventional food products. The agency stated that "*Based on the observations on reticulocytes, platelet counts, Hb levels and PCV in the high-dose LNnT group (5 000 mg/kg body weight per day) and the decrease in the zymogen content in acinar cells in three animals in the high-dose LNnT group, the Panel considers that the no observed adverse effect level (NOAEL) is 2 500 mg/kg body weight per day*." (EFSA, 2015). The Expert Panel noted that hemoglobin levels were 3.2% lower in the high-dose males on day 91 *vs*. control animals, significant differences in hemoglobin levels were not reported in the female LNnT groups and were not reported in male or female treatment groups on day 28. Platelet count was 9.6% lower ($P<.05$) in the high-dose males relative to controls; however, no significant differences were reported for females and no differences or trends towards reduced platelet counts were reported in any group at day 28. The Expert Panel considered differences in various hematological parameters to be a spurious finding of no biological significance. With respect to reduced zymogen content in pancreatic acinar cells of 3 animals in the high-dose group, the findings were of low severity (slight to minimal), within the normal background variation and not reported in the recovery animals. The Expert Panel considers these findings to be incidental and unrelated to LNnT. The Expert Panel also noted that similar changes in pancreatic acinar cells were not reported in subsequent subchronic toxicity studies conducted using LNnT produced by fermentation. It is the opinion of the Expert Panel that the NOAEL of 5,000 mg/kg body weight/day as originally established by the study authors was appropriate. This conclusion is corroborated by the results of additional toxicity studies of 2'-FL not available to EFSA during their review. These studies are discussed below.

The Expert Panel critically evaluated new studies relevant to the safety of LNnT, including a subchronic oral toxicity study in neonatal rats and *in vitro* genotoxicity assays conducted on the material of commerce that is the subject of this GRAS determination (*i.e*., LNnT produced by fermentation) (Penard, 2016; Verbaan, 2016; Verspeek-Rip, 2016). As expected, the results of these studies are consistent with findings from studies reported by Coulet *et al*. (2013) and therefore provide additional evidence to corroborate safety of LNnT for its intended uses in infant formula and conventional foods. New studies examining the toxicity/safety of LNnT published since Glycom's GRAS determination of LNnT in 2014 (*i.e*., GRN 547) were not identified during Glycom's updated search of the scientific literature. A brief summary of the subchronic and genotoxicity studies conducted using LNnT produced by fermentation are presented below.

The final study report of the subchronic study of LNnT, produced by fermentation, was provided to the Expert Panel (Penard, 2016). The subchronic toxicity study was conducted using neonatal (7 days of age) Wistar [Crl:WI(Han)] rats, and utilized the same study design reported previously by Coulet *et al*. (2013). The study was conducted in accordance with Good Laboratory Practice (GLP) and in consideration of internationally accepted guidelines for the toxicity testing of chemicals and food ingredients (*e.g*., OECD 408; FDA Redbook) (OECD, 1998a; U.S. FDA, 2000). Wistar pups were administered LNnT by gavage at doses of 0 (water

vehicle control), 1,000 (low-dose), 2,500 (mid-dose), or 5,000 (high-dose) mg/kg body weight/day of LNnT (94.4% LNnT by assay) or a reference compound, fructo-oligosaccharide (FOS), at 5,000 mg/kg body weight/day for 90 or 91 days. Additional groups of 5 males and 5 females were given the control, LNnT, or FOS doses for 90 days and were terminated after a 28-day recovery period.

No test article-related mortalities occurred during the study. Isolated occurrences of hypersalivation were reported in 1 high-dose male and 3 high-dose females receiving LNnT and were considered by the study authors to be a non-adverse finding. No test article-related ophthalmological findings were reported. No remarkable effects in body weight, body weight gain, or food consumption were reported. No toxicologically relevant effects in tibia length, reflex and physical development, time to sexual maturation, learning capacity, memory, motor activity (as evaluated in the Morris water maze), exploratory behavior, or general movement (as evaluated in the open-field test) were reported at any dose level.

Statistically significant differences in hematology parameters were noted, however these lacked a dose-response relationship, were minimal in magnitude (~10% or lower), and were considered unrelated to LNnT administration. Similarly, no test article-related effects in serum clinical chemistry parameters were reported and any statistically significant differences in serum clinical chemistry parameters were considered to be unrelated to LNnT administration because they were not dose-dependent, seen in only one sex, and/or were within historic control values.

A statistically significant increase in urine volume in high-dose animals and a statistically significant decrease in specific gravity were reported in high-dose animals compared with controls. However, these were deemed by the study investigators to be incidental and of no toxicological relevance due to the lack of dose-response or histopathological changes in the kidney. No treatment-related differences in organ weights, macroscopic observations, or histological observations were reported between rats receiving LNnT and the control and reference groups.

The NOAEL for this study was 5,000 mg/kg body weight/day, the highest dose tested. The Expert Panel concurs with this determination.

Published studies evaluating the mutagenicity/genotoxicity of LNnT (produced by chemical synthesis) were reported by Coulet *et al*. (2013). These studies included the Ames assay (OECD TG 471) and the *in vitro* mouse lymphoma assay (OECD TG 476). The Ames assay was conducted using *Salmonella* tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 102, in the presence and absence of metabolic activation using doses up to 5,000 µg per plate. In the mouse lymphoma assay LNnT was examined for its ability to induce gene mutations at the (TK)-locus of cultured mouse lymphoma L5178Y cells in the presence and absence of metabolic activation at use levels up to the maximum soluble concentration of 4,250 µg/mL. LNnT was non-mutagenic/genotoxic in these studies.

Study reports characterizing the mutagenic/genotoxic effects of LNnT produced by fermentation (94.4% LNnT by assay) in the Ames assay and the *in vitro* micronucleus assay in cultured peripheral human lymphocytes (Verbaan, 2016; Verspeek-Rip, 2016) were critically evaluated by the Expert Panel. Both studies were conducted in compliance with OECD principles of GLP and according to OECD Test Guidelines No. 471 and 487, respectively (OECD, 1997, 1998b). The results of these studies demonstrate that LNnT is not mutagenic or genotoxic when tested at concentrations of up to 5,000 µg/plate both in the presence and absence of metabolic activation.

Two clinical studies in infants (one with LNnT alone and one with LNnT in combination with 2'-fucosyllactose, 2'-FL) and one study in adults were critically evaluated by the Expert Panel (Prieto, 2005; EFSA, 2015; Puccio *et al.*, 2016).

The study by Prieto (2005) was critically evaluated by Glycom and the Expert Panel previously during Glycom's GRAS determination of 2'-FL and is incorporated by reference to GRN 457. Briefly, LNnT produced using a yeast fermentation process (Abbott Laboratories, Columbus Ohio) was provided to 228 healthy male and female infants and toddlers between the ages of 6 to 24 months at an inclusion level of 220 mg/L in formula for 112 days. An additional control group received the same formula without LNnT. No differences in body weight or infant length were reported between the groups, and no indication of adverse effects on *S. pneumonia* colonization or abnormal ear pathologies were reported.

The safety of LNnT (manufactured by Glycom using chemical synthesis) in combination with 2'-FL, was investigated in a randomized, blinded, controlled, multi-center, parallel-design study (Puccio *et al.*, 2016), which was critically reviewed by the Expert Panel. A total of 175 healthy, full-term infants from 0 to 6 months of age were provided a standard infant formula supplemented with LNnT (at a target concentration of 0.5 g LNnT/L reconstituted formula) in combination with 2'-FL (at a target concentration of 1.0 g/L reconstituted formula). No significant differences in formula intake were reported between the test and control groups. The mean weight gain in the test group was determined to be non-inferior to the mean weight gain in the control group in both the intent-to-treat population and the per protocol population. Infants receiving the test formula did not differ from control with regard to weight, length, head circumference, body mass index (BMI) or corresponding z-scores for digestive tolerance. Mean length, head circumference, and body mass index of the infants from enrolment to 4 months of age were not statistically significantly different between the test and control groups and were comparable with the WHO Growth Standard. The incidence of adverse events was not higher in infants fed the test formula compared to the control standard formula.

The safety and tolerability of LNnT was investigated in a randomized, placebo-controlled, double-blind, parallel-design study in which healthy adult volunteers (51 men and 49 women; mean age of 36.0 years) were provided LNnT and 2'-FL alone or in combination at different doses for 2 weeks. Findings from this study were recently evaluated and summarized by EFSA

(2015). LNnT and 2'-FL were provided at bolus daily doses of 5, 10, or 20 g/day. A comparator group receiving 2 g glycose as a control also was included.

All adverse events reported during the study were judged to be "mild" and there were no cases of premature discontinuation from the trial due to adverse events. Most adverse events were judged to be "possibly" related to the test article; however, many symptoms (*e.g*. gas or flatulence) were noted by the study investigators to be common and difficult to ascertain whether they were related to the test article, to normal day-to-day variation, or to increased awareness of gastrointestinal symptoms during the trial period. Nevertheless, a significantly higher incidence of passing gas was reported in individuals consuming 10 or 20 g of LNnT treatment relative to controls. Significantly higher incidences of nausea, rumbling, bloating, passing gas, diarrhea, loose stools and urgency were reported in subjects receiving 20 g per day relative to the placebo.

Hematological and blood biochemistry analyses obtained at the 2-week time-point remained within the normal range for all subjects and any minor changes over the course of the study compared to baseline values were not considered clinically relevant. Gastrointestinal Symptom Rating Scale (GSRS) and Bristol Stool Form Scale (BSFS) scores indicated that both LNnT and 2'-FL were well-tolerated. The results support the tolerability and safety in healthy adult men and women consuming LNnT, either alone or in combination with 2'-FL, at the doses tested. The Expert Panel noted that large bolus intakes of 20 g of LNnT may represent a gastro-intestinal tolerability threshold for some individuals. The Expert Panel further noted that the results of intake estimates demonstrate that usual consumption patterns of LNnT among U.S. consumers from all proposed food uses are well below levels that may produce undesirable gastrointestinal effects.

The potential allergenicity of LNnT was considered during Glycom's GRAS determination. Glycom noted that lactose is used as a raw material during the fermentation process; thus, in accordance with FALCPA (Food Allergen Labeling and Consumer Protection Act of 2004), the labeling of the bulk ingredient 'contains milk' would be required. No other allergenic sources are used during manufacturing and the finished ingredient is free from protein (at a quantitation limit of 0.0017%). Nevertheless, a bioinformatics evaluation of the introduced proteins expressed by the production organism was conducted using the database and search algorithms provided by the most recent version of the Allergen Online tool (version 16) of the University of Nebraska. The Expert Panel reviewed the comprehensive report on the sequence analysis. No full length sequence alignments ≥50% were identified for any new protein. No 80-amino acid segments matched with >35% identity to an allergen, and no 8-mer sequence alignment was identified. The Expert Panel concluded that there is no evidence that indicates that the introduced proteins would cross-react with known or putative allergens.

The Expert Panel critically evaluated the information supporting the safety of the production strain and noted that the development of *E. coli* strain K-12 DH1 has been well characterized

and its genomes have been sequenced and compared to other strains of *E. coli* (Hanahan, 1983; Luli and Strohl, 1990; Bachmann, 1996; Blattner *et al.*, 1997; U.S. EPA, 1997; Lukjancenko *et al.*, 2010). The Expert Panel concluded that the host strain, *E. coli* K-12 and its derivatives were generally recognized as safe and suitable for use as a host organism for the construction of modified microorganisms used for the production of food ingredients. The Expert Panel critically evaluated detailed information regarding the modifications leading to the development of the final LNnT production strain, MP572 and noted that all introduced genes were well characterized with respect to their function, did not have homology to known protein toxins, and as enzymes involved in LNnT biosynthesis, were not reasonably expected to introduce toxicogenic/pathogenic attributes to the host strain. The Expert Panel considered safety concerns related to the introduced genes and corresponding gene expression products to be very low.

Therefore, the basis of GRAS is founded on the substantial chemical and functional equivalence of synthetic LNnT, produced chemically or by fermentation, with the human LNnT.

CONCLUSION

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that lacto-*N*-neotetraose (LNnT), produced by fermentation using a modified strain of *E. coli* K-12 DH1, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable for use as an ingredient in non-exempt term infant formula and specified conventional food and beverage products described in Table A-1.

We, the members of the Expert Panel, have independently and collectively critically evaluated the information summarized above and conclude that LNnT, produced by fermentation using a modified strain of *E. coli* K-12 DH1, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice is Generally Recognized as Safe (GRAS), based on scientific procedures, for use as an ingredient in non-exempt term infant formula and specified conventional food and beverage products as described in Table A-1.

It is our opinion that other qualified experts would concur with these conclusions.

(b) (6)

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11 August 2016
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Attachment A1

Intended Food and Beverage Uses and Use Levels for LNnT in the United States

Table A-1 Intended Food and Beverage Uses and Use Levels for LNnT in the United States

Food Category	Proposed Food-Uses	RACC[a]	Proposed Use Level (g/RACC)	Proposed Maximum Use Level (g/kg or g/L)[b]
Beverages and Beverage Bases	Meal Replacement Drinks, for Weight Reduction	240 mL	0.6	2.5
	Sports, Isotonic, and Energy Drinks	240 mL	0.14	0.58
Dairy Product Analogs	Imitation Milks	240 mL	0.14	0.58
	Non-Dairy Yogurt	225 g	0.6	2.67
Infant and Toddler Foods	Term Infant Formulas	100 mL[c]	0.06	0.60
	Toddler Formulas	100 mL[c]	0.06	0.60
	Other Baby Foods for Infants and Young Children	7 to 170 g	0.02 to 0.68	3.0
	Other Drinks for Young Children	120 mL	0.07	0.58
Grain Products and Pastas	Meal Replacement Bars, for Weight Reduction	30 g	0.6	20.0
Milk, Whole and Skim	Unflavored Pasteurized and Sterilized milk[d]	240 mL	0.14	0.58
Milk Products	Buttermilk	240 mL	0.14	0.58
	Flavored Milk	240 mL	0.14	0.58
	Milk-Based Meal Replacement Drinks, for Weight Reduction	240 mL	0.6	2.5
	Yogurt	225 g	0.6	2.67
Processed Fruits and Fruit Juices	Fruit Juices and Nectars	240 mL	0.14	0.58

LNnT = lacto-*N*-neotetraose; RACC = Reference Amounts Customarily Consumed.
[a] Serving sizes were based on RACCs per Eating Occasion in the United States Code of Federal Regulations (21 CFR §101.12 - U.S. FDA, 2015b).
[b] The proposed maximum use level is presented on a g/kg basis for solids and on a g/L basis for liquids.
[c] RACC not available, 100 mL employed as an approximation.
[d] Milk is a standardized food in the United States. When the milk is fortified with 2'-FL, it will then be classified as a milk product. The intake of the category "unflavored pasteurized and sterilized milks" was used here as a conservative proxy for the dietary pattern of the fortified milk drink product.

Glycom A/S Kogle Allé 4 2970 Hørsholm, Denmark

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JUL 29 2021
OFFICE OF
FOOD ADDITIVE SAFETY

26 July 2021

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: Supplement to GRAS Notice for Lacto-*N*-Neotetraose (LNnT) Produced by Microbial Fermentation (GRN 000659)

Glycom A/S (Glycom), a manufacturer of human-identical milk oligosaccharides (HiMOs), has previously concluded that the company's lacto-*N*-neotetraose (LNnT) produced by microbial fermentation has Generally Recognized as Safe (GRAS) status for use as a food ingredient for addition to non-exempt term infant formula and various conventional food and beverage products across multiple categories. Glycom's GRAS conclusion was notified to the offices of the United States Food and Drug Administration (U.S. FDA) on July 5, 2016 and was filed by the agency under GRN No. 659. In accordance with 21 CFR §170.280, Glycom hereby submits the following supplemental information to GRN 659, which includes a description of an alternative production strain (strain MP572b alternatively to strain MP572). Notably:

1. LNnT manufactured with strain MP572 remains in commercial supply.
2. A review of new information as per Part 3 of this supplement and the results of an updated literature search do not change Glycom's conclusion that LNnT produced by strain MP572 is GRAS.
3. Strain MP572b is positioned as an alternative production strain, engineered to produce higher titres of LNnT. The nature of the modifications was deemed by Glycom to be a significant change in the manufacturing process of LNnT warranting notification to the U.S. FDA.

Further description of this supplement and a narrative supporting that this proposed alternative production process would not change the GRAS status of LNnT are discussed in the attached amendment. Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (4)

Christoph H. Röhrig, Ph.D.
Senior Scientist
Head of Regulatory & Scientific Affairs
Glycom A/S

GRAS STATUS OF LACTO-*N*-NEOTETRAOSE (LNnT)

Supplement to GRN 659

SUBMITTED BY:
Glycom A/S
Kogle Allé 4
2970 Hørsholm
Denmark

Glycom A/S is a wholly owned indirect affiliate of DSM Nutritional Products Ltd, a company with registered address at Wurmisweg 576, 4303 Kaiseraugst, Switzerland.

DATE:
26 July 2021

GRAS Status of Lacto-*N*-neotetraose (LNnT)
Supplement to GRN 659

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GRAS Status of Lacto-*N*-neotetraose (LNnT) Supplement to GRN 659

PART 1. INTRODUCTION

Glycom A/S (Glycom)[1], a manufacturer of human-identical milk oligosaccharides (HiMOs), has previously concluded that the company's lacto-*N*-neotetraose (LNnT) produced by microbial fermentation is Generally Recognized as Safe (GRAS) for use as a food ingredient for addition to non-exempt term infant formula and specified conventional food and beverage products. Glycom's GRAS conclusion was notified to the offices of the United States Food and Drug Administration (U.S. FDA) on 05 July 2016 and was filed without objection by the agency under GRN 659 (U.S. FDA, 2016). Since then, Glycom has developed an alternative production strain, enabling the biosynthesis of LNnT at higher titers and with an alternate carbon source (sucrose). Glycom considers this change to represent a major manufacturing change, and therefore, in accordance with 21 CFR §170.280 (U.S. FDA, 2020), Glycom hereby submits the following supplemental information to GRN 659 (U.S. FDA, 2016), which includes a description of the genetic modifications to the improved production strain (hereafter referred to as *Escherichia coli* K-12 DH1 MDO MP572b, or strain MP572b), as well as corresponding analytical data for three independent production batches demonstrating compliance with the ingredient specifications. A narrative supporting that the addition of strain MP572b as an alternative LNnT production strain would not change the original GRAS conclusions is discussed below in Part 3 of this supplement.

PART 2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

2.1 Changes in the Production Strain

The original Glycom plasmid-based strain *Escherichia coli* K-12 DH1 MDO MP572 used for the manufacture of LNnT has been complemented with an improved fully chromosomal strain *E. coli* K-12 DH1 MDO MP572b that is equipped to biosynthesize LNnT using sucrose as the carbon source. Additional genetic modifications, including the chromosomal integration of the β-1,4-galactosyltransferase gene previously harbored on a plasmid, the introduction of a transporter, and the deletion of genes involved in mixed-acid fermentation, makes it a more robust production strain, resulting in a significantly increased LNnT productivity and an improved resilience towards certain types of stress (low oxygen, high sugar, high osmolarity) encountered in a large-scale manufacturing environment.

The genetic modifications to the *E. coli* K-12 DH1 MDO platform strain to produce the production strain MP572 are described in detail in Section II.B.1.2 of GRN 659 (U.S. FDA, 2016). In brief, the MDO platform strain was transformed to enable LNnT production in a two-step process: by the introduction of β-1,3-*N*-acetylglucosaminyltransferase (*lgtA* gene) that converts lactose into lacto-*N*-triose II (step A, Figure 2.1-1), and a β-1,4-galactosyltransferase (*galT* gene) that converts lacto-*N*-triose II to LNnT (step B).

[1] Glycom A/S is a wholly owned indirect affiliate of DSM Nutritional Products Ltd, a company with registered address at Wurmisweg 576, 4303 Kaiseraugst, Switzerland.

To support an antibiotic resistance marker-free plasmid system in the original MP572 production strain, the *nadC* gene encoding the enzyme quinolinate phosphoribosyltransferase, was deleted from the genome of the platform strain. Finally, the gene encoding the repressor of the Lac operon, *lacI,* was deleted from the genetic background in order to enable the induction of gene expression from the native P*lac* promoter of the *lac* operon without the need of IPTG (isopropyl-β-D-1-thiogalactoside) addition during the fermentation.

An alternative and improved strain was developed to increase LNnT productivity and enhance its resilience. Modifications of MP572 resulting in the alternative production strain MP572b are listed as follows:

- Unlike the original strain MP572, the alternative and improved production strain MP572b does not employ a plasmid-based system; as such, the native *nadC* gene was preserved.

- A single genomic copy of the *galT* gene, previously harbored in the high copy pBS plasmid, was integrated in the genome of the strain MP572b, enabling better control of gene dosage by having a defined number of gene copies in each cell. For optimization of LNnT biosynthesis, two copies of the *lgtA* gene were introduced into the chromosomal DNA of the MDO platform strain at two different targeted loci.

- Three deletions were performed to prevent mixed-acid fermentation under anaerobic conditions and to minimize acetate formation. These included the double knock-outs of lactate dehydrogenase (*ldhA*) and the formate channel and pyruvate-formate lyase (*focA-pflB*), and the single knock-out of the transcriptional repressor regulating the glyoxylate shunt (*iclR*). These modifications aimed to protect the strain against the detrimental effect of overflow metabolism as exemplified by lactate, formate, and acetate formation.

- Deletion of the *hlyE* gene was performed on a precautionary basis to eliminate any hypothetical risk of unintentional expression of the dormant hemolytic toxin cytolysin A.[2]

- Two genetic cassettes carrying *scrYA* genes (from *Klebsiella pneumoniae*) and the *scrBR* genes (*Salmonella* Typhimurium) were introduced at the *pflB-focA* and *ldhA* loci, respectively. The *scrYA* and *scrBR* genes encode enzymes, membrane, and regulatory proteins required for sucrose utilization.

- An extra copy of the inherent wild-type *E. coli lacY* gene encoding lactose permease was integrated to enhance the import of lactose into the cell interior.

- Introduction of the major facilitator superfamily (MFS) transporter *vag* (from *Pantoea vagans*) was performed to enable facilitated secretion of LNnT extracellularly.

- The bacterial hemoglobin (*vgb)* gene (from *Vitreoscilla*) was introduced to enable sustained aerobic metabolism under hypoxic or microaeration conditions potentially encountered in large full-scale fermentation.

[2] Cytolysin A was listed in a EFSA supporting publication from 2017 (de Benito *et al*., 2017) as a typically non-expressed putative toxin of *E. coli* K-12-derived strains.

The production strain *E. coli* K-12 DH1 MDO MP572b has been deposited in a recognized culture collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ). A schematic of the biochemical pathway of the production of LNnT in the production strain *E. coli* K-12 DH1 MDO MP572b is provided in Figure 2.1-1.

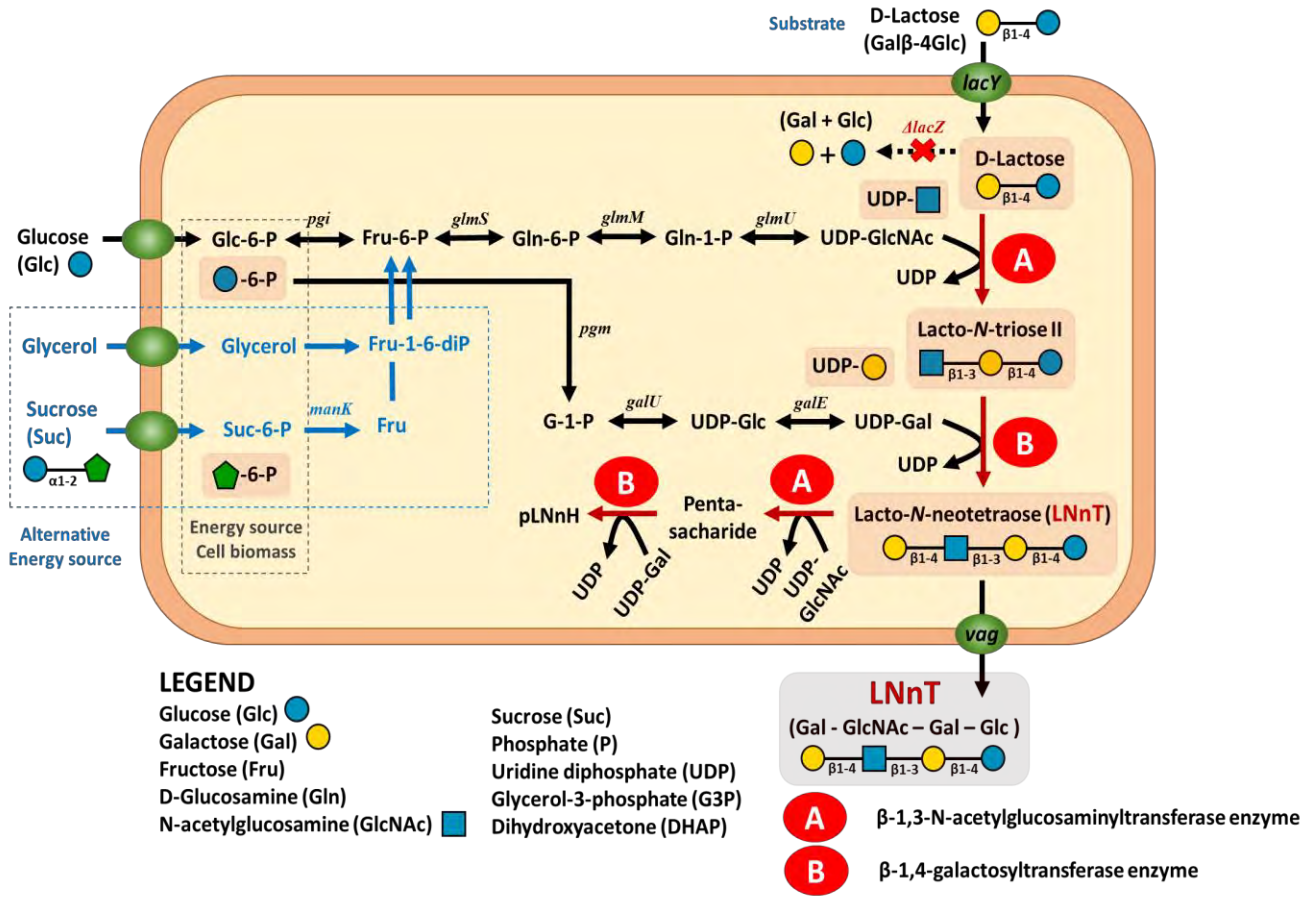

Figure 2.1-1 Schematic of the Biochemical Pathway of LNnT Production in Strain *Escherichia coli* K-12 DH1 MDO MP572b

The enzyme identities are well characterized, and based on their enzymatic functions it was concluded that the introduced genes would not confer toxicogenic/pathogenic properties to the host organism. The genetic modifications made to the production strain result in the expression of proteins that are involved in the normal carbohydrate processing within their donor microbial (bacterial) sources. These proteins are "carbohydrate-active enzymes" ("CAZy"), a panel of enzymes that can degrade, modify, or create glycosidic bonds, and accordingly are involved in the metabolism of complex carbohydrates[3]. When expressed together in the recipient strains, these proteins work in concert to convert the starting carbohydrates (D-lactose and D-glucose[4]) into oligosaccharides that are identical to those in human breast milk. In contrast, bacterial protein toxins (exotoxins) are known to mediate their pathogenic effects by disrupting cellular processes through various mechanisms such as proteolysis (*e.g.*, tetanus and botulinum),

[3] http://www.cazy.org/Welcome-to-the-Carbohydrate-Active.html.
[4] Glycerol and D-sucrose are alternative carbon sources to D-glucose.

ADP-ribosylation (*e.g.*, cholera, pertussis, and diphtheria), or membrane disruptions through pore formation (Finlay and Falkow, 1997; Wilson *et al.,* 2002; Popoff, 2018). Indeed, bioinformatic searches conducted using the amino acid sequences of the proteins introduced to the *E. coli* K-12 DH1 MDO MP572b strain by genetic modification confirmed that there is no relevant homology to known protein toxins or to known allergens. The genetic modifications applied to the platform and production strains were verified by applying whole genome sequencing and colony polymerase chain reaction (PCR) and targeted sequencing methods.

During manufacture, the production strain secretes the LNnT extracellularly, and then is entirely removed through a series of purification steps (as described in Section 2.2.2). Therefore, in this process the production strain is used exclusively as a processing aid.

2.2 Upstream and Downstream Manufacturing

The manufacture of LNnT includes upstream (fermentation) and downstream (purification) stages as described in GRN 659 (U.S. FDA, 2016). Aside from the alternate use of strain MP572b instead of strain MP572 during fermentation, the only additional potential change to the upstream manufacturing process includes the use of sucrose as an alternate carbon source. There are no changes to the downstream manufacturing steps.

2.3 Batch Analyses for LNnT Produced by Microbial Fermentation of *Escherichia coli* K-12 DH1 MDO MP572b

Glycom analyzed three independent production batches produced by *E. coli* K-12 DH1 MDO MP572b. The analytical results are provided in Table 2.3-1 and demonstrate that the LNnT final product produced by strain MP572b is consistent and conforms with the product specifications for LNnT as described in GRN 659 (U.S. FDA, 2016). In addition, analysis for residual DNA from the production strain MP572b was conducted using quantitative polymerase chain reaction (qPCR) (limit of detection: 4 pg/mg) and demonstrated that genetic material from the production strain is not carried over from the fermentation process into the LNnT final product.

Table 2.3-1 Batch Analysis of LNnT Produced by Fermentation Using *Escherichia coli* K-12 DH1 MDO MP572 and MP572b

Specification Parameter	Specification Limit[a]	LNnT from MP572 (GRN 659) (U.S. FDA, 2016)				LNnT from MP572b		
		5247750801	2547750901	2547750902	2547750903	20452003	21067002	21153002
Appearance	Powder, agglomerates, powder with agglomerates	Powder with agglomerates	Slightly agglutinated powder	Slightly agglutinated powder	Powder	Powder with agglomerates	Powder	Powder
Color	White, white to off-white, off-white	White	White	White	White	White	White	White
Identification	RT of standard ± 3%	Complies	Complies	Complies	Complies	Complies	Complies	Complies
Assay (water free) HiMS[b] [w/w%]	≥ 95.0	98.5	97.9	97.2	98.6	102.5	100.6	101.2
Assay (water free) LNnT [w/w%]	≥ 92.0	97.1	94.4	95	97.2	99.8	98.8	99.0
D-Lactose [w/w%]	≤ 3.0	0.25	0.66	0.38	0.21	0.05	0.04	0.06
Lacto-*N*-triose II [w/w%]	≤ 3.0	1.01	1.62	0.65	0.65	2.43	1.68	1.94
para-Lacto-*N*-neohexaose [w/w%]	≤ 3.0	0.13	0.95	1.02	0.45	0.05	< 0.03	< 0.03
LNnT fructose isomer [w/w%]	≤ 1.0	0.03	0.4	0.41	0.29	0.04	< 0.03	< 0.03
pH in 5% solution (20°C)	4.0 to 7.0	5.3	5.8	5.4	6.0	5.1	4.5	5.0
Water [w/w%]	≤ 9.0	6.6	8.0	7.8	7.6	5.6	6.2	5.9
Ash, sulphated [w/w%]	≤ 1.5	< 0.03	< 0.01	0.03	<0.01	< 0.05	< 0.05	< 0.05
Methanol [mg/kg]	≤ 100	57	19	32	22	41	18	9
Residual proteins [w/w%]	≤ 0.01 w/w %	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Heavy Metals								
Lead [mg/kg]	≤ 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.006	< 0.005	< 0.005
Microbiological Criteria								
Salmonella in 25 g	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Total plate count [CFU/g]	≤ 500 CFU/g	<500	<500	<500	<500	<500	<500	<500
Enterobacteriaceae in 10 g	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Cronobacter spp. in 10 g	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Listeria monocytogenes in 25 g	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table 2.3-1 Batch Analysis of LNnT Produced by Fermentation Using *Escherichia coli* K-12 DH1 MDO MP572 and MP572b

Specification Parameter	Specification Limit[a]	LNnT from MP572 (GRN 659) (U.S. FDA, 2016)				LNnT from MP572b		
		5247750801	2547750901	2547750902	2547750903	20452003	21067002	21153002
Bacillus cereus [CFU/g]	≤ 50 CFU/g	< 50	< 50	< 50	< 50	< 50	< 50	< 50
Yeasts [CFU/g]	≤ 10 CFU/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Molds [CFU/g]	≤ 10 CFU/g	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Residual endotoxins [E.U./mg]	≤ 10	0.215	0.015	0.035	0.0003	< 0.00025	< 0.00025	< 0.00025

CFU = colony forming units; E.U. = endotoxin units; HiMS = human-identical milk saccharides; LNnT = lacto-*N*-neotetraose; LOQ = limit of quantitation; RT = retention time.

[a] Specification limits for LNnT described in GRN 659 (U.S. FDA, 2016).

[b] Human-identical milk oligosaccharides is defined as the sum of LNnT, lactose, lacto-*N*-triose II, and *para*-lacto-*N*-hexaose

PART 3. NARRATIVE – IMPACT OF CHANGES ON GRAS STATUS OF LNnT

As described in Part 2 above, a number of refinements in the original MP572 production strain were performed to produce the alternative MP572b production strain and to optimize LNnT biosynthesis. These included the full chromosomal integration of the two glycosyltransferases that are required for LNnT biosynthesis; the introduction of the sucrose operons (enabling the use of sucrose as a carbon source), the introduction of the MFS transporter (facilitating the release of LNnT into the fermentation broth), and the deletion of several genes that equip the production strain with a genetic makeup that can effectively face potential suboptimal fermentation conditions encountered in large-scale production. No changes to the downstream processing of LNnT have been implemented and the final product remains a high purity crystalline ingredient that meets the specifications for LNnT set forth in GRN 659 (U.S. FDA, 2016).

The proposed addition of production strain *E. coli* K-12 DH1 MDO MP572b as an alternative to *E. coli* K-12 DH1 MDO MP572 does not change the GRAS status of the ingredient as previously described in GRN 659 based on the following conclusions:

- Both strains (MP572 and MP572b) originate from the same Glycom platform strain *Escherichia coli* K-12 DH1 MDO.
- *E. coli* MP572b contains genomically integrated recombinant genes, and it does not contain antibiotic resistance markers.
- *E. coli* MP572b is more stable, fully genomic (all genes were introduced to the bacterial chromosome), and it is a reliable production strain that provides much higher titers of LNnT, compared to MP572.
- Using gene sequencing methods, it was confirmed that the genetic modifications did not introduce unknown proteins.
- The LNnT ingredient, produced with *E. coli* MP572b, fully complies with all regulatory and internal specification parameters for LNnT as described in GRN 659 (U.S. FDA, 2016).
- No new unspecified impurities have been detected in LNnT batches (secondary metabolites or by products), and the genetic modifications are sufficiently well characterized that it could be concluded that production of new by-products or secondary metabolites during fermentation of *E. coli* MP572b would not be expected.
- The impurity profile of LNnT produced by *E. coli* MP572b did not contain new or higher concentrations of impurities/by-products relative to levels present in the LNnT test article previously tested in animal toxicity studies reported by Coulet *et al*. (2013) and described in GRN 659 (U.S. FDA, 2016).
- Bioinformatic analyses of all recombinant sequences were performed using Allergen Online (version 20) (FARRP, 2020). No risk of potential allergenicity was identified.

- New qPCR methods for the detection of potential residual DNA from the production strain has been developed and validated. No residual DNA of *E. coli* MP572b has been detected by qPCR in all tested LNnT batches (Batch Nos. 20452003, 21067002, 21153002) to the limit of detection of 4 pg per mg of LNnT product.

Based on the above information Glycom has concluded that the proposed addition of an alternative production strain would not change the GRAS status of LNnT as previously described under GRN 659 (U.S. FDA, 2016), thus LNnT produced from either strain (MP572 and MP572b) would be considered GRAS. It is Glycom's view that other experts qualified by scientific training and experience in food safety evaluation would agree with Glycom's conclusions.

PART 4. LIST OF SUPPORTING DATA AND INFORMATION

Coulet M, Phothirath P, Constable A, Marsden E, Schilter B (2013). Pre-clinical safety assessment of the synthetic human milk, nature-identical, oligosaccharide Lacto-*N*-neotetraose (LNnT). Food Chem Toxicol 62:528-537. DOI:10.1016/j.fct.2013.09.018.

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Wilson JW, Schurr MJ, LeBlanc CL, Ramamurthy R, Buchanan KL, Nickerson CA (2002). Mechanisms of bacterial pathogenicity. Postgrad Med 78(918):216-224. DOI:10.1136/pmj.78.918.216.

From: Roehrig, Christoph
To: Morissette, Rachel
Subject: [EXTERNAL] RE: GRN 659 supplement question
Date: Wednesday, December 1, 2021 10:52:12 AM
Attachments: image008.png
image009.png
image010.png
image011.png
image012.png
image013.png
Response document for questions on GRAS Supplement to GRN 659 - Nov 30"21.pdf

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Dear Rachel,

We did conduct an updated literature search, please find the narrative describing any new studies or information that are relevant to our safety conclusion attached.

I trust this satisfies your request.

Many thanks again.

Kind regards,
Christoph

Christoph Röhrig | Head of HMO Regulatory Affairs | DSM | Kogle Alle 4 | 2970 Hørsholm | Denmark | christoph.roehrig@dsm.com |
Glycom, the leading HMO expert is part of DSM

From: Roehrig, Christoph
Sent: Thursday, 18 November 2021 12:25
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Subject: RE: GRN 659 supplement question

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Dear Rachel,

Many thanks for looking into the GRN 659 supplement and coming back to us with this request. I will confirm with my team and get back to you shortly.

Kind regards,
Christoph

Christoph Röhrig | Head of HMO Regulatory Affairs | DSM | Kogle Alle 4 | 2970 Hørsholm | Denmark | christoph.roehrig@dsm.com |
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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Wednesday, 17 November 2021 16:14
To: Roehrig, Christoph <Christoph.Roehrig@dsm.com>
Subject: GRN 659 supplement question

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Dear Christoph,

We just had one request for the GRN 659 supplement before we move ahead with issuing a no questions letter. We didn't see reference to an updated literature search since GRN 659 in this supplement. Please provide a statement that an updated literature search was conducted and that Glycom did not identify any new studies or issues that would impact your GRAS conclusion, if that is in fact the case. Otherwise, please provide a narrative describing any new studies or information that are relevant to your safety conclusion since GRN 659. We request this information within 10 business days. I understand that the Thanksgiving holiday may impact that, so if you need more time, just let me know.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
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rachel.morissette@fda.hhs.gov

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Glycom A/S Kogle Allé 4 2970 Hørsholm, Denmark

30 November 2021

Dr. Paulette Gaynor
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition (CFSAN)
Food and Drug Administration
5001 Campus Drive
College Park, MD
20740 USA

Dear Dr. Gaynor:

Re: Response to the Questions on Supplement to GRAS Notice for Lacto-*N*-Neotetraose (LNnT) Produced by Fermentation (GRN 000659)

In response to the questions on the Supplement to GRAS Notice for Lacto-N-Neotetraose (LNnT) Produced by Fermentation (GRN 000659), Glycom A/S (Glycom) conducted a comprehensive and detailed updated search of the published scientific literature to obtain the necessary information pertaining to the safety of LNnT with publication dates from 1 October 2014 through 31 July 2021. AdisInsight: Trials, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile® served as the primary sources of published literature pertinent to the safety of LNnT.

The literature search strategy included keywords relevant to LNnT and any of its synonyms as well as keywords relevant in establishing the safety of oral consumption of LNnT including absorption, distribution, metabolism, and excretion, preclinical safety, clinical safety, and genotoxicity. In total, 133 articles were identified from the literature search and the titles, abstracts, and full text of potentially relevant articles were screened. A total of 8 articles were considered to be relevant. Study summaries of the 8 articles identified in the literature search are provided below.

2'-FL and LNnT in a 100% Whey, Extensively Hydrolyzed Formula for Children Aged 2 Months to 4 Years with Cow's Milk Protein Allergy (Nowak-Wegrzyn et al., 2019)

More recently, the hypo-allergenicity of a 100% whey-based extensively hydrolysed formula (EHF) containing 1.0 ± 0.2 g/L 2'-FL and 0.5 ± 0.1 g/L LNnT was conducted in children aged 2 months to 4 years with cow's milk protein allergy (Nowak-Wegrzyn *et al.*, 2019). In this study, participants were subjected to a double-blind, placebo-controlled food challenge (DBPCFC) with the test formula containing 2'-FL and LNnT, and a control formula, in a randomised, cross-over fashion. A 2- to 7-day washout period was included in between the two challenges. The study population consisted of 67 children ages 2 months to 4 years old with cow's milk protein allergy confirmed by physician-supervised clinical testing; 36 children were randomised to receive the test formula containing 2'-FL and LNnT first, and the remaining 31 children received a control formula of commercially available EHF with no HiMOs which has been confirmed as hypo-allergenic. The test formula had a protein content of 2.20 g/100 kcal compared to 2.47 g/100 kcal in the control formula. All other micronutrient and macronutrient profiles were identical. Children who were breastfed at the time of enrolment or had an anaphylactic reaction to cow's milk in the 2 years prior to the study were excluded. The mean age of the children who completed at least one DBPCFC was 24.1±13.2 months, with 12.5% (n=8) of the children at < 12 months of age, 60.9% (n=39) between 12 and 36 months of age, and 26.6% (n=17) at > 36 months of age.

For the DBPCFC, subjects less than 1 year of age receiving an initial dose from a lip smear, followed by oral doses of 5, 10, 20, 30, 30, 35 and 50 mL of the assigned formula at 10- to 15-minute intervals, for a total volume of 180 mL. Subjects over 1 year of age received an initial dose from a lip smear and oral doses of 5, 10, 25, 45, 45, 45 and 65 mL at 10- to 15-minute intervals for a total volume of 240 mL. If a subject successfully completed both challenge sessions with no allergic reaction, a 1-week (7 to 9 days) open challenge with the test formula (minimum 240 mL/day) was performed to assess tolerability and confirm the absence of any delayed allergic reactions. Daily formula intake was recorded, as well as stool characteristics (daily frequency, colour, consistency, and odour), frequency of flatulence, spitting up and/or vomiting, and the occurrence of allergic symptoms and any other adverse event.

A total of 62 subjects completed both DBPCFCs, of whom 61 completed the 1-week open-label home challenge with the test formula. During this open label phase, 2 subjects reported gastrointestinal symptoms, 1 subject vomited on Day 1 of this phase but completed the remainder of the test with no further issues, and 1 subject reported diarrhoea on the last day, which investigators attributed to gastroenteritis. None of the reactions warranted early discontinuation of the test formula. No other significant gastrointestinal symptoms (flatulence, abnormal stool frequency/consistency, increased spitting-up or vomiting) or adverse effects were reported. The authors concluded that EHF supplemented with 2'-FL and LNnT is hypoallergenic and is well-tolerated in children.

Randomised, Double-Blind, Controlled Clinical Study Examining Safety of 2'-FL and LNnT in a Liquid Supplement for Premature Infants (Hascoët et al., 2021)

The effect of a supplement containing 2'-FL and LNnT on growth, safety, and feeding tolerance was examined in a multi-centre, randomised, double-blind, controlled clinical study conducted in France (Hascoët *et al.*, 2021; NCT03607942). In this study, preterm infants (27 to 33 weeks gestation, birth weight < 1,700 g) were randomly allocated to receive either a supplement containing 2'-FL and LNnT in a 10:1 ratio (administered as a total of 0.374 g/kg body weight/day, dissolved in water buffered with a pH adjusting agent) or an isocaloric placebo supplement consisting of only glucose (0.140 g/kg body weight/day) from randomisation (as early as possible) to discharge from the neonatal unit. The primary outcome was feeding tolerance, measured by non-inferiority in days to reach full enteral feeding from birth in the 2'-FL+LNnT group compared to the placebo group (non-inferiority margin of +4 days). Anthropometric z scores were calculated using Fenton growth standards. Other secondary outcomes include faecal markers of gut health/maturation and microbiota.

A total of 43 infants were allocated to the 2'-FL+LNnT supplement group and 43 to the placebo control group. The mean chronological age at the initiation of supplementation were 6.3 days in the 2'-FL+LNnT group and 6.2 days in the placebo group. The mean total duration of intervention was 41 (range: 2 to 80) days in the 2'-FL+LNnT group and 34.5 (range: 2 to 125) days in placebo group. Non-inferiority in time to reach full enteral feeding in the 2'-FL+LNnT group versus the placebo was achieved in the full analysis set (least squares mean difference = 2.16 days; 95% confidence level -5.33, 1.00; upper bound of 95% confidence interval < non-inferiority margin). Similar results were observed in the per protocol set. The adjusted mean time to reach full enteral feeding from birth was 2 days shorter in the 2'-FL+LNnT group compared to placebo (12.2 days versus 14.3 days) but this finding did not reach statistical significance (p=0.177). There was no difference in weight-for-age z-scores between the groups. Length-for-age z-scores were statistically significantly higher in the 2'-FL+LNnT supplement group versus the control group at full enteral feeding days 14 (least squares mean difference of 0.29; p=0.037) and 21 (least squares mean difference of 0.31; p=0.037). Head circumference-for-age z score was significantly higher in the group receiving 2'-FL+LNnT versus the control at discharge (least squares mean difference of 0.42; p=0.007). Gastrointestinal tolerance measures, incidence of gastrointestinal adverse events, incidence of necrotising colitis, and incidence of other illnesses and infections were similar between groups. No cases of illnesses and infections were deemed related to the intervention.

LNnT in Combination with 2'-FL in Infant Formula (Alliet et al., 2016; Steenhout et al., 2016; Puccio et al., 2017)

The safety of LNnT was evaluated in a randomized, blinded, controlled, multi-center, parallel-design study conducted in healthy, full-term infants provided a standard term infant formula supplemented with LNnT (providing 0.5 to 0.6 g LNnT/L reconstituted formula)[1] in combination with 2'-FL (providing 1.0 to 1.2 g 2'-FL/L of reconstituted formula)[2] from 0 to 6 months of age (Alliet *et al.*, 2016; Steenhout *et al.*, 2016; Puccio *et al.*, 2017; full study report provided in Appendix H). Both LNnT and 2'-FL were supplied by Glycom. Infants were aged 0 to 14 days at enrolment (baseline) and clinic visits were scheduled at 1, 2, 3, 4, 6, and 12 months of age. A comparator group receiving a standard whey-predominant starter infant formula without HiMOs was included as controls. Body weight gain through to 4 months was evaluated as the primary endpoint, with secondary endpoints being additional anthropometric measures including body weight, body length, and head circumference, as well as gastrointestinal tolerance, behavioral patterns, and morbidity through to age 12 months. As part of the primary endpoint, weight gain in the test group receiving the formula supplemented with LNnT and 2'-FL was defined as "non-inferior" to controls if the lower bound of the one-sided 97.5% confidence interval on the difference between the test and control groups was greater than the non-inferiority margin of -3 g/day (based on the recommendations from the American Academy of Pediatrics).

A total of 175 infants were enrolled in the study. The mean weight gain in the test group was similar and non-inferior to the mean weight gain in the control group in both the intent-to-treat population and the per protocol population. Mean daily formula consumption was similar between groups at all time points examined.

The proportion of infants who experienced at least one serious adverse event was 6.8% (6 infants) in the group receiving the test formula containing LNnT and 2'-FL and 11.5% (10 infants) in the standard infant formula group. The proportion of infants experiencing at least one adverse event in the first 4 months of the study was similar between the test and control groups. One infant receiving the test formula experienced an adverse event considered to be related to the study formula (the infant developed a cow's milk protein allergy). Twelve infants in the test group and 11 infants in the control group experienced adverse events resulting in the discontinuation of the study formula. Overall, the incidence of adverse events was not significantly different in infants fed the test formula compared to the control standard formula.

The mean length, head circumference, and body mass index of the infants were not statistically significantly different between the test and control groups at any study visit. Furthermore, growth data indicated the formula containing LNnT and 2'-FL supported age-appropriate normal infant growth when compared to the World Health Organization (WHO) Growth Standards. Digestive symptoms (infant flatulence, spitting up, vomiting) and behavior patterns (restlessness/irritability, colic) were comparable between the 2 groups, with the exception of softer stool and fewer night-time wake-ups in infants receiving LNnT and 2'-FL compared to controls. Infants receiving the HiMOs also had significantly fewer parental reports of bronchitis, lower respiratory tract infections, antipyretic use, and antibiotic use compared to controls and endpoints examined. The study authors concluded that "Infant formula with 2'-FL and LNnT is safe, well tolerated, and supports age-appropriate growth."

Additional outcomes from this trial were evaluated including effects on early intestinal microbiota (Alliet *et al.*, 2016; Steenhout *et al.*, 2016). Stool samples were collected at 3 months of age for assessment of microbiota using 16S rRNA gene sequencing and metagenomics, and metabolics signature was assess using proton NMR-based metabolite profiling.

The global average microbial composition for the sub-group of infants with stool samples that followed the study protocol showed similar pattern between control (n=65) and test (n=58) at the genus level, although samples obtained from infants receiving the test formula were closer to breastfed (n=34) than control

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[1] Analytical results indicated a concentration range of 0.52 to 0.61 g/L (SD 0.028 to 0.033 g/L) of LNnT in test formula (unpublished data).

[2] Analytical results indicated a concentration range of 1.04 to 1.14 g/L (SD 0.073 to 0.08 g/L) of 2'-FL in test formula (unpublished data).

samples. Calculations of microbial alpha diversity and comparison of the global microbiota composition confirmed that test was different from control at the genus level ($p<0.001$) and closer to the breastfed reference. Statistical analysis (corrected for false discovery rate) identified several taxa differentially present in control and test including *Bifidobacterium* ($p=0.01$), *Escherichia* ($p=0.008$) and unclassified *Coprobacillaceae* ($p=0.01$). Multivariate analysis identified several influential metabolites that discriminated between test, control and breastfed groups including phenylalanine, isoleucine, tyrosine, fecal organic acids and fucosylated compounds. The values observed for the test formula group were more similar to those observed in the breast fed group compared with control, a finding that suggests reduced protein fermentation. The study authors concluded that "...these findings indicate that the addition of 2'FL and LNnT to a starter infant formula shifts the stool microbiota and metabolic signature towards those observed in breastfed infants, both in several aspects of composition and function."

The study investigators concluded that HMO supplementation was safe and well-tolerated in pre-term infants and that the HMO supplement supported early postnatal growth.

Real-World Study in Infants Fed 2'-FL and LNnT (Román Riechmann et al., 2020)

A non-randomized, open-label, prospective study was conducted in healthy, term infants (Román Riechmann *et al.*, 2020). In this real-world study, infants were enrolled at age 7 days to 2 months and fell into one of three groups: an exclusively formula-fed group, a mixture of formula and human milk fed, or exclusively breastfed infants (serving as a reference population). Infants fed on formula received a study formula *ad libitum* for 8 weeks, comprising of a partially hydrolyzed, 100% whey, term infant formula (67 kcal/100 mL, 1.9 g protein/199 kcal, 11.5 g carbohydrates/100 kcal, 5.1 g lipids/100 kcal, 1.0 g 2'-FL/L, and 0.5 g LNnT/L). The formula also included *Lactobacillus reuteri* (dose not reported), vitamins, and minerals.

Anthropometry measures (weight, length, head circumference) were measured at baseline and at Week 8. Z-scores for weight-for-age, length-for-age, head circumference-for-age, and body mass index-for-age were calculated. Gastrointestinal symptoms were evaluated via the Infant Gastrointestinal Symptom Questionnaire (IGSQ). Adverse events were recorded from the time of enrolment through the end of study.

A total of 66 exclusively formula fed, 48 mixed fed, and 45 exclusively breastfed infants were included in the analyses. When comparing baseline characteristics of the enrolled infants, the exclusively formula fed group was slightly younger at enrolment ($p < 0.01$) and had a higher proportion of male infants ($p > 0.05$) compared to the mixed-fed and breastfed group. Consistent with the slightly younger age group, baseline weight and length were slightly lower in the exclusively formula-fed group. Other baseline anthropometric characteristics were comparable across groups.

Through the study, age-appropriate growth was observed in all groups. Differences in baseline weight and length did not persist by Week 8; there were no significant differences between any groups for any of the anthropometric measures. The composite IGSQ scores showed low gastrointestinal distress in all groups at all time points. No significant differences were observed in 4 of the subdomains of gassiness, fussiness, crying, and spitting-up/vomiting. In the last subdomain of stooling, the formula-fed and mixed feeding group exhibited a statistically significant different score at baseline compared to exclusively breastfed infants. This was significantly improved at Week 8 in exclusively formula-fed infants, with scores moving closer to the stooling profile of the exclusively breastfed group. Stooling scores in mixed fed infants remained significantly different at Week 8.

Three patients experienced potentially product-related adverse events, including two instances of cow's milk intolerance (one in exclusively formula fed group, one in the mixed-feeding group= and one instance of irritability in the exclusively formula fed group. No seriously adverse events were attributed to the study feeding. The authors noted that the incidence of adverse events was low overall and was not significantly different between the groups.

Supplementation of LNnT in Combination with 2'-FL in Obese Children Aged 5 to 12 years old [Holm et al., 2019 [unpublished]; Fonvig et al., 2021]

In older children, a single-centre, randomised, controlled, double-blinded, parallel intervention study was conducted to determine the effect of 8 weeks supplementation of either 2'-FL alone or a mixture of 2'-FL and LNnT in a 4:1 mass ratio on faecal microbiota (primary objective) and safety/tolerability (secondary objectives) in 5 to 12 year-old children (Holm et al., 2019 - Unpublished). The study population consisted of children aged 6.4 to 12.7 years at enrolment who were admitted to a childhood obesity treatment program. Except from the excess body weight (body mass index z-score ≥ 2.3), the children were healthy and showed no sign of illness or metabolic disorders. A total of 75 eligible children (43 females) were randomised to receive a daily dose of 4.5 grams of either 2'-FL alone, a mix of 2'-FL and LNnT in a 4:1 mass ratio ("Mix"), or placebo of powdered glucose (25 children in each group) for 8 weeks. The participants were asked to dissolve the investigational product in at least 50 mL of liquid and consume it in the morning with breakfast. Data on effect during the intervention period was collected at baseline, at an intermediate visit (after 4 weeks of intervention), and at the end of the 8-week intervention.

The results show that the abundance of Bifidobacterium in faecal samples was increased from baseline to the intermediate visit and from baseline to the end-of-intervention in the 2'-FL group and from baseline to the intermediate visit in the Mix group. There were no statistically significant differences in the magnitude of this effect between these two intervention groups. No such changes in the abundance of Bifidobacterium were observed in the placebo group. In addition, no statistically significant difference in Bifidobacterium abundance was seen from the intermediate visit to the end-of-intervention for the 2'-FL group and for the Mix group, indicating that the full bifidogenic effect is reached after 4 weeks of intervention. The bifidogenic effect was primarily mediated through an increased abundance of B. adolescentis.

Furthermore, this study shows that a daily intake of 4.5 grams of either 2'-FL or the Mix is safe for use in children. Blood samples were collected at baseline, end-of-intervention (week 8), and during final study visit at 10±1 month after the end-of-intervention visit. All measurements were generally within the normal range expected for the age group at all timepoints assessed; any measurements that deviated from the normal range were minor and were considered clinically irrelevant by the investigators. A few minor statistically significant changes in safety biomarkers (i.e., haematology and clinical chemistry parameters measured in blood) were observed between the baseline and end-of-intervention visit. However, these changes remained within the normal variation and were considered clinically irrelevant by the investigators, with no consistent changes observed in any of the intervention groups. No clinically relevant changes in anthropometric measures, body composition, or vital signs (resting blood pressure) were reported.

An analysis of Adverse Events (AEs) indicates no clinically relevant differences between the groups. A total of 75 AEs were reported by 37 subjects. The number of children experiencing at least one AE throughout the study was similar across the intervention groups (12, 13 and 13 subjects in the placebo, 2'-FL, and Mix group, respectively). The number of AEs also occurred with similar frequency across the intervention groups (24, 24 and 27 in the placebo, 2'-FL, and Mix group, respectively). Of the 75 AEs reported, 53 AEs were considered unlikely to be related to the investigational product, 20 AEs were considered possibly related, and 2 AEs (both occurring in the Mix group) were considered "unknown", whereby it was not possible to rate the severity or relationship to the investigational product by the investigator. Of the 20 possibly-related AEs, 15 were considered to be mild; these were mostly gastrointestinal in nature, and occurred across all groups including the placebo (6, 2 and 7 in the placebo, 2'-FL, and Mix group, respectively). The remaining 5 AEs were deemed moderate in severity, and they all occurred as a single incidence in 1 subject in the Mix group (abdominal cramps, abdominal pain, diarrhoea, reflux, and flatulence). No serious AEs occurred. Overall, the reported AEs raised no safety concerns.

The investigational products did not provoke digestive intolerance in any of the groups as measured by the Gastrointestinal Symptom Rating Scale (GSRS), indicating that both 2'-FL and the Mix are well tolerated in children. Mean scores for the individual symptoms remained at levels between "No discomfort at all" and "Mild discomfort". Except from a statistically significant reduction in urgent need to have a bowel movement from baseline to end-of-intervention in the 2'-FL group, the fluctuations in total GSRS score and scores for individual symptoms were not statistically significant for any of the intervention groups. The

Bristol Stool Form Scale (BSFS) scores also showed no between-group differences in proportion of abnormal bowel movements, indicating that the investigational product did not induce digestive distress.

Overall, the results from this study demonstrate that supplementation with HiMOs in children (2'-FL alone or in combination with LNnT in a 4:1 ratio) exerts a bifidogenic effect which is primarily mediated through an increased abundance of B. adolescentis, and that these HiMOs are safe for use without provoking digestive intolerance. This is similar to what has been shown in previous studies with HiMOs in infants and adults.

Randomised, Placebo-Controlled, Double-Blind, parallel-design study in healthy adult (Elison et al., 2016)

The safety and tolerability of LNnT produced by Glycom was investigated in a randomized, placebo-controlled, double-blind, parallel-design study in which healthy adult volunteers (51 men and 49 women; mean age of 36.0 years) were provided either LNnT or 2'-FL alone, or in combination at different doses for 2 weeks (Elison *et al.*, 2016). A comparator control group receiving glucose as a placebo was also included. The intervention groups used in the study are summarized in Table 1 below. All interventions were provided as daily bolus doses. Test articles were provided in powder form and participants were instructed to dissolve the powder in approximately 250 mL of water prior to intake in the morning with breakfast. Compliance was evaluated using a subject diary in which subjects were instructed to record the intake of the test article, which was confirmed by the collection of empty and un-opened bottles at the end of the intervention period.

Table 1 Interventions Used in the Two-Week Healthy Adult Study (Elison *et al.*, 2016)

Group No.	Daily Dose of LNnT (grams)	Daily Dose of 2'-FL (grams)
1	0	20
2	0	10
3	0	5
4	20	0
5	10	0
6	5	0
7	6.67	13.33
8	3.33	6.67
9	1.67	3.33
Control	2 grams Dextropure (glucose)	

2'-FL = 2'-*O*-fucosyllactose; LNnT = lacto-*N*-neotetraose

Adverse events were monitored during the study. Blood samples were collected at baseline and at the end of the intervention period (2 weeks) and evaluated for standard hematological and blood biochemistry parameters. Feces were collected at baseline and at the end of the intervention period (2 weeks) and evaluated for calprotectin, secretory IgA, glucose, galactose, lactose, and short-chain fatty acids). Fecal DNA was also extracted to assess microbiota composition (by 16S rRNA sequencing) at baseline, during the first week of supplementation, and at Week 2. Gastrointestinal symptoms were evaluated using the Gastrointestinal Symptom Rating Scale (GSRS) and changes in bowel habits were assessed using the Bristol Stool Form Scale (BSFS).

All adverse events reported during the study were judged to be "mild" and there were no cases of premature discontinuation from the trial due to adverse events. Most adverse events were judged to be "possibly" related to the test article; however, many symptoms were noted by the study investigators to be common and difficult to ascertain whether they were related to the test article, to normal day-to-day variation, or to increased awareness of gastrointestinal symptoms during the trial period.

Hematological and blood biochemistry analyses obtained at the 2-week time point remained within the normal range for all subjects and any minor changes over the course of the study compared to baseline values were not considered clinically relevant. The GSRS scores indicated that LNnT and 2'-FL were well tolerated. When compared to the placebo control, individuals receiving the highest dose of LNnT (20 g) reported an increased bloating and passing of gas, and harder stools and individuals receiving 10 g LNnT reported increased passing of gas after the 2-week intervention period. However, scores generally remained at a level of "mild discomfort" or less. The BSFS scores indicated a mild tendency to softer stools in individuals provided the high dose of LNnT or 2'-FL over the course of the study compared to baseline, but differences were small and clinically irrelevant.

The microbiota profiling results demonstrate that LNnT and 2'-FL can change the intestinal microbiota composition with the increase of Actinobacteria and *Bifidobacterium* being the major effect of treatment. LNnT and 2'-FL reduced the relative abundance of Firmicutes and Proteobacteria compared to placebo. These changes in the gut microbiota composition are considered to be favorable (Brown *et al.*, 2012).

Overall, the results support that the consumption of LNnT and 2'-FL, either alone or in combination, at the doses tested, was safe and well tolerated in healthy adult men and women. Acute intake of a bolus dose of 20 g of LNnT (or 2'-FL) may represent a gastrointestinal tolerability threshold for some individuals; however, bolus exposures of 20 g of LNnT are highly unlikely to be experienced by the consumer given the proposed use-levels and consequently required food intakes that would lead to such intakes.

Randomised, Placebo-Controlled, Double-Blind, Study in Irritable Bowel Syndrome Patients (Iribarren et al., 2020)

The influence of LNnT administration (in combination with 2'-FL) was explored in a Phase II randomised, placebo-controlled, double-blind study in adults with irritable bowel syndrome (IBS) (Iribarren *et al.*, 2020). Patients aged 18 to 75 years diagnosed with at least moderate-severity irritable bowel syndrome according to the Rome IV criteria were randomly allocated to receive either a placebo (glucose), or 5 or 19 g/day of a 4:1 ratio of 2'-FL and LNnT for 4 weeks. Patients were followed for an additional 4 weeks after the cessation of the intervention. All subjects were advised to maintain their usual diet and medication regimen throughout the study. During clinic visits at screening, baseline, end of intervention (Week 4) and study end (Week 8), patients completed validated clinical questionnaires to assess the severity of gastrointestinal and psychological symptoms. Body weight, height, adverse events, changes in medication or diet, and compliance were also recorded, and a physical examination was performed by the study physician. Faecal samples were also collected during these visits and subject to microbiota profiling.

The primary endpoint of the study was to determine the daily dose of 2'-FL+LNnT manifesting an increase in Bifidobacterium spp. abundance without aggravating gastrointestinal symptoms, as assessed by the Gastrointestinal Symptom Rating Scale for IBS (GSRS-IBS). Secondary efficacy endpoints included IBS severity [as measured by the IBS Symptom Severity Scale (IBS-SSS)], bowel habits (stool consistency), and anxiety and depression.

In total, 60 patients (20 patients in each arm) were included in the intention-to-treat analysis. Two patients (one from the placebo group and one from the 10 g 2'-FL+LNnT/day group) discontinued prematurely after 2 weeks of intervention due to increased IBS symptoms. Anthropometric and IBS classifications did not differ between the three groups at baseline; however, patients allocated to receive 5 g 2'-FL+LNnT/day demonstrated a lower GSRS-IBS total score at baseline compared to the other groups (p=9.93). After 4 weeks of intervention, the group receiving 10 g 2'-FL+LNnT/day exhibited a statistically significant higher abundance of faecal bifidobacteria as compared to the groups receiving placebo and 5 g 2'-FL+LNnT/day, as well as when compared to baseline; however, these differences did not persist into Week 8 (*i.e.*, following cessation of the intervention). Patients receiving 5 g 2'-FL+LNnT/day did not show a change at Week 4 but had a decreased bifidobacteria abundance at Week 8 compared to baseline. No changes were observed in the placebo group at any timepoint. The severity of overall or individual gastrointestinal symptoms did not differ between the groups at the Week 4 or Week 8 timepoints.

The study authors concluded that daily intake of 10 g of 2'-FL+LNnT over 4 weeks was well tolerated and did not induce worsening of IBS symptoms, bowel habits, anxiety, or depression, and that most patients were able to complete the 4 weeks of intervention without significant side effects.

Prospective, Open-label, Single-arm Study in Irritable Bowel Syndrome Patients (Palsson *et al.*, 2020)

The safety and tolerability of LNnT (in combination with 2'-FL) was investigated in a multicentre, open-label trial in which adult volunteers with irritable bowel syndrome (70.7% women; mean age of 44 years) were provided the mixture for 12 weeks (Palsson *et al.*, 2020). Volunteers were instructed to consume one 5 g pack of 2'-FL+LNnT (4:1 ratio of 2'-FL and LNnT) per day, either mixed in food, beverage, or taken on its own. Of the 317 patients included in the analysis, 245 reported full adherence to the study intervention. A baseline assessment was conducted to determine stool consistency (measured by British Stool Form Scale), IBS symptom severity, IBS gastrointestinal symptom rating scale, and IBS quality of life. These surveys were also conducted 4, 8, and 12 weeks after the beginning of intervention. The occurrence of adverse events was also monitored throughout the intervention period.

The overall safety of the 2'-FL+LNnT was reported as well tolerated, with the occurrence adverse events remaining low (*i.e.*, 46 patients reported a combined total of 87 adverse events). Upon reviewing the reported adverse events, the investigators considered 65 adverse events (reported by 33 patients) to be possibly or probably related to the test article. Most adverse events were related to passing gas, abdominal distension, and abdominal pain, in order of occurrence frequency. Eight participants dropped out of the study due to adverse events. One serious adverse event occurred in the test period; however, it was reviewed by the medical safety officer and concluded to be unrelated to the test article.

The study authors therefore concluded that daily administration of 2'-FL+ LNnT (4:1 ratio of 2'-FL and LNnT) is safe and well tolerated in adults with IBS.

I trust that this information satisfies your request.

Sincerely,

(b) (4)

Christoph H. Röhrig, Ph.D.
Senior Scientist
Head of Regulatory & Scientific Affairs
Glycom A/S

From: Roehrig, Christoph
To: Morissette, Rachel
Subject: [EXTERNAL] RE: one more question for GRN 659 supplement
Date: Thursday, December 2, 2021 1:37:29 AM
Attachments: image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

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Dear Rachel,

Yes, I confirm that we agree to the statement. My apologies that we overlooked to express it explicitly.

Many thanks.

Kind regards,
Christoph

Christoph Röhrig | Head of HMO Regulatory Affairs | DSM | Kogle Alle 4 | 2970 Hørsholm | Denmark | christoph.roehrig@dsm.com |
Glycom, the leading HMO expert is part of DSM

From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Wednesday, 1 December 2021 20:21
To: Roehrig, Christoph <Christoph.Roehrig@dsm.com>
Subject: one more question for GRN 659 supplement

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Dear Christoph,

One more question.

Can you please confirm that Glycom agrees with the following statement?

Glycom states that it did not identify any data or information that would contradict the safety conclusion from GRN 000659 or this supplement.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

I GOT MY COVID-19 VACCINATION

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From:	Lau, Annette
To:	Morissette, Rachel; Miks, Marta
Cc:	Roehrig, Christoph
Subject:	RE: [EXTERNAL] RE: follow-up for GRN 659 supplement
Date:	Friday, December 10, 2021 6:04:40 AM
Attachments:	image001.png image002.png image003.png image004.png image008.png image010.png image012.png image014.png image016.png image018.png image021.png image023.png image025.png image027.png image030.png Response document for questions on GRAS Supplement to GRN 659 - Dec 9"21.pdf

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Dear Dr. Morissette,

On behalf of Christoph and Glycom A/S, I am providing responses to the Agency's questions in the attachment.

I hope you will have a wonderful holiday season ahead and if we do not speak beforehand, have a wonderful new year.

Most kindly,
Annette

Annette Lau | Senior Regulatory Affairs Manager | Kogle Alle 4 | 2970 Hørsholm | Denmark | T +45 6043 7274 | annette.lau@dsm.com | Stay connected:
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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, 9 December 2021 04.52.pm
To: Lau, Annette <Annette.Lau@dsm.com>; Miks, Marta <Marta.Miks@dsm.com>
Cc: Roehrig, Christoph <Christoph.Roehrig@dsm.com>

Subject: RE: [EXTERNAL] RE: follow-up for GRN 659 supplement

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Thank you. Looking forward to receiving your response to our questions.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

I GOT MY COVID-19 VACCINATION

From: Lau, Annette <Annette.Lau@dsm.com>
Sent: Thursday, December 9, 2021 10:26 AM
To: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>; Miks, Marta <Marta.Miks@dsm.com>
Cc: Roehrig, Christoph <Christoph.Roehrig@dsm.com>
Subject: [EXTERNAL] RE: follow-up for GRN 659 supplement

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Confidential

Dear Rachel,

Your email was well-received and I confirm that we will revert with the responses to the Agency's questions on the supplement to GRN 659 in Christoph's absence.

Warmly,
Annette

Annette Lau | Senior Regulatory Affairs Manager | Kogle Alle 4 | 2970 Hørsholm | Denmark | T +45 6043 7274 | annette.lau@dsm.com | Stay connected:
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From: Morissette, Rachel <Rachel.Morissette@fda.hhs.gov>
Sent: Thursday, 9 December 2021 03.56.pm
To: Miks, Marta <Marta.Miks@dsm.com>; Lau, Annette <Annette.Lau@dsm.com>
Subject: FW: follow-up for GRN 659 supplement

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Hi Marta and Annette,

Would you be able to address our questions below in Christoph's absence?

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
rachel.morissette@fda.hhs.gov

FDA U.S. FOOD & DRUG ADMINISTRATION

From: Morissette, Rachel
Sent: Tuesday, December 7, 2021 7:46 AM
To: 'Roehrig, Christoph' <Christoph.Roehrig@dsm.com>
Subject: follow-up for GRN 659 supplement

Dear Christoph,

We are currently working on the response letter for the GRN 659 supplement and our chemist just asked me to confirm the following two items with Glycom:

1. Please state whether the information provided for the analysis of three batches of LNnT are for non-consecutive batches.

2. Please confirm whether there are any expected changes in the estimated dietary exposure to LNnT from the intended uses and with respect to the information provided in GRN 000659.

Best regards,

Rachel

Rachel Morissette, Ph.D.
Regulatory Review Scientist

Division of Food Ingredients
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Glycom A/S Kogle Allé 4 2970 Hørsholm, Denmark

10 December 2021

Rachel Morissette, Ph.D.
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration

Dear Dr. Morissette:

Re: Response to the Follow-Up on the Supplement to GRAS Notice for Lacto-*N*-Neotetraose (LNnT) Produced by Fermentation (GRN 000659)

In response to the email sent by the Agency on 7 December 2021, Glycom A/S herein confirms the following:

1. Please state whether the information provided for the analysis of three batches of LNnT are for non-consecutive batches.

   Response: Glycom A/S confirms that the three batches of LNnT described in the supplement were non-consecutive manufacturing batches.

2. Please confirm whether there are any expected changes in the estimated dietary exposure to LNnT from the intended uses and with respect to the information provided in GRN 000659.

   Response: Glycom A/S does not expect any changes in the estimated dietary exposure to LNnT when compared to the information provided in GRN 000659.

We trust that this information satisfies the Agency's questions and we look forward to your response.

Sincerely,

DocuSigned by:
5CCC25CCA11A454...

Annette Lau
Senior Regulatory Affairs Manager
Glycom A/S

annette.lau@dsm.com
+45 6043 7274