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February 21, 2018

Dr. P. Gaynor
Office of Food Additive Safety (HFS-206)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

Subject: GRAS Notification - 3'-sialyllactose Sodium Salt

Dear Dr. Gaynor,

On behalf of GeneChem, Inc., we are submitting a GRAS notification for 3'-sialyllactose (3'-SL) Sodium Salt as a food ingredient. The enclosed document provides notice of a claim that the food ingredient, 3'-sialyllactose Sodium Salt, described in the enclosed notification is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for addition to infant formula and other foods. We believe that this determination and notification are in compliance with Pursuant to 21 C.F.R. Part 170, subpart E.

We enclose an original copy of this notification for your review. Please feel free to contact me if additional information or clarification is needed as you proceed with the review. We would appreciate your kind attention to this matter.

Sincerely,

(b) (6)

Susan Cho, Ph.D.
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Agent for GeneChem, Inc.

GRAS Notification of 3′-Sialyllactose (3′-SL) Sodium Salt

Prepared for GeneChem Inc.
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PART 1. SIGNED STATEMENTS AND A CERTIFICATION

Pursuant to 21 CFR Part 170, subpart E, GeneChem Inc. (hereinafter referred to as "GeneChem") submits a Generally Recognized as Safe (GRAS) notice and claims that the use of 3′-sialyllactose sodium salt (3′-SL sodium salt; powder form) in foods, as described in Parts 2 through 7 of this GRAS notice, is not subject to premarket approval requirements of the FD&C Act based on its conclusion that the substance is GRAS under the conditions of its intended use.

1.A. Name and Address of the Notifier

Company: GeneChem, Inc.
Address: Migun Techno World II, A-201,
187 Techno 2-ro, Yuseong-gu,
34025, Daejeon, Republic of Korea.
Tel.: +82-42-716-0998
Fax: +82-70-8280-2282

1.B. Common or Trade Name

Common name: 3′-sialyllactose sodium salt or 3'-SL

1.C. Applicable Conditions of Use of the Notified Substance

1.C.1. Foods in Which the Substance is to be Used

GeneChem's 3′-SL sodium salt is intended for use in non-exempt term infant formulas (milk-, soy-, amino acid-, and hydrolyzed protein-based). In addition, for general population, 3'-SL sodium salt will be used in dairy product analogs, infants and toddler foods, milk (whole and skim), milk products, grain products, beverages and beverage bases, and sugar substitute (herbal extract liquid).

1.C.2. Levels of Use in Such Foods

GeneChem's 3′-SL sodium salt is intended for use in non-exempt term infant formulas at a maximum use level of up to 238 mg/L (ready-to-drink or reconstituted formula), corresponding to 230 mg/L 3'SL in ready-to-drink or reconstituted formula. This maximum use level of 3′-SL sodium salt in term infant formulas is based on providing a similar level of 3′-SL as that which occurs in mature human breast milk, which typically ranges from 42-840 mg/L. Typical infant formula is estimated to contain 17-19 mg/L of 3′-SL. The addition of 3′-SL sodium salt to term infant formulas is consistent with efforts to produce infant formula that closely match the nutrient composition of human milk. To determine the use levels of 3'-SL in term infant formulas, average values were obtained as 197 mg/L for American mothers' milk and 299 mg/L for European mothers' milk. Thus, GeneChem's 3′-SL is intended for use in term infant formulas at a maximum use level of up to 230 mg/L (ready-to-drink or reconstituted formula) or 28 mg per serving.

For general population excluding infant formula applications, 3'-SL will be used in other foods at 24 to 3,000 mg per serving (Table 1). Corresponding 3'-SL sodium salt concentrations will be 24.8 to 3,104 mg per serving of foods.

Table 1. Summary of the Proposed Uses and Use Levels for 3'-SL in Conventional Food and Beverage Products and Infant Formula.

Food Category	Proposed Food-Uses	RACC[a]	Proposed Maximum Use Level		
			3'-SL		3'-SL sodium salt
			mg/RACC	mg/kg or mg/L[b]	mg/kg or mg/L[b]
Dairy Product Analogs	Imitation milks	240 mL	28	117	121
	Non-dairy yogurt	225 mL	120	533	552
Infant and Toddler Foods	Term infant formulas	122 mL[c]	28	230	237
	Toddler formulas	100 mL[c]	24	240	248
	Cereals for babies and toddlers, instant	15 g	24	1600	1,656
	Cereals for babies, jarred	110	24	218	226
	Cereal bar with fruit fillings	40	24	600	621
	Cookies and finger foods for babies	30	24	800	828
	Vegetables for babies, junior and toddlers	60-110	24	218-400	226-414
	Fruits and fruit sauce for infants, junior, and toddlers	60-125	24	192-400	199-414
Milk, Whole and Skim	Unflavored pasteurized and sterilized milk[d]	240 mL	28	117	121
Milk Products	Flavored milk	250 mL	28	112	116
	Yogurt, frozen	75-118	120	1017-1600	1,052-1,656
	Yogurt	225 g	120	533	552
Grain Products	Meal replacement bars for weight reduction	40 g	1,000	25,000	25,868
Beverages and Beverage Bases	Sports, isotonic drinks	240 mL	28	117	121
	Herbal tea, presweetened with low calorie sweetener or sugar	240 mL	3,000	12,500	12,934
	Cappucino, non fat, with dairy milk, sweetened	240 mL	120	500	517

Sugar Substitute	Sugar substitute, herbal extract powder or liquid		10%		

RACC = Reference amounts customarily consumed; U.S. = United States.

[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, 2015a)

[b] The proposed maximum use level is presented on a mg/kg basis for solids and on a mg/L basis for liquids.

[c] RACC not available, 100 mL employed as an approximation.

[d] Milk is a standardized food in the United States.

[c] RACC not available, 100 mL employed as an approximation.

1.C.3. Purpose for Which the Substance is Used

The substance will be used as a food ingredient for term infant formulas and other foods. GeneChem does not intend to add 3'-SL sodium salt to any meat and/or poultry products that come under USDA jurisdiction.

1.C.4. Description of the Population Expected to Consume the Substance

The population expected to consume the substance consists of non-exempt full-term infants and general populations.

1.D. Basis for the GRAS Determination

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

1.E. Availability of Information

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Susan Cho at NutraSource, Inc. at the address above. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

1.F. Availability of FOIA Exemption

None of the data and information in Parts 2 through 7 of this GRAS notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. §552.

1.G. Certification

We certify that, to the best of our knowledge, our GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

1.H Name, Position/Title of Responsible Person Who Signs Dossier, and Signature

(b) (6)

Date: February 21, 2018
Name: Jinsuk Woo, Ph.D.
Title: CEO

Address of Correspondence:
Susan S. Cho, Ph.D.,
NutraSource, Inc.
Agent for GeneChem

1.I. FSIS/USDA Statement

GeneChem does not intend to add 3'-SL sodium salt to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

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

2.A. Scientific Information About the Identity of the Notified Substance

2.A.1. Identity of the Notified Substance

2.A.1.1. Common or Trade Name

3'-sialyllactose sodium salt
Common Abbreviation: 3'-SL (3'SL, 3-SL, 3SL) Na or 3'-SL (3'SL, 3-SL, 3SL)
Trade name: *Siallac*3®

2.A.1.2. Chemical Names

IUPAC Names
3'-SL Na: α-D-N-Acetylneuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose monosodium salt

3'-SL: α-D-*N*-Acetylneuraminyl-(2→3)-β-D-galactopyranosyl-(1→4)-D-glucopyranose

Alternative Denotations
3'-SL Na: 3'-Sialyllactose monosodium salt; (2, 3')-α-Sialyllactose sodium salt; 3'-α-Sialyllactose sodium salt; α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-D-Glc sodium salt; 3'-*N*-Acetylneuraminyl-D-lactose sodium salt; 3'-Sialyl-D-lactose sodium salt

3'-SL: 3-Sialyllactose; (2, 3')-α-Sialyllactose; 3'-α-Sialyllactose; α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-D-Glc; 3'-*N*-Acetylneuraminyl-D-lactose; 3'-Sialyl-D-lactose

2.A.1.3. Chemical Abstract Service (CAS) Registry Number

3'-SL Na: 128596-80-5
3'-SL: 35890-38-1

2.A.1.4. Empirical Formula

3'-SL Na: $C_{23}H_{38}NO_{19} \cdot Na$
3'-SL: $C_{23}H_{39}NO_{19}$

2.A.1.5. Molecular Weight

3'-SL Na: 655.5
3'-SL: 633.5

2.A.1.6. Structural Formula

Structural formula of 3'-SL monosodium salt is shown in Figure 1. 3'-SL is composed of *N*-acetylneuraminic acid (or sialic acid) and lactose. Figure 2a) and 2b) show the structural formulas of 3'-SL and related compounds.

3'-SL Sodium Salt (*Siallac*3®)

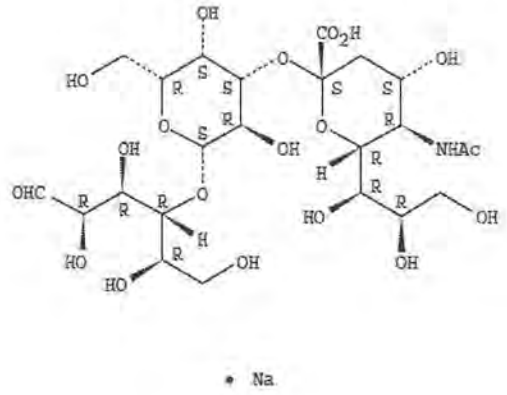
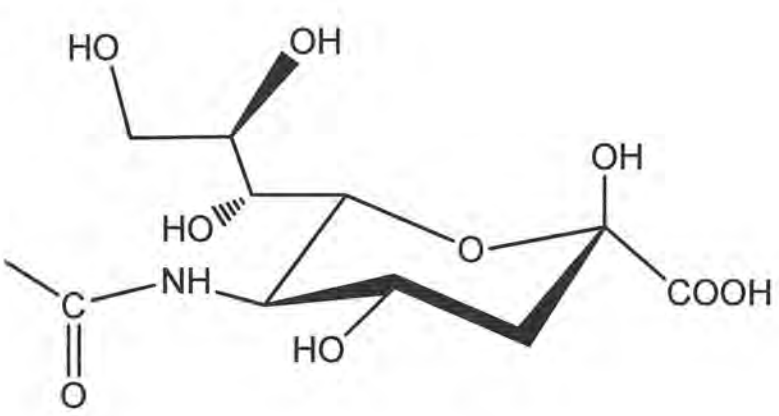
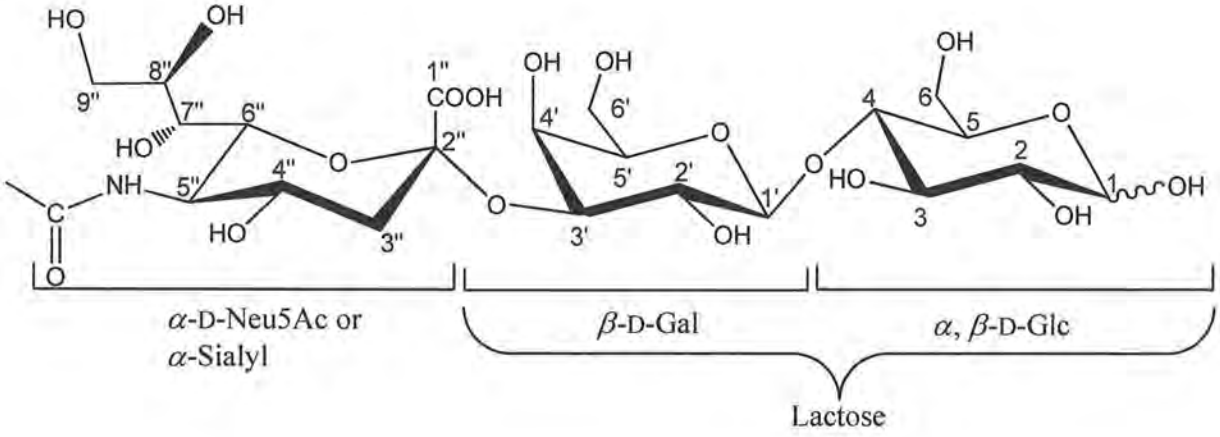


Figure 1. Structural Formula of 3'-SL Monosodium Salt (CAS Registry Figure)

a)



b)



3'-SL Sodium Salt (*Siallac3*®)

c)

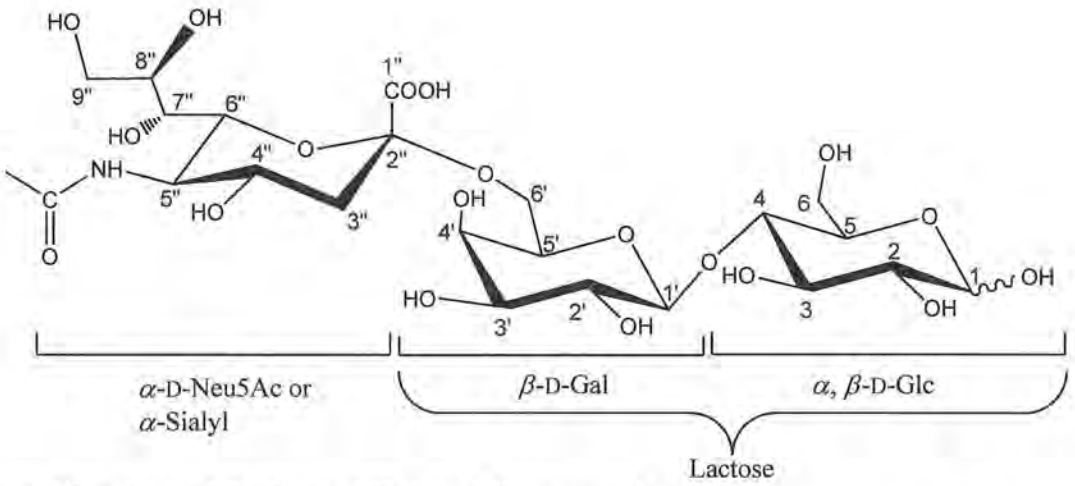


Figure 2. Structure of 3'-SL and Related Compounds.
a) *N*-acetylneuraminic acid, b) 3'-SL, c) 6'-SL (from ten Bruggencate et al., 2014).

2.A.1.7. Chemical and Physical Characteristics

Appearance:	white powder
Melting point:	200~210°C
pH:	5.5 – 7.0

2.A.1.8. Background

Sialyllactose (SL) is a functional human milk oligosaccharide (HMO) that exists in small amounts in beestings (cow's foremilk), but not in commercialized milk products, whereas it is abundant in human milk. The presence of HMOs in breast milk has been associated with a variety of nutritional effects including the establishment and maintenance of healthy intestinal bacterial microflora that is rich in bifidobacteria, reducing the adhesion of pathogens to the intestinal wall, providing nutritional support to the neonatal immune system, and potentially supporting the maintenance of normal cognitive, learning and memory functions of the brain (Bode et al., 2012; ten Bruggencate et al., 2014).

SL has a combined structure of lactose and *N*-acetylneuraminic acid (NeuAc, also called sialic acid) and exists as two forms, 3′-SL and 6′-SL in human milk. Approximately 50–70% and 10–30% of HMOs are fucosylated or sialylated, respectively, and less than 10% are neither fucosylated nor sialylated (Ninonuevo et al, 2006). The most abundant sialylated oligosaccharides in human milk are 3′-SL, 6′-SL, disialyllactose-N-tetraose (DSLNT), and sialyllacto-N-tetraose.

Approximately 200 molecular species of milk oligosaccharides have been identified, based on the extension of lactose. Tables 2-1, 2-2, and 2-3 show concentrations of 3'-SL and 6'-SL in human milks. It is noteworthy that human milk has similar concentrations of 6'-SL and 3'-SL at or after 30 days postpartum but has higher concentrations of 3'-SL than 6'-SL at less than 30 days postpartum (Tables 2-1 and 2-2). On the other hand, cow milk contains higher concentration of 3'-SL; the ratio of 3'-SL to 6'-SL concentration ranges from 1.8:1 to 5:1 (Table 3).

3'-SL was detected in all samples ranging from 42 to 840 mg/kg with mean concentrations at earlier stages of lactation being higher than those at later stages. Typical infant formula is estimated to contain 17-19 mg/L of 3'-SL. The addition of 3'-SL to term infant formulas is consistent with efforts to produce infant formula that closely match the nutrient composition of human milk.

Tables 2-1 and 2-2 summarizes 3'-SL concentrations of human colostrum and mature milk collected from breastfeeding women in various international cohorts (Term-studies - Asakuma et al., 2007; Austin et al., 2016; Bao et al., 2007; Bode et al., 2012; Spevacek et al., 2015; Coopa et al., 1999; Martin-Sosa et al., 2003; Monti et al., 2015; McGuire et al., 2017; Smilowitz et al., 2013; Sprenger et al., 2017; Thurl et al., 2010; Preterm studies - Gabrielli et al., 2011; van Niekerk et al., 2014; Xu et al., 2017). In the literature, concentrations of 3'-SL in human milk were estimated to be in the range of 42 and 840 mg/L.

A decrease ($P < 0.05$) in 3'-SL concentration was observed during the course of lactation. The most prominent effects were found in a study by Thurl et al. (2010); the concentrations of 3'-SL were 350 mg/L for milk collected on postnatal day 3, 270 mg/L on day 30, and 230 - 240 mg/L on Days 60 - 90. Although the data analyses with term and preterm milks were conducted separately in this review, no clear effects of gestational age on 3'-SL concentrations were found (Spevacek et al., 2015).

The comparison of the 3'-SL concentrations in secretor and non-secretor milk were varied. The study by van Niekerk et al. (2014) showed that secretor mothers produce high amounts of 3'-SL compared with non-secretor mothers. However, a study by Xu et al. (2017) reported that the concentration of total SL was 26% lower on postnatal day 120 in secretor compared with non-secretor mothers ($P < 0.05$) although fucosylated HMO concentrations were 14–39% higher at all times tested in milk from secretor mothers. The study by Spevacek et al. (2015) found comparable values between milks from secretor and non-secretor mothers.

To determine the use level of 3'-SL in term infant formulas, the 0.5-8 month data from term studies, specifically those of American and European mothers, were considered. We chose the addition level of 230 mg/L of 3'-SL resulting in the final concentrations of 247-249 mg/L in the formula since a typical un-supplemented infant formula contains 17-19 mg/L of 3'-SL.

Table 2-1. Concentrations of 3'-SL in Human Milk (mg/L); Term Studies

Reference	Country	Colostrum or milk at less than day 35 post-partum		**Human milk at 0.5-8 months post-partum**	
Americans					
McGuire et al., 2017	Washington, USA, N=41			0.5-5 mo	356 ± 25
	CA, USA, N=19			0.5-5 mo	300 ± 35
Smilowitz et al., 2013	USA, N=52				72 ± 28
Bao et al., 2007	USA, N=10	Colostrum, Day 2-4	71-133		
		Day 3-5	97 ± 38	Day 49	78
		Day 6-21	76 ± 14	Day 67	42
Spevacek et al., 2015	USA, term mothers, N=15	Day 0-5	228 ± 63		
		Day 14	165 ± 38		
		Day 28	146 ± 32		
Hong et al., 2014	USA, secretor mothers, N=10	Day 35	53 ± 8		
	USA, non secretors, N=10	Day 35	49 ± 10		
Sample Number Weighted Average of 0.5-8 mo					197
Average of 3 Studies (0.5-8 mo post-partum)					197
Europeans					
Thurl et al., 2010	Germany, N=14-21	Day 3	350	Day 60	230
		Day 8	300	Day 90	240
		Day 15	270		
		Day 22	260		
		Day 30	270		
Martín-Soba et al., 2003	Spain, N=12			Mature	250
McGuire et al., 2017	Spain, N=41			0.5-5 mo	385 ± 27
	Sweden, N=24			0.5-5 mo	296 ± 41
Coppa et al., 1999	Italy, N=18	Day 4	90 ± 60	Day 60	130 ± 120
		Day 10	100 ± 70	Day 90	90 ± 50
		Day 30	90 ± 40		
Monti et al., 2015	Italy, N=2			Time, NS	196- 840
Kunz et al., 1999	Germany, N=10	Days 2-28	270 ± 80		
Sample Number Weighted Average					284
Average of 5 Studies (0.5-5 mo post-partum)					299
Asians					
Asakuma et al., 2007	Japan N=10	Day 1	362 ± 103		
		Day 2	269 ± 70		

		Day 3	258 ± 80		
Austin et al., 2016	China N=88-90	Day 5-11	110 ± 35	1-2 mo	80 ± 22
		Day 12-30	94 ± 25	2-4 mo	79 ± 20
				4-8 mo	83 ± 28
Sprenger et al., 2017	Singapore, low 2'-FL conc. in milk, N=16	1 mo	238	2 mo	219
		1 mo	259±88	2 mo mean (range) 4 mo mean (range)	243±86 (130-477) 221±90 (25-462)
	Singapore, high 2'-FL conc. in milk, N=34	1 mo	217±74	2 mo mean (range) 4 mo mean (range)	195±60 (87-328) 198±59 (98-325)
Sample Number Weighted Average of 0.5-8 mo					147
Average of 2 Studies (0.5-8 mo post-partum)					148
Africans					
McGuire et al., 2017	Ethiopia, Rural N=40			0.5-5 mo	262
	Ethiopia, Urban N=40			0.5-5 mo	333
	Gambia, Rural N=40			0.5-5 mo	295
	Gambia Urban N=40			0.5-5 mo	320
	Ghana, N=40			0.5-5 mo	392 ± 35
	Kenya, N=42			0.5-5 mo	335 ± 28
Bode et al., 2012	Zambia, HIV transmitted, N=81	1 mo	143		
	Zambia, HIV non-transmitted, N=86	1 mo	140		
	Zambia, HIV uninfected, N=36	1 mo	114		
Sample Number Weighted Average of 0.5-8 mo					323
Average of 4 Studies (0.5-8 mo post-partum)					332
Latin Americans					
McGuire et al., 2017	Peru, N=43			0.5-5 mo	335 ± 32
Sample Number Weighted Average of 0.5-8 mo					**254**
Average of all Studies (0.5-8 mo post-partum)					**266**

Table 2-2. Concentrations of 3'-SL in Human Milk (mg/L); Pre-term Studies

Reference	Country	Colostrum or milk at less than day 30 post-partum		Human milk at 0.5-8 months post-partum	
Americans					
Spevacek et al., 2015	USA, term mothers, N=15	Day 0-5	228 ± 70		NA
		Day 14	184 ± 32		
		Day 28	177 ± 51		
Europeans					
Gabrielli et al., 2011	Italy, N=63 25 to 30 weeks of gestation (mean gestational age: 27.9 weeks)	Day 4	220 – 280		NA
		Day 10	260 – 310		
		Day 20	170 – 280		
		Day 30	150 – 230		
Africans					
van Niekerk et al., 2014*	S. Africa; HIV infected secretor mothers	Days 4-28 N=22	~400		NA
	HIV uninfected secretor mothers	Days 4-28 N=21	~350		
	HIV infected non-secretor mothers	Days 4-28 N=19	~200		
	HIV uninfected non-secretor mothers	Days 4-28 N=20	~200		

*Values are approximate values since they were read from graphs. Mothers gave birth to a premature infant with a birth weight of 500 to 1,250 g. NA=not available.

Tables 2-3 and 2-4 summarizes 6'-SL concentrations of human colostrum and mature milk collected from breast-feeding women in various international cohorts. Although the data analyses with term and preterm milks were conducted separately in this review, no clear effects of gestational age on 3'-SL concentrations were found (Spevacek et al., 2015). Comparing 3'SL concentrations in secretor and nonsecretor milk showed mixed results (Spevacek et al.,2015; van Niekerk et al., 2014; Xu et al., 2017); there is no clear evidence that 6'-SL concentrations of milk from secretor mothers are higher than those from nonsecretors.

Table 3 summarizes 3'-SL concentrations of mature bovine milk.

Table 2-3. Concentrations of 6'-SL in Human Milk (mg/L), Term Studies

Reference	Country	Colostrum or milk at less than day 35 post-partum		**Human milk at 0.5-8 months post-partum**	
Americans					
McGuire et al., 2017	Washington, USA, N=41			0.5-5 mo	255 ± 20
	CA, USA, N=19			0.5-5 mo	186 ± 31
Smilowitz et al., 2013	USA, N=52				119 ± 55
Bao et al., 2007	USA, N=10	Colostrum, Day 2-4	215-391		
		Day 3-5	335 ± 33	Day 49	277
		Day 6-21	396 ± 54	Day 67	63
Spevacek et al., 2015	USA, N=10	Day 0-5	519 ± 152		
		Day 14	557 ± 139		
		Day 28	367 ± 108		
Hong et al., 2014	USA, secretor mothers, N=10	Day 35	28 ± 6		
	USA, non secretors, N=10	Day 35	25±10		
Sample Number Weighted Average			361		173
Average of Studies (0.5-5 mo post-partum)			361		170
Europeans					
Thurl et al., 2010	Germany, N=14-21	Day 3	1,310	Day 60	630
		Day 8	1,770	Day 90	470
		Day 15	1,570		
		Day 22	1,420		
		Day 30	1,350		
Martin-Soba et al., 2003	Spain, N=12			Mature	250
McGuire et al., 2017	Spain, N=41				319 ± 25
	Sweden, N=24			0.5-5 mo	127 ± 15
Coppa et al., 1999	Italy, N=18	Day 4	590 ± 150	Day 60	300 ± 110
		Day 10	550 ± 180	Day 90	240 ± 100
		Day 30	440 ± 140		
Monti et al., 2015	Italy, N=2			Age, NS	46 - 98
Kunz et al., 1999	Germany, N=10	Days 2-28	380 ± 50		
Sample Number Weighted Average					285
Average of Studies (0.5-5 mo post-partum)					273
Asians					
Asakuma et al., 2007	Japan N=10	Day 1	342 ± 120		
		Day 2	371 ± 115		
		Day 3	369 ± 86		
Austin et al., 2016	China N=88-90	Day 5-11	330	1-2 mo	140
		Day 12-30	250	2-4 mo	78
				4-8 mo	42

Sprenger et al., 2017	Singapore N=50	1 mo	528	2 mo	272
				3 mo	135
Weighted Average					
Average of Studies (1-8 mo post-partum)					
Africans					
McGuire et al., 2017	Ethiopia, N=80			0.5-5 mo	237 - 345
	Gambia, N=80			0.5-5 mo	293 - 371
	Ghana, N=40			0.5-5 mo	564 ± 56
	Kenya, N=42			0.5-5 mo	276 ± 22
Sample Number Weighted Average					366
Average of Studies (0.5-5 mo post-partum)					366
Latin Americans					
McGuire et al., 2017	Peru, N=43			0.5-5 mo	403 ± 40
Sample Number Weighted Average of 0.5-8 mo					269
Average of all Studies (0.5-8 mo post-partum)					245

Table 2-4. Concentrations of 6'-SL in Human Milk (mg/L), Pre-term Studies

Reference	Country	Colostrum or milk at less than day 30 post-partum		Human milk at 0.5-8 months post-partum	
Americans					
Spevacek et al., 2015	USA, N=10	Day 0-5	545 ± 291		NA
		Day 14	722 ± 279		
		Day 28	659 ± 418		
Europeans					
Gabrielli et al., 2011	Italy, N=61	Day 4	420 - 650		NA
		Day 10	480 - 870		
		Day 20	290 - 770		
		Day 30	160 - 590		
Africans					
van Niekerk et al., 2014*	S. Africa; HIV infected secretor mothers	Days 4-28 N=22	~400		NA
	HIV uninfected secretor mothers	Days 4-28 N=21	~350		
	HIV infected non-secretor mothers	Days 4-28 N=19	~200		
	HIV uninfected non-secretor mothers	Days 4-28 N=20	~200		

NA=not available.

Table 3. Concentrations of SLs in Mature Bovine Milk (mg/L)

References	Sample	3'-SL Concentrations in Mature Milk	6'-SL Concentrations in Mature Milk
Fong et al., 2011 and Urashima et al., 2013	Mature milk 1 (n=6) (from Martín-Sosa et al., 2003)	94 – 119	67 – 88
	Mature milk 2 (n=4) (from McJarrow and van Amelsfort-Schoonbeek, 2004)	35 – 50	14 – 25
	Mature milk 3 (n=4) (from Nakamura et al., 2003)	30	25
Martín-Sosa et al., 2003	Mature milk (n=6)	120	90
	Late Lactation milk (n=6)	90	70
Kelly et al., 2013	Friesian cows (n=5253)	49.3 ± 12.4	11.1 ± 3.4
	Friesian-Jersey crossbred cows (n=6854)	58.4 ± 17.1	11.7 ± 3.4
	Jersey cows (n=3400)	71.8 ± 21.5	12.7 ± 4.0
Sample Number Weighted average		58.3	11.8
Average of studies		71.1	39.7

2.A.1.9. Potential Toxicants in the Source of the Notified Substance

Potential toxicants have not been identified.

2.A.1.10. Particle Size

NLT 99±0.1% passes through an 80 mesh.

2.B. Method of Manufacture

2.B.1. Manufacturing Process

The production of 3'-SL from its precursor *N*-acetyl-D-mannosamine (ManNAc) has been well studied (Auge et al., 1984). However, the substrate ManNAc is too expensive to be commercially produced in a large scale at a reasonable cost. Recently, GeneChem has solved this problem by using a much cheaper starting material, *N*-acetyl-D-glucosamine (GlcNAc) (99% purity) and a one-pot reaction process.

The main production process of GeneChem is composed of two steps: the first step is one-pot reaction using raw materials and enzymes. The next step is the purification of 3'-SL sodium salt using various filtrations and anion exchange column chromatography.

3'-SL sodium salt is manufactured in compliance with current Good Manufacturing Practices (cGMP) and the principles of Hazard Analysis and Critical Control Points (HACCP).

<u>One-Pot Multienzyme Synthesis</u>

Production of 3'-SL derived from *N*-acetyl-D-glucosamine, cytidine 5'-monophosphate (CMP), and lactose utilizing efficient one-pot multienzyme system is shown in Figure 3. The constituents of the reaction are *N*-acetyl-D-glucosamine, sodium pyruvate, lactose monohydrate, cytidine 5'-monophosphate (CMP), acetyl phosphate, adenosine 5'-triphosphate disodium salt hydrate, magnesium chloride hexahydrate, sodium hydroxide (NaOH) and purified enzymes. Amount of each enzyme extract is determined by the initial rate of CMP-*N*-acetylneuraminic acid (CMP-NeuAc) formation equating the initial rate of the desired 3'-SL formation. Due to the high energy CMP-*N*-acetylneuraminic acid produced, the pH should be controlled with NaOH.

In GeneChem's manufacturing process, *N*-acetyl-D-glucosamine-2-epimerase is used to convert *N*-acetyl-D-glucosamine to *N*-acetyl-D-mannosamine. Then, *N*-acetyl-D-mannosamine passes through several steps to produce *N*-acetylneuraminic acid. Finally, NeuAc is combined with cytidine triphosphate (CTP) to produce CMP-*N*-acetylneuraminic acid. *N*-acetylneuraminic acid (sialic acid) from CMP-*N*-acetylneuraminic acid is conjugated to lactose by sialyltransferase to produce the final product, 3'-SL.

The resulting mixture is heated rapidly to denature the enzymes used in the reaction, and then cooled down to <35 °C. The resulting mixture is centrifuged to remove the undissolved proteins and debris. The precipitated sludge is re-suspended in water, and then centrifuged again. The combined supernatant solution is passed through a 0.45 μm filtration to remove the remaining debris.

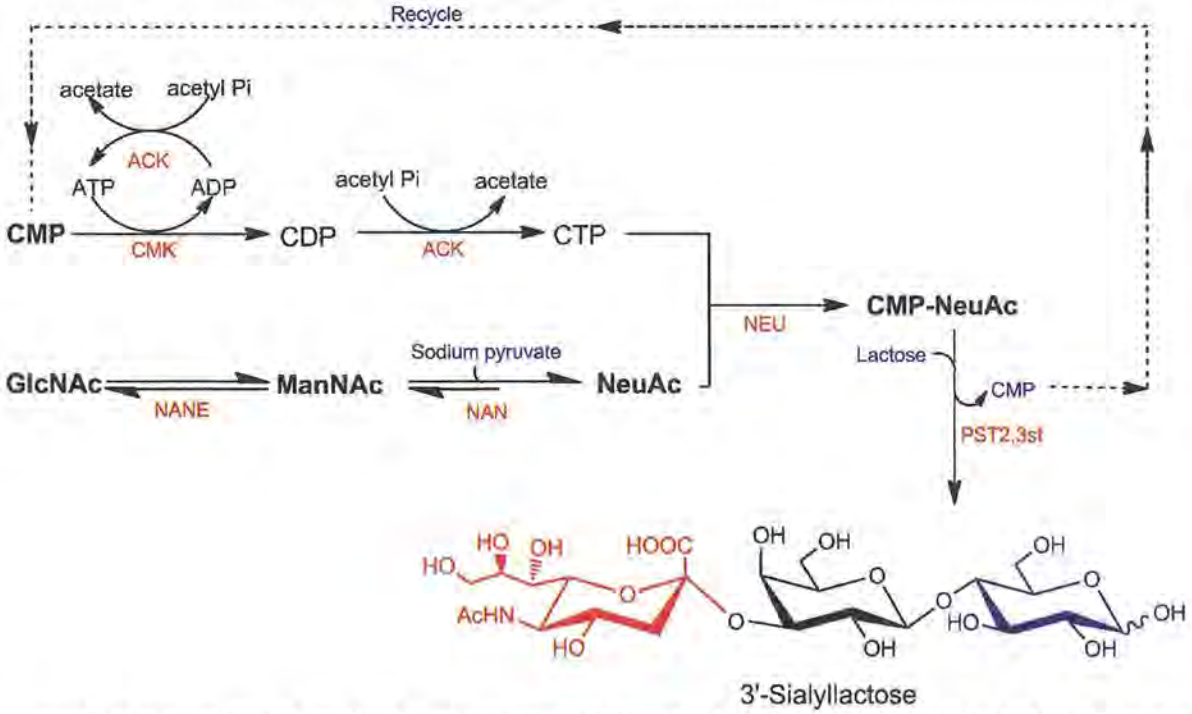


Figure 3. Production of 3'-SL Derived from *N*-Acetyl-D-Glucosamine (GlcNAc), Cytidine 5'-Monophosphate (CMP), and Lactose Using Purified Enzymes

Where CTP=cytidine triphosphate; ManNAc =*N*-acetyl-D-mannosamine; NeuAc = *N*-acetylneuraminic acid (sialic acid).

Purification of 3'-SL

The crude 3′-SL sodium salt is subjected to extensive purification using multiple filtration steps. 3'-SL sodium salt is a high purity powder (>98%), and has been determined to be free of quantifiable protein and residual DNA from the microorganism as determined by enzyme specific enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (qPCR), respectively.

The filtered reaction mixture containing the product is passed through a ultra-filtration system (with 10 KDa molecular weight cutoff membrane) to remove the remaining proteins. Nano filtration is carried out to remove salts and impurities < 400 MW from the solution. Deionized water is added to the nano filtration system to maintain the load volume. The solution from nano filtration is loaded onto the first ion exchange column to remove the charged nucleotides, salts (chloride) and residual proteins.

The second nano filtration is carried out to remove remaining salts and organic impurities (removal of *N*-acetylneuraminic acid and *N*-acetyl-D-glucosamine) < 400 MW from the solution. Deionized water (DI water) is added again to the system to maintain the load volume. The solution from the previous nano filtration is loaded onto second ion exchange column and then eluted with sodium chloride (NaCl) to isolate the desired product, 3′-SL sodium salt. To remove salts less than 100 MW from the previously obtained solution, another nano filtration is carried out. The recovered solution from the previous nano filtration is passed through a filtration system with activated charcoal to remove color. The colorless solution containing the 3′-SL sodium salt is concentrated using vacuum evaporator. The solution filtered through microfiltration (MF, 0.22 μm) and ultrafiltration membrane filter is then dried using freeze dryer. The resulting pure white powder product is milled, transferred to a sterilized storage bag, and kept out of direct sunlight, in a cool and dry place. Figure 4 summarizes the manufacturing process of 3′-SL sodium salt including purification steps.

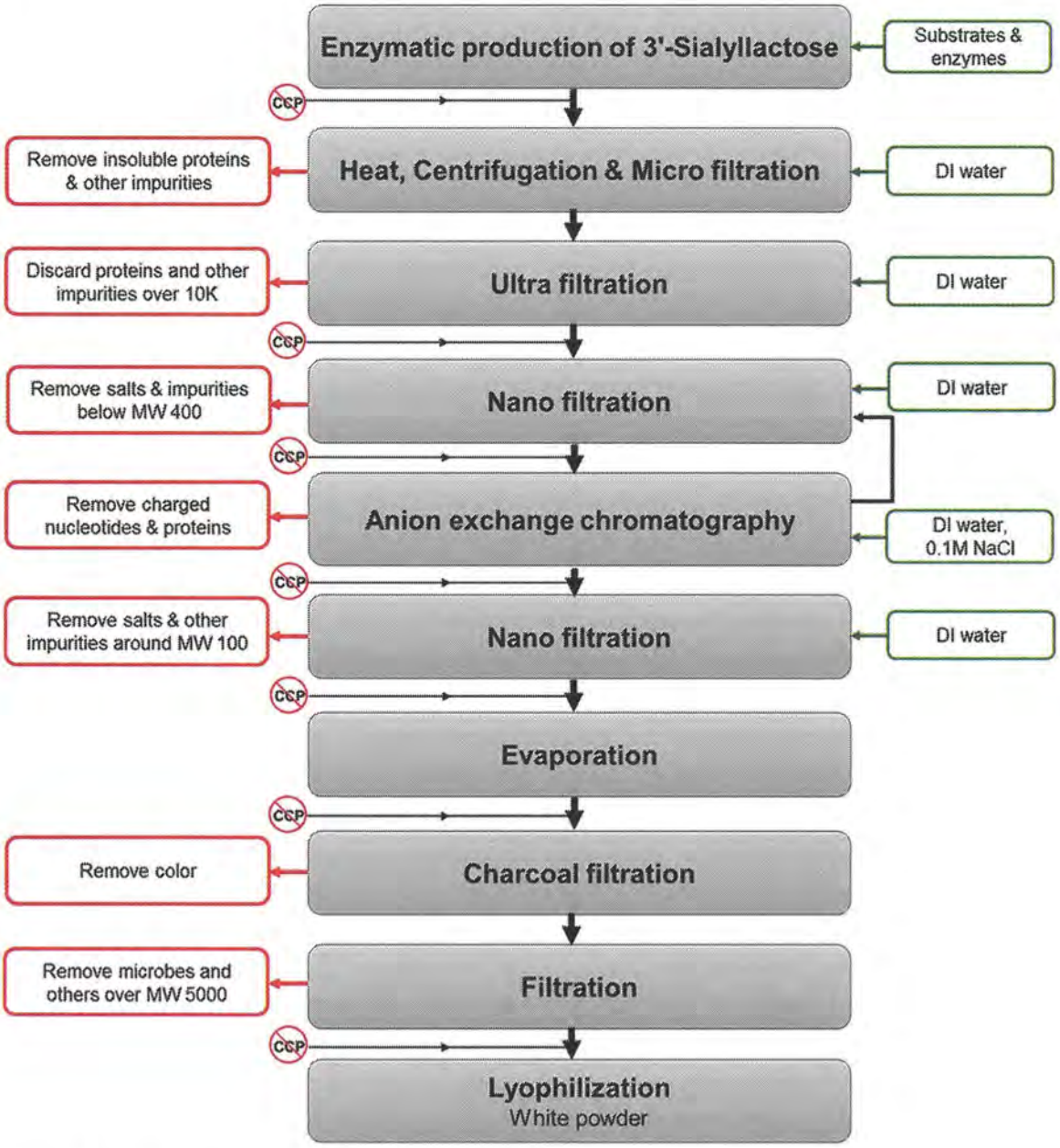


Figure 4. Flow Diagram of 3′-SL Sodium Salt Manufacturing Process

2.B.2. Quality Control of Raw Materials

Raw Materials and Processing Aids

All raw materials and processing aids are food-grade and are safe.

In-Process Control

GeneChem's 3′-SL sodium salt is manufactured consistent with the principles of Hazard Analysis and Critical Control Points (HACCP).

List of the Raw Materials and Processing Aids

Tables 4 and 5 list raw materials and processing aids, respectively, used in enzymatic production of 3'-SL sodium salt.

Table 4. Raw Materials for Enzymatic Production of 3'-SL Sodium Salt

Material	Function
Raw Material Substrates	
N-Acetyl-D-glucosamine	Substrate/source raw material
Sodium pyruvate	Substrate/source raw material
Lactose monohydrate	Substrate/source raw material
Other Components and Processing Aids	
Enzymes	Biological Catalyst
Cytidine 5'-monophosphate	Substrate/ producing the high energy compound
Acetyl phosphate**	Substrate/ producing the high energy compound
Adenosine 5'-triphosphate disodium salt hydrate	Substrate/ producing the high energy compound
Magnesium chloride hexahydrate	Maintains enzyme activity
Sodium hydroxide (NaOH)	Adjusting pH

**The half-life of acetyl phosphate in aqueous solution at 30.5°C and pH 7.2 is 8 hours (Hirschbein et al., *J. Org. Chem.* 1982). Therefore, acetyl phosphate used in the reaction decomposed to acetate and phosphate during the production process.

Table 5. Processing Aids for Purification of 3'-SL Sodium Salt

Materials	Function
Micro-filtration 0.45 µm membrane	Removal of insoluble matter and protein
Ultra-filtration 10K membrane	Remove high molecular weight impurities
1st Nano-filtration membrane	Removal of small molecules below 400
2nd Nano-filtration membrane	Removal of small molecules below 100
1st Ion-exchange resin	Removal of charged impurities
2nd Ion-exchange resin	Removal of charged and neutral impurities. Producing the pure product.
Sodium chloride (NaCl)	Eluent for resin
Activated charcoal	Decolorant
Micro-filtration (0.22 µm) and Ultra-filtration (MF/UF) membrane	Product polishing

2.C. Specifications and Product Analysis

2.C.1. Specifications for GeneChem's 3'-SL Sodium Salt

The product specifications for 3'-SL sodium salt are detailed in Table 6. All methods of analyses are nationally or internationally recognized or have been validated by GeneChem. The product is ≥98% pure on a dry weight basis, as measured by high performance anion-exchange chromatography with pulsed amperometric detection

(HPAEC-PAD). Appropriate limits for heavy metals and microbial impurities have been established.

Table 6. Specifications of GeneChem's 3'-SL Sodium Salt

Parameter	Specification	Method	Detection Limit
Appearance	White powder	Visual	
Solubility	Clear colorless solution at 20 mg/mL in water	Visual	
Purity	≥ 98%	HPAEC-PAD	
Moisture, %	≤ 6	KFSC 7/1/ 1.1 / 1.1.1 / 1.1.1.1	
Ash, %	≤ 8.5	KFSC 7/1 / 1.1 / 1.1.2	
Fat, g/100 g	≤ 0.5	KFSC 7/1/1.1/1.1.5/1.1.5.1	
Protein, g/100 g	≤ 0.1	KFSC 7/1/1.1/1.1.3/1.1.3.3	
Sodium, %	≤ 3.5	KFSC 7/1/1.2/1.2.1/1.2.1.6 (ICP)	
Arsenic, ppm	≤ 0.2	KFSC 7/7/7.1/7.1.2/7.1.2.3 (ICP)	0.002 ppm
Cadmium, ppm	≤ 0.1	KFSC 7/7/7.1/7.1.2/7.1.2.2 (ICP)	0.004 ppm
Lead, ppm	≤ 0.1	KFSC 7/7/7.1/7.1.2/7.1.2.1 (ICP)	0.004 ppm
Mercury, ppm	≤ 0.5	KFSC 7/7/7.1/7.1.2/7.1.2.5	0.0002 ppm
Gene residue	Negative	qPCR	0.007 ng/g
Endotoxins, EU/g	≤ 300	Endotoxin Kit (Endosafe®-PTS™)	1-0.01 EU/g
Total plate counts, CFU/g	≤ 200	KFSC 7/3/3.5.1 (Evaluation of Dry Rehydratable Film Method)	
Coliform, CFU/g	Negative	KFSC 7/3/3.7/3.7.1 (*BGLB method)	
Salmonella, CFU/g	Negative	KFSC 7/3/3.11	
Yeasts and Molds, CFU/g	≤ 200	KFSC 7/3/3.10	
Listeria monocytogenes	Negative	KFSC 7/3/3.15	
Enterobacter sakazakii (Cronobacter spp.)	Negative	KFSC 7/3/3.21	

* BGLB method; brilliant green lactose bile method; KFSC=Korean Food Standards Codex; CFU = colony forming units; EU = endotoxin unit.

2.C.2. Product Analysis

Analysis of 5 non-consecutive batches of GeneChem's 3'-SL sodium salt demonstrated that the manufacturing process produces a consistent product that is in compliance with the established specifications. A summary of the results of the product analysis are shown in Table 7.

Table 7. Product Analysis for 5 Non-Consecutive Batches of 3'-SL Sodium Salt

Tests	Batch Number				
	150622-01	160509-01	160510-01	160515-01	160601-01
Appearance	Complied	Complied	Complied	Complied	Complied
Solubility	Complied	Complied	Complied	Complied	Complied
^{1}H NMR Spectrum	Complied	Complied	Complied	Complied	Complied
Mass Spectrum	Complied	Complied	Complied	Complied	Complied
Purity (HPLC), %	98.8	99.08	99.25	99.05	98.68
Moisture, %	1.83	3.21	2.72	2.95	2.65
Ash, %	7.59	7.14	7.01	7.14	7.43
Fat, %	0.26	0.25	0.25	0.25	0.25
Protein, %	0.0	0.0	0.0	0.0	0.0
Sodium, %	1.79	1.71	2.47	2.12	2.45
Arsenic, ppm	ND	ND	ND	ND	ND
Cadmium, ppm	ND	ND	ND	ND	ND
Lead, ppm	ND	ND	ND	ND	ND
Mercury, ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Gene residue	Negative	Negative	Negative	Negative	Negative
Endotoxins, EU/g	<50	<50	<50	59.9	<50
Total Plate Counts, CFU/g	180	Negative	5	5	Negative
Coliform, CFU/g	Negative	Negative	Negative	Negative	Negative
Salmonella, CFU/g	Negative	Negative	Negative	Negative	Negative
Yeasts and Molds, CFU/g	Negative	Negative	Negative	Negative	Negative
Listeria monocytogenes	Negative	Negative	Negative	Negative	Negative
Enterobacter sakazakii (*Cronobacter* spp.)	Negative	Negative	Negative	Negative	Negative

ND= Not Detected; CFU = colony forming units; EU = endotoxin unit.

2.C.3. Identification Methods

Nuclear magnetic resonance (NMR), mass spectrometric (MS) analysis, and Fourier transform infrared spectroscopy (FT-IR) confirmed that the 3'-SL sodium salt manufactured by GeneChem is chemically and structurally identical to those published in the literature.

Nuclear Magnetic Resonance (NMR) Analysis of GeneChem's 3'-SL Sodium salt

Platzer et al. (1989) compared and confirmed the chemical structure of 3'-SL and 6'-SL from Sigma by COSY, COSY LR, RECSY and COSY LR-R determinations at 500 MHz NMR spectroscopy (Table 8). The structure of 3'-SL was confirmed by comparison of ^{1}H and ^{13}C NMR spectra of SL originated from caprine colostrum or bovine milk (Sabesan et al., 1986). Kjærulff (2014) verified the structure of 3'-SL using ^{1}H-, ^{13}C, and 2D NMR (Table 8). The purified 3'-SL sodium salt produced by GeneChem was dissolved in deuterium oxide (D_2O) and subjected to NMR analyses using a 900 MHz NMR Spectrometer (Figures 5 and 6; Tables 9-1, and 9-2). Characteristic proton chemical shifts of glucose, galactose and sialic acid were obtained, identical to those of Sigma's product (A8681).

Table 8. ^{1}H Chemical Shift Assignments for 3'-SL Sodium Salt (Literature vs. Commercial Standard)

Atom assignment	^{1}H Chemical Shift (ppm), multiplicity		
	Kjærulff, 2014	Platzer et al., 1989	Sigma standard
H_2O	4.79	4.79	4.61
α-Glc			
1α	5.21, d	5.219	5.01, d
2α	3.58, m	3.58	
3α	3.82, m	3.84	
4α	3.67, m	3.67	
5α	3.95, m	3.96	
6α	3.87, m	3.87±0.02	
6α	3.92, m	3.87±0.02	
β-Glc			
1β	4.65, d	4.661	4.46, d
2β	3.27, dd	3.281	3.09, dd
3β	3.64, m	3.63	
4β	3.65, m	3.65	
5β	3.59, m	3.6	
6β	3.82, m	3.8	
6β	3.97, m	3.96	
β-Gal			
1'	4.52, d	4.53	4.33, d
2'	3.57, m	3.57	
3'	4.10, bd	4.117, 4.113	3.92, bd
4'	3.95, m	3.956	3.77, m
5'	3.70, m	3.71	
6'	3.71, m	3.72	
6'	3.76, m	3.78	
α-NeuAc			
1"			
2"			

3"	1.79, dd	1.8	1.61, dd
3"	2.75, dd	2.757	2.56, dd
4"	3.68, m	3.68	
5"	3.84, m	3.84	
6"	3.62, m	3.62	
7"	3.59, m	3.58	
8"	3.88, m	3.88±0.02	
9"	3.64, m	3.69	
9"	3.86, m	3.88±0.02	
CH_3	2.02, s	2.03	1.825

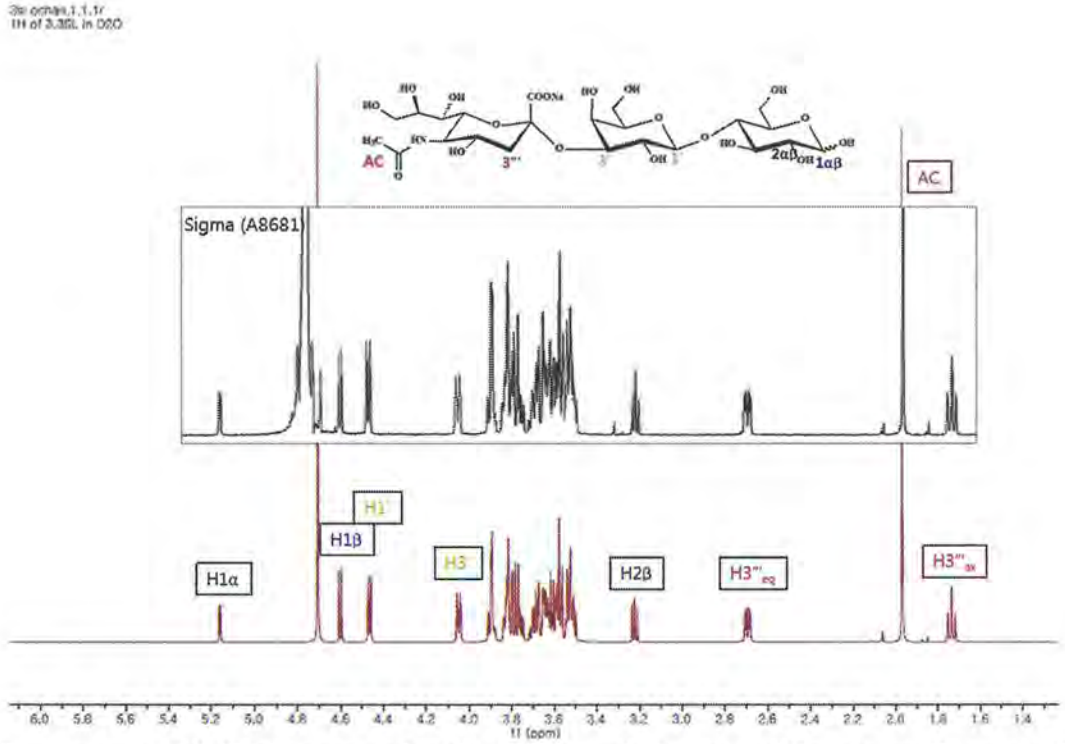


Figure 5. ^{1}H NMR Spectra of 3'-SL Sodium Salt (GeneChem's vs. Sigma's Standard)

The 3'-SL sodium salt produced by GeneChem was dissolved in deuterium oxide (D_2O) and analyzed by ^{13}C-NMR using a 900 MHz NMR Spectrometer (Korea Basic Science Institute, Ochang Headquarters). Identity of the product was confirmed by comparison with literature spectroscopic data (Figure 6; Tables 9-1 and 9-2).

3'-SL Sodium Salt (*Siallac*3®)

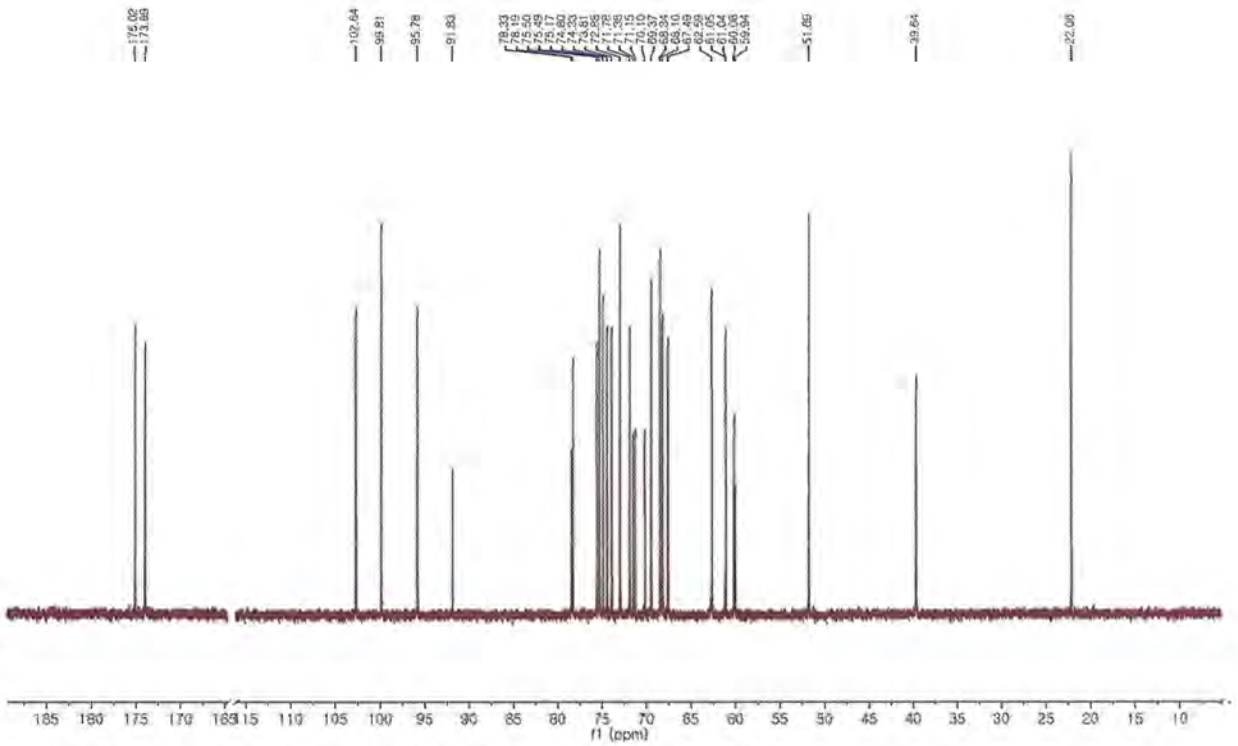


Figure 6. ^{13}C NMR Spectrum of GeneChem's 3'-SL Sodium Salt

Table 9-1. ^{13}C Chemical Shift Assignments for 3'-SL Sodium Salt (Literature vs. GeneChem's)

Atom assignment	^{13}C Chemical Shift (ppm),		
	Kjærulff (PhD Thesis, 2014)	Sabesan et al., (*J. Am. Chem. Soc.* 1986)	GeneChem Inc.
α-Glc			
1α	92.7		91.83
2α	72		71.38
3α	72.2		71.78
4α	79.1		78.33
5α	70.9		70.10
6α	60.7		60.08
β-Glc			
1β	96.6	96.26	95.78
2β	74.6	74.33	74.33
3β	75.2	74.84	74.80
4β	79.0	78.86	78.19
5β	75.6	75.29	75.17
6β	60.9	60.65	61.04

3'-SL Sodium Salt (*Siallac*3®)

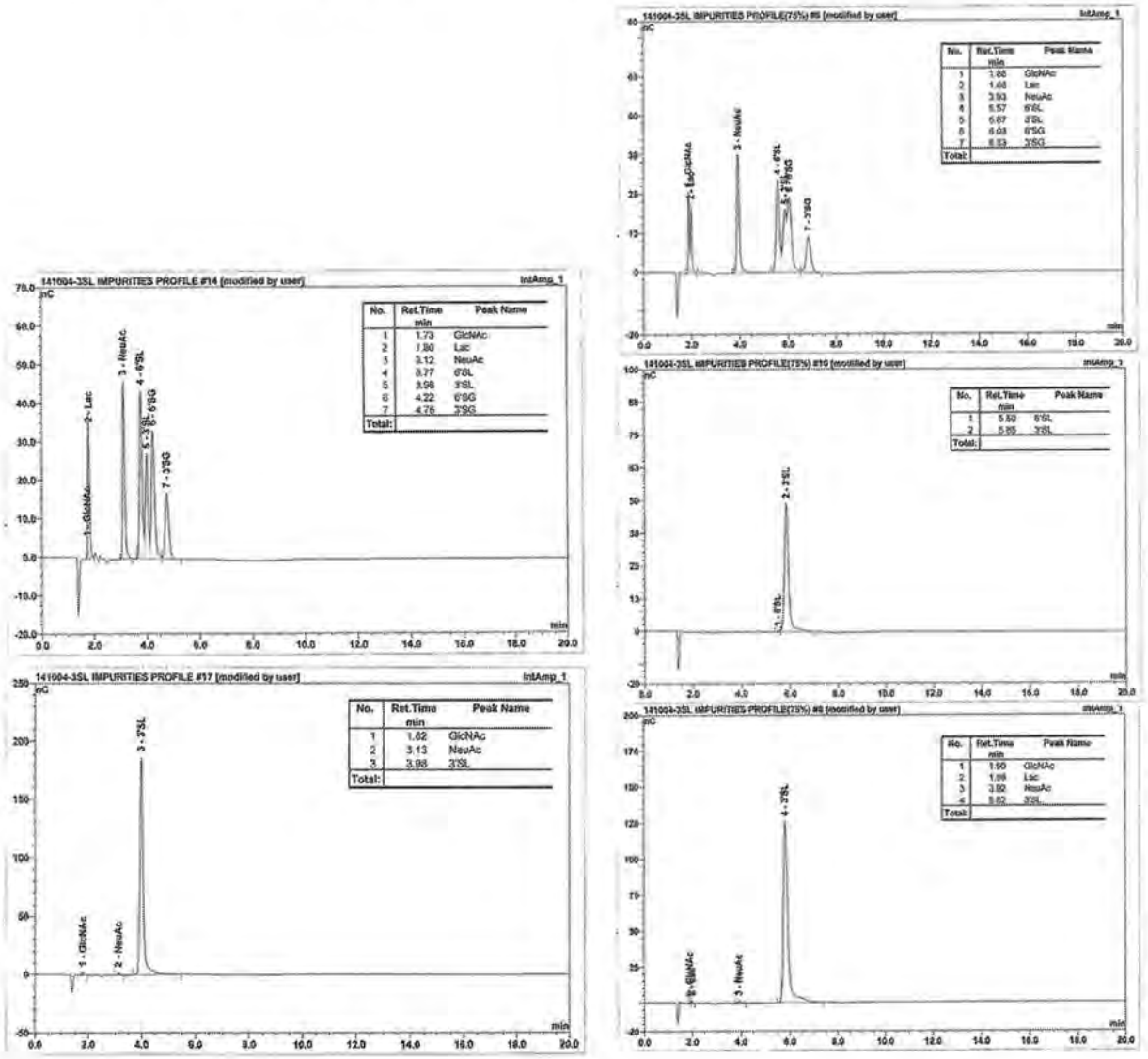
β-Gal			
1'	103.5	103.17	102.64
2'	70.2	69.86	71.15
3'	76.3	76.03	75.50
4'	68.3	68.00	68.10
5'	76.0	75.65	75.49
6'	61.9	61.50	61.05
α-NeuAc			
1"	174.7	174.23	173.89
2"	100.6	100.32	99.81
3"	40.5	40.19	39.64
4"	69.2	68.79	69.37
5"	52.5	52.22	51.69
6"	73.7	73.39	73.81
7"	68.9	68.67	68.34
8"	72.6	72.25	72.88
9"	63.4	63.14	62.59
CO	175.8	175.48	175.02
CH_3	22.9	22.55	22.08

Table 9-2. Summary of the Analytical Data for the ^{13}C NMR Analysis of GeneChem's 3'-SL Sodium Salt

Atom assignment	^{1}H chemical shift [ppm], No. of proton	^{13}C chemical shift [ppm]	^{13}C Chemical Shift [ppm], Literature	HMBC correlations
α-Glc				
1α	5.15, 1H	91.83	92.7	2α, 4α
2α	3.58, 1H	71.38	72.0	
3α	3.83, 1H	71.78	72.2	
4α	3.67, 1H	78.33	79.1	
5α	3.90, 1H	70.10	70.9	3α
6α	3.85, 1H	60.08	60.7	
6α	3.90, 1H	60.08		
β-Glc				
1β	4.60, 1H	95.78	96.6	
2β	3.22, 1H	74.33	74.6	1β, 3β
3β	3.64, 1H	74.80	75.2	5β
4β	3.65, 1H	78.19	79.0	
5β	3.59, 1H	75.17	75.6	4β
6β	3.82, 1H	61.04	60.9	5β
6β	4.04, 1H	61.04		
β-Gal				
1'	4.46, 1H	102.64	103.5	5α/5β
2'	3.57, 1H	71.15	70.2	1', 3'
3'	4.45, 1H	75.50	76.3	2", 2'
4'	4.04, 1H	68.10	68.3	2', 3'
5'	3.71, 1H	75.49	76.0	6'
6'	3.74, 1H	61.05	61.9	
6'	3.77, 1H	61.05		5'
α-NAcNeu				
1"	-	173.89	174.7	-
2"	-	99.81	100.6	-
3"	1.74, 1H	39.64	40.5	1", 2", 4", 5"
3"	2.69, 1H	39.64		2", 4", 5"
4"	3.68, 1H	69.37	69.2	
5"	3.83, 1H	51.69	52.5	CO, 4", 6"
CO	-	175.02	175.8	-
CH_3	1.96, 1H	22.08	22.9	CO
6"	3.62, 1H	73.81	73.7	2", 4", 8"
7"	3.56, 1H	68.34	68.9	8", 9"
8"	3.88, 1H	72.88	72.6	7"
9"	3.65, 1H	62.59	63.4	
9"	3.81, 1H	62.59		

Analysis of 3′-SL Sodium Salt Using HPAEC-PAD

Standards of 3′-SL sodium salt and 6′-SL sodium salts were purchased from Sigma-Aldrich Co. LLC, USA, and NeuAc was purchased from Carbosynth Limited, UK. The HPAEC analyses were conducted using an isocratic elution program in a solvent system containing (a)100 mM sodium hydroxide and 100mM sodium acetate and (b)100 mM sodium hydroxide and 75mM sodium acetate at a flow rate of 1 mL/min, using a Dionex CarboPac™ PA100 Analytical (4.6 X 250 mm, Thermo Scientific) (Figure 7). The HPAEC chromatograms also confirmed the identify of 3′-SL sodium salt.



(a) (b)

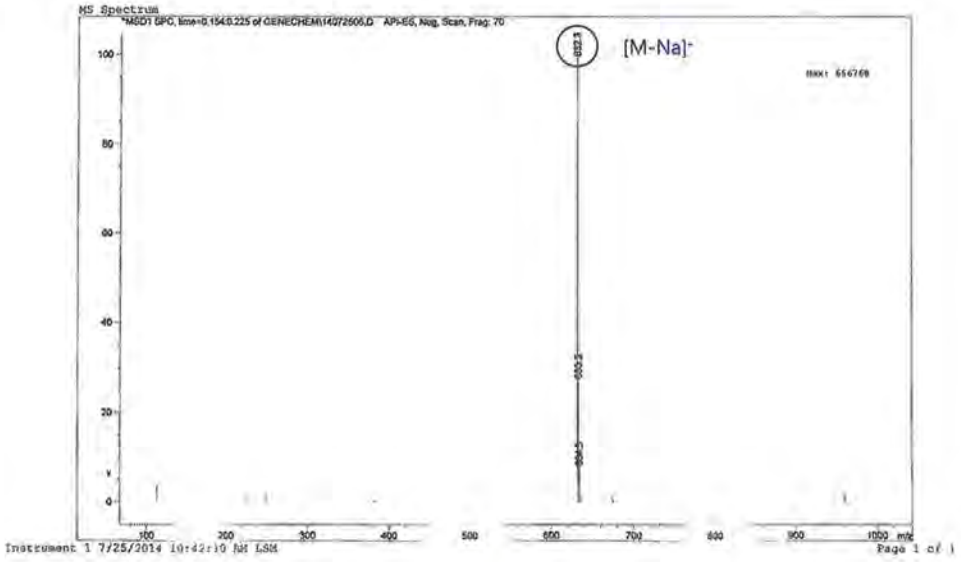
Figure 7. HPLC Chromatograms of 3′-SL Sodium Salt Under Two Different HPLC Conditions.

(a) Standard (top) and GeneChem (bottom) both with retention time 3.98 min, and (b) Standard (top), Sigma (middle), and GeneChem (bottom) with retention times 5.87min, 5.85, and 5.82 min respectively.

Mass Spectrometric Analysis of 3'-SL Sodium Salt

The mass analysis was performed using Mass 1100 + G1958 model (Agilent Technologies Inc., USA). MS spectra confirmed the expected mass of 3'-SL sodium salt. Molecular weight of 3'-SL sodium salt is 655.54 (Molecular Formula: $C_{23}H_{38}NO_{19}Na$). The recorded values were 632.3 for $[M-Na]^-$ in negative-ion mode, and 656.2 for $[M + H]^+$, and 678.3 for $[M + Na]^+$ in positive-ion mode (Figure 8).

a)



b)

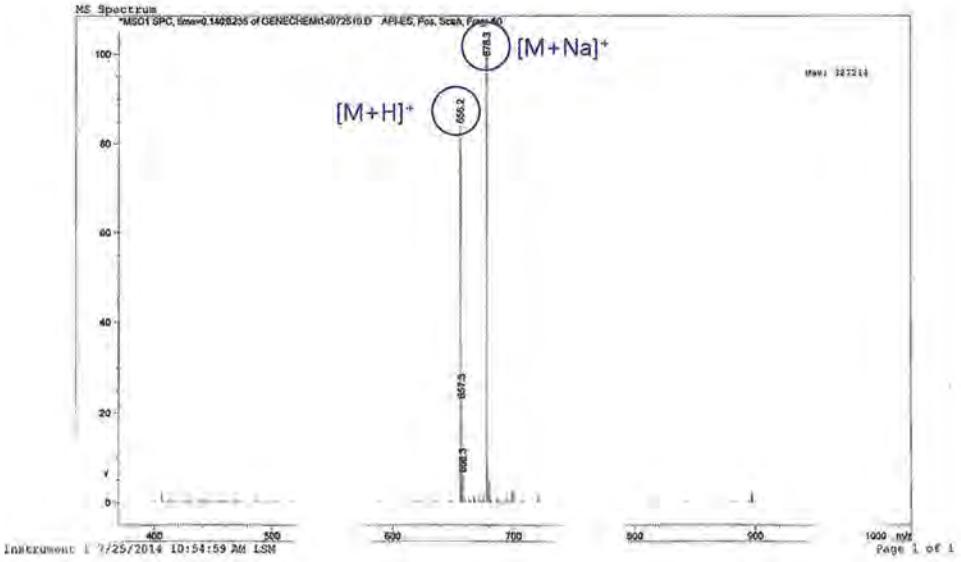


Figure 8. Mass Spectra of GeneChem's 3'-SL Sodium Salt. a) Negative mode and b) Positive mode.

Fourier Transform Unfrared Spectroscopy (FT-IR) Analysis for 3′-SL Sodium Salt

FT-IR (Nicolet 380 FT-IR (Thermo, USA)) showed the characteristic spectrum of C-O bond, N-H bond and O-H bond existing in 3′-SL (Figure 9).

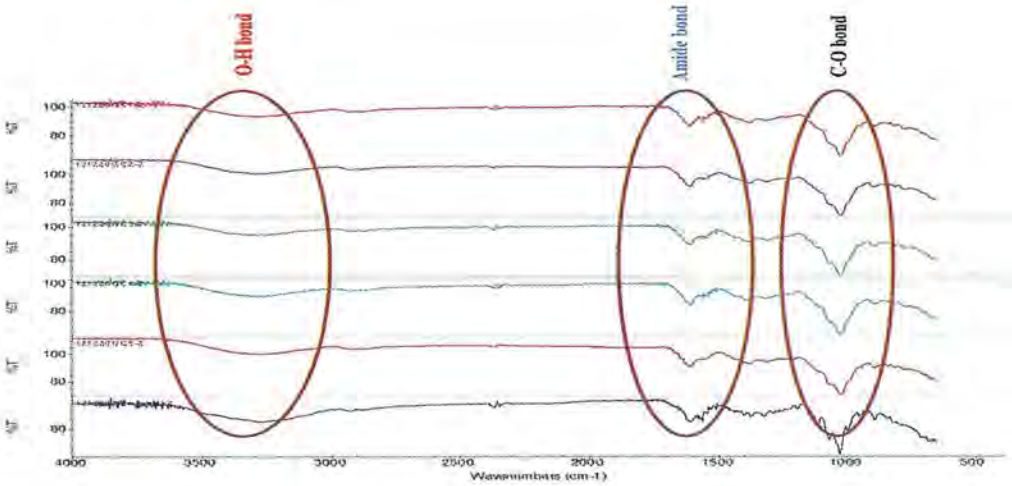


Figure 9. FT-IR Spectra of GeneChem's 3′-SL Sodium Salt

2.C.4. Potential Impurities in the Notified Substance

Impurities which may potentially remain in the 3′-SL product include lactose, CMP, and N-acetylglucosamine, the raw materials used in the production process that may be carried over into the final 3′-SL product. However, these concentrations result in quantitatively insignificant carry-over into the finished infant formula.

Absence of Host Organism, Introduced Antibiotic Resistant Genes and Enzyme Residues

The microorganism used in the enzyme preparation is efficiently removed by the ultrafiltration step. Additionally, during downstream processing, various sequential purification processes are also applied to ensure microbiological purity.

The absence of the microorganisms in the ingredient is demonstrated by microbial testing for *E. coli* during batch analyses according to nationally-recognized methods (Korean Food Standards Codex 9 / 3.7.1). The absence of the microorganism and residual protein in the ingredient is also supported by the analysis of residual DNA in batches of the final ingredient. The absence of residual DNA from the microorganism is confirmed by validated quantitative PCR (qPCR) methods. Further, absence of enzyme residues in the final product is confirmed by ELISA test.

Microbial Endotoxins

Regulatory threshold levels for food regarding endotoxin contamination currently do not exist. Typical ranges of endotoxin load have been reported for cow's milk (Gehring et al., 2008), and infant formula powder (Townsend et al., 2007). The endotoxin

specification for 3'-SL sodium salt is set to not contribute additional exposure to endotoxins that would result in exposures above the usual levels that are expected for infant formula powder currently on the market (Townsend et al., 2007). Batch analyses of 3'-SL sodium salt demonstrate compliance to the endotoxins specifications.

2.C.5. Stability

2.C.5.1. Bulk Stability (GeneChem)

The shelf-life of 3'-SL sodium salt bulk powder is supported by the data available to date from a one-year long-term stability study (25±2°C, 25±6% relative humidity) on 3'-SL sodium salt powder. No significant change was observed in the assay value for 3'-SL sodium salt for up to 12 months of storage (Table 10-1).

The accelerated stability test was performed for 3 months in which packages containing 3'-SL sodium salt were sealed and stored at 40±2°C in a climatic chamber. The chemical analyses were performed at regular intervals and the analytical data available to date are presented in Table 10-2. No significant changes were observed under accelerated storage conditions for up to 3 months of storage.

Table 10-1. Stability of 3'-SL Sodium Salt Powder at Accelerated Storage Condition (Storage Condition: 25±2°C (77°F), Humidity: 25±6%)

	Initial value	1 month	3 month	6 month	12 month
Purity (HPLC)	98.81%	98.48%	99.67%	99.28%	99.70%
Appearance	Complied	Complied	Complied	Complied	Complied
Odor	Complied	Complied	Complied	Complied	Complied
Solubility	Complied	Complied	Complied	Complied	Complied

Table 10-2. Stability of 3'-SL Sodium Salt Powder at Accelerated Storage Condition (Storage Condition: 40±2°C (104°F), Humidity: 24±8%)

	Initial value	1 day	10 day	1 month	3 month
Purity (HPLC)	98.63%	98.61%	98.75%	98.40%	99.26%
Appearance	Complied	Complied	Complied	Complied	Complied
Odor	Complied	Complied	Complied	Complied	Complied
Solubility	Complied	Complied	Complied	Complied	Complied

2.C.5.2. Stability Under the Intended Use Conditions

Stability in Powdered Infant Formula

The stability of 3'-SL sodium salt in a representative infant formula was assessed by HPAEC-PAD in a 2 year study in which 3'-SL sodium salt was added to infant formula powder. At room temperature, 3'-SL sodium salt had a good stability up to 24 months. At accelerated temperature, 3'-SL sodium salt was stable for up to 18 months (at 24 months, the color was changed with decreased 3'-SL content) (Tables 11-1 and 11-2; Figure 10).

Table 11-1. Stability of 3'-SL Sodium Salt in Powdered Infant Formula at Room Temperature (Storage Condition: 25±2°C (77°F), Humidity: 25±6%)

The duration of storage	0 mo	6 mo	12 mo	18 mo	24 mo
Content (mg/L)	536.9	548.4	538.7	543.9	516.0
Appearance	Pale yellow powder				
Odor	Slight characteristic odor				
Comparison with control 3'-SL %	95.3	100.8	97.6	99.6	104.5

Table 11-2. Stability of 3'-SL Sodium Salt in Powdered Infant Formula at Accelerated Storage Condition (Storage Condition: 40±2°C (104°F), Humidity: 24±8%)

	0 month	6 month	12 month	18 month	24 month
Content (mg/L)	552.9	554.3	537.8	519.4	453.9
Appearance	Complied	Complied	Complied	Slightly brown	Brown
Odor	Complied	Complied	Complied	Complied	Complied
Comparison with control 3'-SL %	98.1	103.7	102.6	100.7	88.5

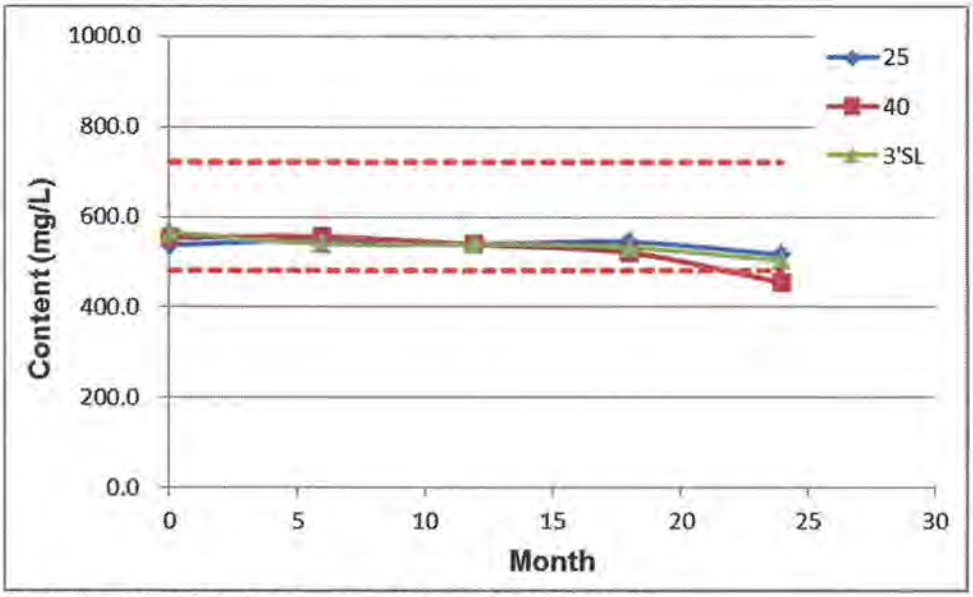


Figure 10. Stability of 3'-SL Sodium Salt in Infant Formula

Stability of 3'-SL Sodium Salt in Milk

The stability test results show that 3'-SL sodium salt was stable in milk for 45 days at 4±2°C and 25±2°C. (Tables 12-1 and 12-2; Figure 11). The appearance (color, odor, etc.) of the milk sample was not changed much during the testing period. The stability tests were conducted using commercial ready-to drink milk and all test samples were analyzed for 3'-SL sodium salt content in duplicate.

Table 12-1. Stability of 3'-SL Sodium Salt in Milk at Low Temperature (Storage Condition: 4±2 °C (39.2 °F), Humidity, 26±3 %)

	1 day	3 day	7 day	15 day	30 day	45 day
Content (mg/L)	520.8	637.3	591.8	597.6	539.8	530.0
Appearance	Complied	Complied	Complied	Complied	Complied	Complied
Odor	Complied	Complied	Complied	Complied	Complied	Complied

Table 12-2. Stability of 3'-SL Sodium Salt in Milk at Room Temperature (Storage Condition: 25±2°C (77°F), Humidity, 25±6%)

	1 day	3 day	7 day	15 day	30 day	45 day
Content (mg/L)	516.8	632.5	599.5	605.0	548.3	575.5
Appearance	Complied	Complied	Complied	Complied	Complied	Complied
Odor	Complied	Complied	Complied	Complied	Complied	Complied

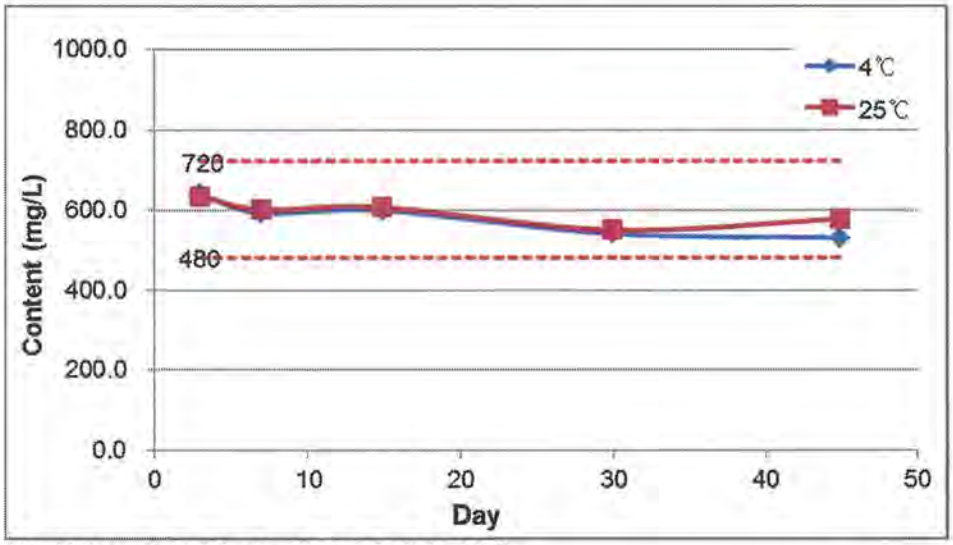


Figure 11. Stability of 3'-SL Sodium Salt in Milk

Stability of 3'-SL Sodium Salt in Yogurt

The stability test was carried under the condition explained below. At 4°C, although the contents of 3'-SL sodium salt in yogurt slowly decreased as time passed (Tables 13-1 and 13-2; Figure 12), the result showed that the concentration of 3'-SL sodium salt in yogurt was still within the target stability range (80 %~120 %) for 45 days. The

appearance (color, odor, etc.) of the yogurt sample did not change much during the testing period. The stability tests were conducted using commercial yogurt from the market and all test samples were analyzed for 3'-SL sodium salt content in duplicate.

At 25±2°C, the concentration of 3'-SL sodium salt in yogurt was out of target stability range after 15 days. The overall result showed that 3'-SL sodium salt in yogurt was less stable than in water or milk, probably due to the fact that some microorganisms present in yogurt digest 3'-SL sodium salt (Yu et al., 2013).

Table 13-1. Stability of 3'-SL Sodium Salt in Yogurt at Low Temperature (Storage Condition: 4±2°C (39.2°F), Humidity, 26±3 %)

	1 day	3 day	7 day	15 day	30 day	45 day
Content (mg/L)	610.1	595.8	560.3	565.4	544.3	537.3
Appearance	Complied	Complied	Complied	Complied	Complied	Complied
Odor	Complied	Complied	Complied	Complied	Complied	Complied

Table 13-2. Stability of 3'-SL Sodium Salt in Yogurt at Room Temperature (Storage Condition: 25±2°C (77°F), Humidity, 25±6%)

	1 day	3 day	7 day	15 day	30 day	45 day
Content (mg/L)	621.2	581.1	519.5	510.3	454.1	397.5
Appearance	Complied	Complied	Complied	Complied	Complied	Complied
Odor	Complied	Complied	Complied	Complied	Complied	Complied

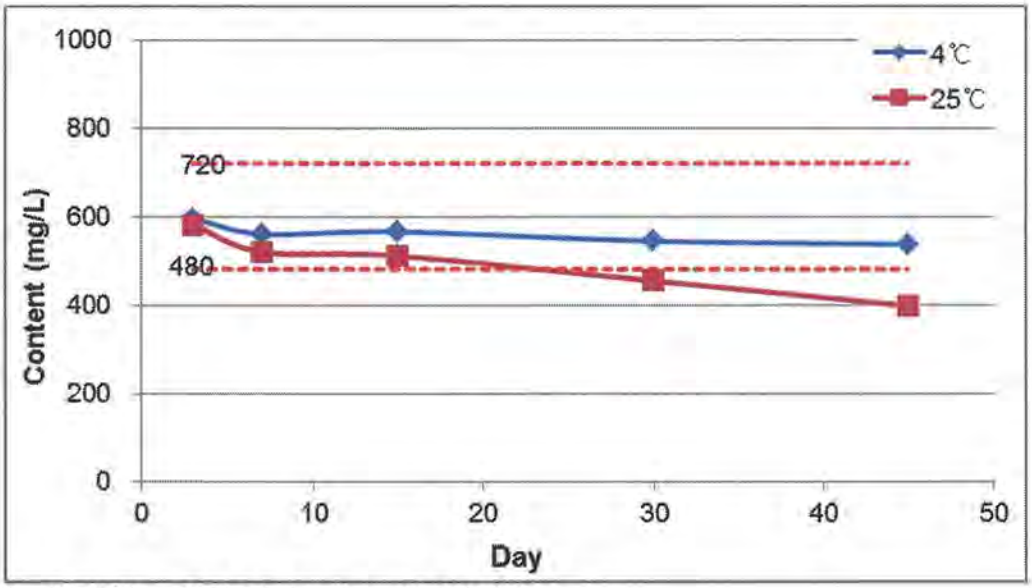


Figure 12. Stability of 3'-SL Sodium Salt in Yogurt

PART 3. DIETARY EXPOSURE

3.A. EDIs Under the Intended Use

3.A.1. EDIs of 3'-SL in Infants

3'-SL is intended for use in term infant formulas (milk-, soy-, amino acid-, and hydrolyzed protein-based) at a maximum use level of up to 230 mg/L in ready-to-drink or reconstituted formula. This maximum use level of 3'-SL in term infant formulas is based on providing a similar level of 3'-SL as that which occurs in mature human breast milk, which ranges from 42 to 840 mg/L at 2 weeks to 5 months after postpartum (Bao et al., 2007; ten Bruggencate et al., 2014; details are shown in Part 3.B.).

EDIs of Infant Formula

Table 14 presents the data on infant formula intakes by age, which range from 1,077 to 1,219 g/person/day. On a body weight basis, these intakes correspond to 118 to 226 g/kg body weight (bw)/day.

EDIs of 3'-SL from the Proposed Use in Infant Formula Only

Estimates for the daily intake of 3'-SL from its use in only term-infant formulas are summarized in Table 15. From the use of 3'-SL in only infant formula, in all-user infants aged 0 to 11.9 months old, the estimated mean and 90th percentile intakes of 3'-SL were determined to be at 187 and 278 mg/person/day, respectively. On a body weight basis, these intakes were determined to be 25.9 and 43.1 mg/kg bw/day, respectively. The all-user estimated mean and 90th percentile intakes of 3'-SL were greatest in infant aged 3 to 5.9 months old at 204 and 293 mg/person/day, respectively (Table 15). On a body weight basis, the greatest intake was observed to occur in infants aged 0-2.9 months at 34.8 and 54.2 mg/kg bw/day, respectively.

Table 14. EDIs of Infant Formula

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
g/person/day						
0-2.9 mo	509 ± 47	1095 ± 44	66.5	140	766 ± 34	1212 ± 44
3-5.9 mo	609 ± 56	1128 ± 44	71.8	151	849 ± 38	1219 ± 85
6-8.9 mo	629 ± 27	1069 ± 48	81.2	162	775 ± 23	1077 ± 37
9-11.9 mo	495 ± 41	1012 ± 46	68.6	115	721 ± 23	1156 ± 107
0-11.9 mo	563 ± 26	1096 ± 18	72.2	568	780 ± 17	1157 ± 30
g/kg bw/day						
0-2.9 mo	96.3 ± 8.8	204.4 ± 7.1	66.5	140	144.9 ± 6.6	225.8 ± 8.0
3-5.9 mo	85.6 ± 8.1	170.4 ± 7.7	71.8	151	119.2 ± 5.4	175.5 ± 6.7
6-8.9 mo	74.0 ± 3.6	133.4 ± 8.1	81.2	162	91.1 ± 3.0	140.8 ± 7.5
9-11.9 mo	52.8 ± 4.1	76.6 ± 2.4	68.6	115	76.6 ± 2.4	118.3 ± 6.4
0-11.9 mo	77.9 ± 4.4	168.3 ± 5.4	72.2	568	107.8 ± 3.9	179.7 ± 5.1

bw = body weight; mo = months; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Table 15. EDIs of 3'-SL from the Proposed Use in Infant Formula Only

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
mg/person/day						
0-2.9 mo	117.1 ±10.8	251.8 ±10.1	66.5	140	176.2 ±7.8	278.8 ±10.1
3-5.9 mo	146.2 ±13.4	259.4 ±10.1	71.8	151	195.3 ±8.7	280.4±19.5
6-8.9 mo	144.7 ±6.2	245.9 ±11.0	81.2	162	178.2 ±5.3	247.7 ±8.5
9-11.9 mo	114.8 ±9.4	232.8 ±10.6	68.6	115	165.8 ±5.3	265.9 ±24.6
0-11.9 mo	129.5 ±5.9	252.1 ±4.1	72.2	568	179.4 ±3.9	266.1 ±6.9
mg/kg bw/day						
0-2.9 mo	22.1 ± 2.0	47.0 ± 1.6	66.5	140	33.3 ± 1.5	51.9 ± 1.8
3-5.9 mo	19.7 ± 1.9	39.2 ± 1.8	71.8	151	27.4 ± 1.2	40.4 ± 1.5
6-8.9 mo	17.0 ± 0.8	30.7 ± 1.8	81.2	162	20.9 ± 0.7	32.4 ± 1.7
9-11.9 mo	12.1 ± 0.9	17.6 ± 0.6	68.6	115	17.6 ± 0.6	27.2 ± 1.5
0-11.9 mo	18.0 ±1.0	38.7 ± 1.2	72.2	568	24.8 ± 0.9	41.3 ± 1.2

bw = body weight; mo = months; NHANES = National Health and Nutrition Examination Survey.

EDIs of 3'-SL from the Combined Use in Infant Formula and Other Foods and Beverages

Table 16-1 present the EDIs of 3'SL from the combined use of infant formula and other foods and beverages in all infants (combining infant formula-fed and breast-fed) by age. When both formula-fed and breast-fed infants are combined in all-user infants aged 0 to 11.9 month old, the mean and 90th percentile EDIs from formula and other foods were determined to be 197 to 315 mg/person/day, respectively. On a body weight basis, these intakes correspond to 26.5 to 42.7 mg/kg body weight (bw)/day.

In all formula-fed infants aged 0 to 11.9 months old (Table 16-2), the estimated mean and 90th percentile intakes of 3'-SL from foods were determined to be 232 to 326 mg/person/day (or 31.1 and 43.9 mg/kg bw/day), respectively. The all-user estimated mean and 90th percentile intakes of 3'-SL were greatest in formula-fed infants aged 6 to 8 months old at 255 and 338 mg/person/day, respectively. On a body weight basis, the greatest intake was observed to occur in formula-fed infants aged 0-2.9 months at 40.1 and 56.6 mg/kg bw/day, respectively.

In breast-fed infants (Table 16-3), all-user infants had EDI ranging from 87 to 210 mg/person/day (or 12.4 and 30.6 mg/kg bw/day), respectively.

Table 16-1. EDIs of 3'-SL from the Proposed Use in Both Infant Formula and Other Foods and Beverages - All Population

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
mg/person/day						
0-2.9 mo	123.0	266.2	67.5	123	182.3	274.5
3-5.9 mo	167.9	318.8	86.6	149	194.0	329.8
6-8.9 mo	215.6	323.0	97.8	165	220.6	323.1
9-11.9 mo	184.4	306.2	99.5	152	185.3	306.4
0-11.9 mo	172.1	305.4	87.4	589	196.8	315.0
mg/kg bw/day						
0-2.9 mo	23.1	48.4	67.5	123	34.3	54.3
3-5.9 mo	24.0	42.4	86.6	149	27.7	42.7
6-8.9 mo	25.7	39.8	97.8	165	26.3	39.8
9-11.9 mo	19.8	35.5	99.5	152	19.8	35.5
0-11.9 mo	23.2	41.7	87.4	589	26.5	42.7

bw = body weight; mo = months; NHANES = National Health and Nutrition Examination Survey.

Table 16-2. EDIs of 3'-SL from the Proposed Use in Both Infant Formula and Other Foods and Beverages - Infant Formula Users

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
mg/person/day						
0-2.9 mo	215.6	284.2	100	83	215.6	284.2
3-5.9 mo	247.3	373.2	100	111	247.3	373.2
6-8.9 mo	254.7	337.8	100	127	254.7	337.8
9-11.9 mo	209.2	319.4	100	128	209.2	319.4
0-11.9 mo	232.2	326.3	100	449	232.2	326.3
mg/kg bw/day						
0-2.9 mo	40.1	56.6	100	83	40.1	56.6
3-5.9 mo	35.5	49.8	100	111	35.5	49.8
6-8.9 mo	30.1	41.3	100	127	30.1	41.3
9-11.9 mo	22.2	37.2	100	128	22.2	37.2
0-11.9 mo	31.1	43.9	100	449	31.1	43.9

Table 16-3. EDIs of 3'-SL from the Proposed Use in Both Infant Formula and Other Foods and Beverages – Breast Milk-Fed Infants

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
mg/person/day						
0-2.9 mo	27.9	115.4	34.0	40	82.0	188.8
3-5.9 mo	50.9	166.6	66.8	38	76.2	186.0
6-8.9 mo	94.3	238.9	90.9	38	103.8	240.4
9-11.9 mo	83.4	184.9	97.7	24	85.4	188.6
0-11.9 mo	54.4	168.4	62.8	140	86.7	209.6
mg/kg bw/day						
0-2.9 mo	5.7	19.3	34.0	40	16.8	44.0
3-5.9 mo	7.0	22.4	66.8	38	10.4	26.9
6-8.9 mo	11.9	30.3	90.9	38	13.1	31.6
9-11.9 mo	9.7	23.9	97.7	24	10.0	24.8
0-11.9 mo	7.8	23.4	62.8	140	12.4	30.6

3.A.2. EDIs 3'-SL from All Proposed Uses in Toddler and General Foods and Beverages (Age 1 year and Older)

Estimates for the daily intake of 3'-SL from its use in toddler and general foods are summarized in Table 17. Table 17-1 presents the data on a per person basis by population group. Table 17-2 presents these data on a per kilogram body weight basis.

In all-users aged 1 year and above, the estimated mean and 90th percentile intakes of 3'-SL from foods were determined to be at 72.5 and 129.3 mg/person/day, respectively. On a body weight basis, these intakes were determined to be 1.8 and 3.6 mg/kg bw/day, respectively. From the use of 3'-SL in foods and beverages, the all-user estimated mean and 90th percentile intakes of 3'-SL were greatest in toddlers at 75 and 139 mg/person/day, respectively. On a body weight basis, these intakes correspond to 5.7 and 11.5 mg 3'-SL/kg bw/day, respectively.

Table 17-1. EDIs of 3'-SL from All Proposed Food and Beverage Uses, mg/person/day

Population Group	Age Group (Years)	All-Person Consumption (mg/person/day)		All-Users Consumption (mg/person/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Toddlers	1 to 3	71.8	138.8	95.9	1,001	74.9	138.8
Children	4 to 12	54.4	109.9	93.1	2,581	58.4	114.3
Female Teenagers	13 to 18	42.6	98.2	71.0	587	60.1	109.7
Male Teenagers	13 to 18	57.4	107.8	79.4	636	72.3	117.2

Female Adults of child bearing age	19 to 40	48.6	78.4	61.0	940	79.7	105.7
Female Adults	19 to 64	44.8	82.1	62.9	2,156	71.1	110.5
Male Adults	19 to 64	54.4	104.7	60.6	1,958	89.8	152.5
Elderly Adults	Over 65	33.3	86.2	73.0	1,361	45.6	104.4
Total Population	All Ages	50.6	105.9	69.8	10,869	72.5	129.3

bw = body weight; NHANES = National Health and Nutrition Examination Survey.

Table 17-2. EDIs of 3'-SL from All Proposed Food and Beverage Uses, mg/kg bw/day

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90th Percentile	% Users	n	Mean	90th Percentile
Toddlers	1 to 3	5.5	11.4	95.4	993	5.7	11.5
Children	4 to 12	1.9	4.0	92.6	2,575	2.0	4.1
Female Teenagers	13 to 18	0.73	1.6	69.8	578	1.0	2.0
Male Teenagers	13 to 18	0.82	1.8	79.3	633	1.0	2.0
Female Adults of child bearing age	19 to 40	0.67	1.1	60.4	927	1.1	1.6
Female Adults	19 to 64	0.62	1.2	62.6	2,135	0.98	1.6
Male Adults	19 to 64	0.64	1.2	60.3	1,950	1.0	1.7
Elderly Adults	Over 65	0.46	1.2	72.1	1,342	0.63	1.4
Total Population	All Ages	1.3	2.5	69.38	10,795	1.8	3.6

bw = body weight; NHANES = National Health and Nutrition Examination Survey.

3.B. Food Sources of 3'-SL

Table 3 (page 17; Part 2.A.1.8) summarizes 3'-SL concentrations of bovine milk collected from various cohorts (Fong et al., 2011; Kelly et al., 2013; Martín-Sosa et al., 2003; Urashima et al., 2013). In the literature, concentrations of 3'-SL in bovine milk were estimated to be in the range of 30 and 139 mg/L (Table 3). To determine the EDI of naturally occurring 3'-SL in dairy products, average values were obtained as 58.5 mg/L (sample number weighted average) or 104 mg/L (average of studies). Thus, for the purpose of EDI calculation of 3'-SL from the diet, 80 mg/L was chosen as an average concentration of 3'-SL in bovine milk

3.C. EDI from Diets

Typical infant formula is estimated to contain 17-19 mg/L of 3'-SL. The addition of 3'-SL sodium salt 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. Addition of 3'-SL sodium salt at concentration of 230 mg/L to typical infant formulas will result in the final 3'-SL concentrations of 247-249 mg/L.

For other age groups, EDIs of 3'-SL from the diet were calculated based on the assumption that all dairy foods would contain 3'-SL at a concentration of 65 mg/L or kg (Fong et al., 2011; Kelly et al., 2013; Martín-Sosa et al., 2003). Table 18 shows EDIs of dairy foods in Americans. Table 19 shows EDIs of 3'-SL from the diet (or dairy foods). As shown in Table 19, EDIs of 3'-SL sodium salt from the background diet are much smaller than those under the intended use. For example, in all-users of dairy foods, the 90th percentile EDI from the diet was estimated to be 40.6 mg/person/day. This level corresponds to 0.92 mg/kg bw/day.

Table 18. EDIs of Dairy Foods in Americans

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
g/person/day						
2-5 yr	382.7±14.9	765.0±22.8	95.6	1,371	413.4 ±14.0	775.8 ±26.6
6-12 yr	313.4±9.7	680.6±25.5	85.5	2,047	366.6±9.8	701.4 ±20.8
13-18 yr	258.9±10.3	650.3±17.7	71.3	1,259	362.9 ±10.8	733.9 ±39.7
19-99 yr	163.3±4.0	475.7±12.1	66.3	6,277	246.1±6.5	558.2 ±11.2
2-99 yr	197.0±4.0	533.7±4.4	70.0	10,954	281.6±5.2	625.2 ±14.6
g/kg bw/day						
2-5 yr	23.6±0.82	51.1±1.90	95.6	1,360	25.5±0.8	52.08±1.2
6-12 yr	9.6±0.34	21.5±0.58	85.5	2,043	11.2±0.4	23.0±0.8
13-18 yr	4.0±0.18	10.3±0.50	71.3	1,247	5.6±0.21	12.1±0.8
19-99 yr	2.1±0.06	6.0±0.13	66.3	6,223	3.2±0.09	7.4±0.2
2-99 yr	4.1±0.11	11.0±0.33	70.0	10,873	5.9±0.13	14.1±0.5

bw = body weight; NHANES = National Health and Nutrition Examination Survey.

Table 19. EDIs of Naturally Occurring 3'-SL from All Dairy Food Uses

Population Group	All-Person Consumption		All-Users Consumption			
	Mean	90th Percentile	% Users	n	Mean	90th Percentile
mg/person/day						
2-5 yr	24.9 ± 1.0	49.7 ± 1.5	95.6	1,371	26.9 ± 0.9	50.4 ± 1.7
6-12 yr	20.4 ± 0.6	44.2 ± 1.7	85.5	2,047	23.8 ± 0.6	45.6 ± 1.4
13-18 yr	16.8 ± 0.7	42.3 ± 1.2	71.3	1,259	23.6 ± 0.7	47.7 ± 2.6
19-99 yr	10.6 ± 0.3	30.9 ± 0.8	66.3	6,277	16.0 ± 0.4	36.3 ± 0.7
2-99 yr	12.8 ± 0.3	34.7 ± 0.3	70.0	10,954	18.3 ± 0.3	40.6 ± 0.9
mg/kg bw/day						
2-5 yr	1.5 ± 0.05	3.3 ± 0.12	95.6	1,360	1.7 ± 0.05	3.4 ± 0.08
6-12 yr	0.62 ± 0.02	1.4 ± 0.04	85.5	2,043	0.73 ± 0.03	1.5 ± 0.05
13-18 yr	0.26 ± 0.01	0.67 ± 0.03	71.3	1,247	0.36 ± 0.01	0.79 ± 0.05
19-99 yr	0.14 ± 0.00	0.39 ± 0.01	66.3	6,223	0.21 ± 0.01	0.48 ± 0.01

2-99 yr	0.27 ± 0.01	0.72 ± 0.02	70.0	10,873	0.38 ± 0.01	0.92 ± 0.03

bw = body weight; NHANES = National Health and Nutrition Examination Survey.

Summary of Consumption Data

Infants: EDIs of 3'-SL from Infant Formula Use Only

From the use of 3'-SL in only infant formula, the estimated mean and 90th percentile intakes of 3'-SL were determined to be at 187 and 278 mg/person/day, respectively, in all-user infants aged 0 to 11.9 months old. On a body weight basis, these intakes correspond to 25.9 and 43.1 mg/kg bw/day, respectively. The all-user estimated mean and 90th percentile intakes of 3'-SL were greatest in infant aged 3 to 5.9 months old at 204 and 293 mg/person/day, respectively (Table 15). On a body weight basis, the greatest intake was observed to occur in infants aged 0-2.9 months at 34.8 and 54.2 mg/kg bw/day, respectively.

Infants: EDIs of 3'-SL from the Use of Infant Formula and Other Foods

In all formula-fed infants aged 0 to 11.9 months old (Table 16-2), the estimated mean and 90th percentile intakes of 3'-SL from the use of both infant formula and other foods and beverages were determined to be 232 to 326 mg/person/day (or 31.1 and 43.9 mg/kg bw/day), respectively.

In breast-fed infants (Table 16-3), all-user infants had EDIs ranging from 87 to 210 mg/person/day (or 12.4 and 30.6 mg/kg bw/day), respectively.

When both formula-fed and breast-fed infants were combined, the mean and 90th percentile EDIs from the use of both formula and other foods and beverages were determined to be 197 to 315 mg/person/day, respectively, in all-user infants aged 0 to 11.9 months old. On a body weight basis, these intakes correspond to be 26.5 to 42.7 mg/kg bw/day, respectively.

Toddlers and Other Age Groups

In all-users aged 1 year and older, the estimated mean and 90th percentile intakes of 3'-SL from the use of toddler and general foods and beverages were estimated to be at 72.5 and 129.3 mg/person/day, respectively. On a body weight basis, these intakes correspond to 1.8 and 3.6 mg/kg bw/day, respectively. The all-user estimated mean and 90th percentile intakes were greatest in toddlers at 75 and 139 mg/person/day, respectively. On a body weight basis, these intakes correspond to 5.7 and 11.5 mg 3'-SL/kg bw/day, respectively.

These EDIs are within safe intake levels (details are described in Part 6).

Part 4. SELF-LIMITING LEVELS OF USE

No known self-limiting levels of use are associated with GeneChem's 3′-SL sodium salt ingredient.

PART 5. THE HISTORY OF CONSUMPTION OF THE SUBSTANCE FOR FOOD USE

The statutory basis for the conclusion of GRAS status of 3'-SL sodium salt in this document is not based on common use in foods before 1958. The GRAS determination is based on scientific procedures. As described in Part 3 of this document, 3'-SL is a naturally occurring food component in human and bovine milk. It is reasonable to conclude that it was present in foods consumed by infants and other human populations prior to 1958.

PART 6. THE BASIS FOR OUR CONCLUSION OF GRAS STATUS

6.A. Regulatory Status

Several sources of HMOs have been evaluated by the FDA and other global regulatory agencies over the past 5 years for incorporation of HMO products in infant formulas for consumption by term infants. Relevant U.S. GRAS notifications include 2'-O-fucosyllactose (GRN 546, FDA 2015b; GRN 571, FDA 2015d; GRN 650, FDA 2016a) and lacto-*N*-neotetraose (GRN 547, FDA, 2015c; GRN 659, FDA 2016b). FDA had no questions on the use levels of these HMOs similar to those found in human milks.

These HMOs (degree of polymerization [DP] unit of 3) are considered as dietary fiber. The Institute of Medicine, the Academy of Sciences, has recommended that Americans increase the consumption of dietary fiber and has not established Tolerable Upper Intake Levels of dietary fiber for any age/gender groups or special populations (IOM, 2002).

6.B. 3'-SL Part of GeneChem's 3'-SL is Structurally Identical to that Present in Human Milk

As presented in Parts 2.A and 2.C, 3'-SL part of GeneChem's 3'-SL sodium salt is chemically and structurally identical to the 3'-SL which is found in human milk, and therefore, the safety of GeneChem's 3'-SL sodium salt for all intended uses is supported by the known consumption of 3'-SL from human breast milk in infants.

A summary of the levels of 3'-SL in human breast milk is provided in Part 2. The safety of GeneChem's 3'-SL sodium salt is further supported by the results from animal toxicological studies and human clinical studies, which are summarized in Part 6.C to 6.F.

6.C. Safety of 3'-SL Sodium Salt

This section comprises the pivotal studies for the safety assessment of GeneChem's 3'-SL sodium salt. To identify other data and information relevant to the safety of infant formula and food uses of 3'-SL sodium salt, a comprehensive search of the published scientific literature was conducted through January 2018. Published studies identified during the literature search consisted of studies relating to the metabolic fate and safety of 3'-SL sodium salt.

6.C.1. Metabolism (adopted from ten Bruggencate et al., 2014)

6.C.1.1. Digestion of SL in the Upper Gastrointestinal Tract

It is generally accepted that most of the oligosaccharides resist the pH of the stomach in infants; they are resistant to enzymatic hydrolysis in the small intestine and are thus largely undigested and unabsorbed (Brand-Miller et al., 1998). Chaturvedi et al. (2001) investigated the fate of human milk oligosaccharides during transit through the alimentary canal by determining the degree to which breast-fed infants' urine and fecal oligosaccharides resembled those of their mothers' milk. Oligosaccharide profiles of milk from 16 breast-feeding mothers were compared with profiles of stool and urine from their infants. Results were compared with endogenous oligosaccharide profiles obtained from the urine and feces of age-, parity-, and gender-matched formula-fed infants. Among

breast-fed infants, concentrations of oligosaccharides were higher in feces than in mothers' milk, and much higher in feces than in urine. Urinary and fecal oligosaccharides from breast-fed infants resembled those in their mothers' milk. Those from formula-fed infants did not resemble human milk oligosaccharides and were found at much lower concentrations. Most of the human milk oligosaccharides survived transit through the gut, and some were absorbed and then excreted into the urine intact. Thus, it is likely that most oligosaccharides will pass through the intestinal tract and enter the colon intact (Brand-Miller et al., 1998, Newburg et al., 2000). Indeed, in a model mimicking the physiological pH of the gastric fluid of the infant's stomach, Gnoth et al. (2000) demonstrated that acidic oligosaccharides, including SL, show minor changes in their structure and concluded that <5% of the HMO amount would be digested in the intestinal tract. Furthermore, it has been demonstrated that a mixture of SL and sialyllactitol is not hydrolyzed during retention in the stomach (Nöhle and Schauer, 1984). The majority of HMOs seem to reach the colon, where they are available for fermentation by the microbiota, and as much as 40–50% may pass unaltered into the feces (Sabharwal et al., 1991; Albrecht et al., 2010).

6.C.2. Absorption, Distribution, and Excretion of SL

A small fraction of milk oligosaccharides, including SL, is absorbed (partly intact) by the paracellular route, transported via blood, and excreted in urine (Gnoth et al., 2001).

Studies in human infants and rats demonstrate that HMOs are orally absorbed intact to a small extent (Goehring et al., 2014; Ruhaak et al., 2014; Santos-Fandila et al., 2014). A recent study in rats showed that, from the HMOs fed to rat pups, only 3'-SL was absorbed and detected in the serum and urine (Jantscher-Krenn et al., 2013). This is in contrast to findings in human studies, where the HMOs detected in urine of infants are more diverse after applying ^{13}C-labeled glucose or ^{13}C-galactose as an oral bolus given to their lactating mother (Rudloff et al., 2012). This may be related to the fact that the oligosaccharides in human milk are more diverse than those in rat milk, which contains mainly 3'-SL (Urashima et al., 2001).

In fasted mice, it was shown that 50% of orally administered (^{14}C labelled) SL was excreted unchanged in urine within 24 hours (Duncan et al., 2009; Nöhle and Schauer, 1984). Furthermore, Nöhle and Schauer (1984) demonstrated that only 1% of the labelled SL was still detectable in the body after 24 hours, indicating that only a minor fraction is metabolized upon absorption.

In infant urine, HMOs are detected in small amounts, in a range of 50–500 mg/day, which corresponds to less than 10% of the daily HMO intake (Rudloff et al., 2012; Coppa et al., 1990). This suggests that a larger fraction of the HMOs is absorbed in humans than in mice. HMOs are also detectable in the urine of the mother (500–800 mg/day; Rudloff et al., 1996). The excretion of oligosaccharides in the mother's urine during lactation suggests that the oligosaccharides synthesized by the mother not only enter the breast milk but also become available systemically as well, as reflected by urinary excretion. This has been suggested to protect the mother against urinary tract infections (Coppa et al., 1990).

Sialic acid is detected in various organs, including the brain (Wang et al., 2009) and is found in human milk, plasma, and urine. Moreover, sialic acid is present in many other body fluids, including saliva, gastric juice, and tears, in the form of glycoproteins or as terminal sugars of oligosaccharide chains of mucins (Wang et al., 2003). Both the bound and free sialic acid content of saliva is higher in breastfed infants than in bottle-fed infants (Tram et al., 1997). This suggests that sialylated oligosaccharides present in breast milk may act as a source of sialic acid for the newborn.

6.D. Mutagenicity and Genotoxicity of GeneChem's 3'SL Sodium Salt

As summarized in Table 20, GeneChem's 3'-SL sodium salt was found to be non-mutagenic or genotoxic (Kim et al., 2018).

Table 20. Mutagenicity and Genotoxicity Studies of GeneChem's 3'-SL Sodium Salt

Test System	Dose	Results	Reference
Bacterial reverse mutation test: *S. typhimurium* (TA98, TA100, TA1535, TA1537) and *E. coli* WP2*uvrA* (pKM101)	8.2 - 5,000 µg/plate	Not mutagenic	Kim et al., 2018
In vitro chromosome aberration test: Chinese Hamster Lung (CHL/IU) cells	5 - 5,000 µg/mL	Not clastogenic	
In vivo micronucleus test: ICR mice	500, 1,000, and 2,000 mg/kg bw	Not genotoxic	

6.D.1. Bacterial Reverse Mutation Test of GeneChem's 3'-SL Sodium Salt

The potential mutagenicity of GeneChem's 3'-SL sodium salt (purity of 98.9 %) was evaluated in the bacterial reverse mutation assay (Ames test) using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* (WP2*uvrA* (pKM101)) in the presence or absence of metabolic activation (S9) (Kim et al., 2018). In the first dose range finding study, bacterial strains were treated with 3'-SL sodium salt at concentrations of 8.18, 20.5, 51.2, 320, 800, 2,000, and 5,000 µg/plate using pre-incubation method. In the second experiment, the concentrations of 313, 625, 1,250, 2,500 and 5,000 µg/plate were tested using pre-incubation method. Also, the negative and positive control (in the absence of metabolic activation 2-nitrofluorene for TA98, sodium azide for TA100 and TA1535, 9-aminoacridine (TA1537), or 2-[2-furyl]-3-[5-nitro-2-furyl] acrylamide for WP2*uvrA* (pKM101); in the presence of metabolic activation 2-aminoanthracene for all strains) groups were used in both experiments. The growth inhibition and deposition of the test substance was not evident at any dose levels of all strains in the absence and presence of metabolic activation. Thus, 3'-SL sodium salt was determined to be non-mutagenic in the Ames test at concentrations up to 5,000 µg/plate.

6.D.2. *In Vitro* Chromosome Aberration Test of GeneChem's 3'-SL Sodium Salt

This study was designed to evaluate the potential of the test substance, 3'-SL sodium salt, to induce chromosomal aberrations in Chinese Hamster Lung (CHL/IU) cells (Kim et al., 2018). To evaluate the ability of 3'-SL sodium salt to induce chromosomal aberrations in cultured CHL/IU cells with and without S9 metabolic activation, two separate chromosome aberration assay tests *in vitro* were conducted. DMSO served as both the diluent for 3'-SL sodium salt and the negative control substance. Mitomycin C and Benzo[a]pyrene were used for positive controls in the absence or presence of S9 metabolic activation, respectively. In the growth inhibition study, concentrations of 5, 10, 50, 100, 250, 500, 1,000, 2,500, and 5,000 μg/mL were tested with and without S9 activation. Cytotoxicity was not evident in the short time treatment without and with metabolic activation and continuous treatment without metabolic activation. In the second experiment, the concentrations of 1,250, 2,500, and 5,000 μg/mL were tested. Also, the negative and positive controls were used. The result of the main study showed that the frequency of cells with structural and numerical chromosome aberrations was less than 5% at all dose levels of the test substance in the short time treatment with and without metabolic activation, and in the continuous treatment ($p<0.05$). The deposition of the test substance was not evident at any dose levels in the short time treatment without and with metabolic activation, and continuous treatment without metabolic activation. In contrast, the incidence of structurally aberrant cells was obviously increased in the positive control of all groups, demonstrating the sensitivity of the test system. Based on the results of this study, it was concluded that 3'-SL sodium salt was not clastogenic under the conditions of this study.

6.D.3. *In Vivo* Mouse Micronucleus Test of GeneChem's 3'-SL Sodium Salt

3'-SL sodium salt was tested for its ability to induce micronuclei in polychromatic erythrocytes (PCE) of the bone marrow of treated Imprinting Control Region (ICR) mice (Kim et al., 2018). The doses of 3'SL used in the study were 500, 1000, and 2000 mg/kg body weight (bw). Fifty-four male and female mice aged 8 weeks were treated by oral gavage with 3'-SL sodium salt dissolved in saline over 3 consecutive days. Saline was used as a vehicle control. Mitomycin C (2 mg/kg, i.p.) was administered as a positive control. Animals were observed for clinical signs and mortality immediately (0 hour), at 2 hours, and on days 1, 2, and 3 post-dosing. All doses were well tolerated, and no clinical signs were observed. We collected bone marrow cells at 24, 48, and 72 hours after dosing and evaluated the frequency of micronuclei.

No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes (MNPCE) in polychromatic erythrocytes (PCE) were observed in any test substance groups compared with the negative control group. A significant increase in the incidence of MNPCE in PCE was observed in the positive control group compared with the negative control group. There were no statistically significant differences in the ratio of PCE to total erythrocytes in any test substance groups compared with the negative control value. Body weights of mice were comparable among the groups before and after the treatment with the test substance. It was concluded that 3'-SL sodium salt did not induce micronuclei in the bone marrow cells of mice under the conditions of this study.

6.E. Toxicity Studies of GeneChem's 3'-SL Sodium Salt in Animals

Table 21 summarizes the results from a series of oral toxicity studies conducted on GeneChem's 3'-SL sodium salt in rats, piglets, and beagle dogs (Donovan, 2017; Kim et al., 2018). Mean lethal dose (LD_{50}) was greater than 20 g/kg bw. A 90 day oral subchronic toxicity study showed that the No Observed Adverse Effect Level (NOAEL) was greater than 2,000 mg/kg, the highest dose tested, in rats.

Table 21. Summary of Animal Toxicity Studies of GeneChem's 3'-SL Sodium Salt

Animal	Dose	Duration of study	Results	Reference
Acute toxicity study				
Rat (M25, F25)	0, 5, 10, 15 and 20 g/kg	Single day dose	Mean lethal dose (LD_{50}) and the Maximum Tolerance Dose (MTD) were greater than 20 g/kg bw.	Kim et al., 2018
Subacute toxicity study				
Beagle dogs (M2, F2)	3 single doses (0, 500, 1,000, and 2,000 mg/kg)	Dose escalating single doses at 4 day interval	There were no deaths in any animals in any dosing groups. A diarrhea was observed in two males and one female at approximately 4 h after the third dosing 2,000 mg/kg bw. No treatment-related abnormalities were noted in body weights. The MTD of the test substance was greater than 2,000 mg/kg bw, the highest dose tested.	Kim et al., 2018
Rat (M20, F20)	0, 500, 1,000, and 2,000 mg/kg	4 weeks	No treatment-related abnormalities were found in clinical signs, body weights, food consumption, hematology, clinical chemistry, organ weights and necropsy in any animals in the dosing groups. The NOAEL was greater than 2,000 mg/kg bw, the highest dose tested.	Kim et al., 2018
Neo-natal piglet (48)	0, 140, 200, and 500 mg/L	3 weeks	No effect on food consumption, growth and body weight, hematological parameters, electrolytes and minerals, and serum enzymes. All doses were well tolerated.	Donovan et al., 2017
Neo-natal	0, 2,000, and 4,000 mg/L	3 weeks	No adverse effects on feed intake, growth, fecal consistency, and colonic	Jacobi et al., 2016

piglet (54)			microbiome. All doses were well tolerated.	
Subchronic toxicity study				
Rat (M40, F40)	0, 500, 1,000, and 2,000 mg/kg	13 weeks	No treatment-related abnormalities were noted in clinical signs, body weight, food consumption, ophthalmic examination, urinalysis, hematology, clinical chemistry and gross post mortem and histopathological examinations. The NOAEL was greater than 2,000 mg/kg bw, the highest dose tested.	Kim et al., 2018

6.E.1. Acute Toxicity of GeneChem's 3'-SL Sodium Salt in Rats

Kim et al. (2018) evaluated acute toxicity of 3'-SL sodium salt after a single day oral administration in rats. In this study, 3'-SL sodium salt was administered to 50 young Sprague-Dawley rats (6 weeks of age; each group of 10 rats consisted of 5 male and 5 female rats) by oral gavage at a single day dose of 0, 5, 10, 15, or 20 g/kg bw and observed for 14 days to monitor changes in body weight, clinical signs, food and water consumption. At the end of the study, animals were sacrificed, and major organs were examined macroscopically and microscopically. No animal died during the 14 days observation period and no clinical signs of abnormality were observed at any dose level. Furthermore, no significant differences in mean body weight, food and water intake, and organ weights were found among the four test and control groups. No treatment-related abnormalities were observed upon macroscopic or microscopic examinations. The researchers concluded that the lethal dose (LD_{50}) and the maximum tolerance dose (MTD) of 3'-SL sodium salt was far above 20 g/kg bw, the highest dose tested.

As shown in Table 22, the LD_{50} of 3'-SL sodium salt in rats are comparable with or higher than those of other carbohydrates such as polydextrose (>18.9 g/kg bw; Burdock and Flamm, 1999), glucose (25.8 g/kg bw, Sax 1984) and fructose (14.7 g/kg bw) in rats, and is much higher than that of table salt (3.0 g/kg bw, Sax 1984). A compound which has a LD_{50} value of >5 g/kg bw in rats is classified as 'practically non-toxic' and acompound with a LD_{50} value of >15 g/kg bw as 'relatively harmless' (Altug, 2003). 3'-SL sodium salt, like other oligosaccharides, belongs to the group which has the lowest toxicity rating.

Table 22. Comparison of LD_{50} Values in Rats

Compounds	LD_{50}, g/kg bw	Reference
3'-SL sodium salt	>20	Kim et al., 2017
Polydextrose	>18.9	Burdock and Flamm, 1999
Beta-D-fructose	14.7	Sax, 1984
Alpha-D-glucose	25.8	Sax, 1984
Sucrose	29.7	Sax, 1984
Maltose	34.8	Sax, 1984
Table salt	3.0	Sax, 1984
Alcohol	7.1	Sax, 1984

6.E.2. Subacute Toxicity of GeneChem's 3'-SL Sodium Salt in Rats

A 28-day study was conducted in 40 six-week old Sprague-Dawley rats (10/group) to investigate the potential toxic effects of 3'-SL sodium salt at a daily dose of 0, 500, 1,000, or 2,000 mg/kg bw (Kim et al., 2018). No treatment-related abnormalities were found in clinical signs, body weights, food consumption, hematology, clinical chemistry, organ weights, and necropsy in any animals in the dosing groups. Histopathological examination also found no treatment-related abnormalities in the highest dose group. The authors concluded that the No Observed Adverse Effect Level (NOAEL) was greater than 2,000 mg/kg bw, the highest dose tested.

6.E.3. Subacute Toxicity of GeneChem's 3'-SL Sodium Salt in Beagle dogs

Subacute oral toxicity of 3'-SL sodium salt was evaluated in beagle dogs in a dose-escalating manner. In this study, single doses of 3'-SL sodium salt was sequentially administered at 0, 500, 1,000, and 2,000 mg/kg bw at four day intervals (Kim et al., 2018). Clinical signs and body weights were observed in the following 14 day period. There were no deaths in any animals in any dosing groups during the study. No treatment-related effects on clinical signs were evident in males and females at 500 and 1,000 mg/kg bw. However, a transient diarrhea was observed in two males and one female at approximately 4 hours after the third dosing of 2,000 mg/kg bw. No test substance-related toxicity effects on body weights were observed. Based on these results, maximum tolerance dose of 3'-SL sodium salt was determined to be greater than 2,000 mg/kg bw in male and female beagle dogs under the conditions of this study.

6.E.4. Subacute Toxicity of 3'-SL Sodium Salt in Piglets

An Unpublished Piglet Study by Donovan (2017)

Donovan (2017) evaluated a subacute toxicity study of GeneChem's 3'-SL sodium salt in neonatal piglets to investigate the effect of 3'-SL sodium salt on the health and development during the first 3 weeks of postpartum. The addition of 3'-SL sodium salt at the dose of 140, 200, or 500 mg/L (or up to 483 mg 3'-SL/L) was well tolerated and supported normal growth patterns. Details of study methods are described below.

Study Design:

The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois, Urbana-Champaign. Two-day old piglets (n=48) were obtained from the University of Illinois Swine Research Center and were housed individually at the ERML animal facility. The piglets received an IM injection of 1 mL iron dextran and 0.3 mL Excede antibiotic for swine (Zoetis Animal Health, Parsippany, NJ) within 24 h of birth. In addition, piglets received another dose of antibiotic on days 7 and 14 of the study. Piglets were randomized to four dietary treatments: formula alone (CON, N=12), formula + 140 mg 3'SL sodium salt/kg body weight (BW) (LOW, n=12), formula + 200 mg 3'SL sodium salt/kg BW (MOD, n=12) or formula + 500 mg 3'SL sodium salt/kg BW (HIGH, n=12). Test ingredient (>98% purity; GeneChem, Inc., Daejeon, South Korea) was dissolved in water and appropriate volumes were added to each diet. A commercially-available non-medicated sow-milk replacer formula (Advance Liqui-Wean, Milk Specialties Co., Dundee, IL, USA), which was formulated to meet or exceed 2012 National Research Council requirements for 3-5 kg piglets, was prepared daily at the concentration of 183 g/L and piglets were fed at the rate of 300 and 360 mL/kg BW on days of study 1-5 and 6-12 respectively. Formula was delivered to piglets 10 times per day via a peristaltic pump. Piglet BW was recorded daily to determine milk volume of formula to be dispensed to individual animals.

Sample Collection:

On day 8 of the study (pig age=10 days old), blood was collected via jugular vein in either plain, K_2EDTA, or Na Citrate-laced vacutainers (BD Biosciences, Franklin Lakes, NJ) to perform chemistry, CBC, and coagulation time analysis, respectively. On day 22 (pig age= 24 day-old), blood was collected via cardiac puncture after animals were sedated with an intramuscular injection of Telazol® (Tiletamine HCl and Zolazepam HCl, 3.5 mg/kg BW each, Pfizer Animal Health, Fort Dodge, IA). Piglets were then euthanized by an intravenous injection of 72 mg/kg BW sodium pentobarbital (Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI). Urine samples were collected via cystocentesis at terminal necropsy for urinalysis. Spleen, kidneys, heart, lungs, and liver were immediately removed and weighed before a section was submerged in 10% buffered formalin for histopathological analysis. The small intestine was excised between the pyloric sphincter and ileocecal valve for measurement of total intestinal length. Then it was cut at 10% and 85% from the proximal end to give 3 segments corresponding to the duodenum, jejunum, and ileum, respectively. In addition, the large intestine was excised, and length was taken from the cecum to the most distal part of the descending colon. Sections of the duodenum, jejunum, ileum, cecum, and ascending and descending colon were fixed in formalin. Other samples collected and fixed, but not weighed, were stomach, mesenteric lymph nodes, pancreas, and gallbladder.

Microscopic Histological Analysis:

Microscopic histological analyses were performed on tissue samples obtained from piglets fed the CON and HIGH diets by a certified pathologist at the Diagnostic Lab of the College of Veterinary Medicine at the University of Illinois. Parameters identified were: lymphoplasmacytic inflammation in the stomach, extramedullary hematopoiesis in cecum, spleen, liver, and gallbladder, spleen congestion, glycogen depletion in the liver,

kidney hemorrhage, and neutrophilic inflammation in the cecum, and ascending and descending colon. Findings were reported as absent, minimal, mild, moderate or marked.

Large intestine content pH:

The pH of ascending and descending colon and cecum contents was measured immediately after collection.

Clinical Chemistry Analyses and Urinalysis:

Serum chemistries and coagulation time (partial prothrombin time [PT] and activated partial thromboplastin time [aPTT]) were determined using an Olympus AU680 chemistry analyzer (Beckman Coulter, Brea, CA) and a STA-Compact coagulation analyzer (Diagnostica STAGO, France), respectively, at the Clinical Pathology lab (College of Veterinary Medicine at the University of Illinois). Serum was obtained after respective vials were centrifuged at 2,200 x g for 20 minutes at 4°C in a benchtop centrifuge (CS-6R Centrifuge, Beckman Coulter Life Sciences, Indianapolis, IN). Chemistry analyses included serum concentration of minerals (calcium, phosphorus, magnesium), electrolytes (sodium, potassium, chloride), protein (total protein, albumin and globulin), metabolites (glucose, total cholesterol, triglycerides, creatinine, urea, total bilirubin and bicarbonate), the enzymes alkaline phosphatase (ALP), aspartate transaminase (AST), gamma glutamyltransferase (GGT), creatine phospholinase (CPK) and glutamate dehydrogenase (GLDH). In addition, cell blood count (CBC) was performed on CELL-DYN® 3700 (Abbott, Abbott Park, Illinois) while trained technicians at the College of Veterinary Medicine performed differential analysis. The variables evaluated in our study were red blood cells count (RBC), hemoglobin concentration, hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and corpuscular hemoglobin concentration mean. Total white blood cell (WBC) count and differential WBC analyses (neutrophils and lymphocytes) were also performed. Platelet indices were analyzed and included platelet count and mean platelet volume (MPV).

Urinalysis was performed with the CLINITEK Advantus® Urine Chemistry Analyzer (Siemens Healthcare, Germany), which provided the automated reading of pH, protein, glucose, ketones, bilirubin and blood. Specific gravity was measured on a refractometer and analysis of urine sediments and cells were done microscopically by Clinical Pathology Lab technicians.

Statistical analysis

Univariate analysis: Analysis of variance (ANOVA) was conducted using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) to differentiate dietary treatment effects on young pigs.

Results and Discussion

Food consumption, growth, and body weight were unaffected. Three animals from the LOW group were removed from the study due to the development of diarrhea. It was not considered treatment-related since diarrhea was not observed in animals in MOD and HIGH groups. Although there were some pathological findings on samples of control and

high dose groups, it does not appear that they were correlated with dietary treatment. Clinical chemistry, hematological parameters and urine parameters were unaffected by the addition of 3'SL sodium salt to formula. Serum enzymes were also unaffected, with the exception of alkaline phosphatase levels, which differed between 140 and 200 mg/L groups. It is unclear the reason for the difference, however, levels in all groups were within acceptable range for young piglets (CON vs. LOW vs. MOD vs. High [Reference range]: Day 22, 515^{ab} vs. 595^{a} vs. 466^{b} vs. 562^{ab} [110-1292 U/L]; different letters represent statistical significance at $p<0.05$). In addition, the differences in alkaline phosphatase levels did not have the dose-response relationship and they were in small magnitude, thus, it was not considered to be of toxicological significance. The researcher concluded that supplementation of formula with 3'SL sodium salt at concentrations of up to 500 mg 3'-SL sodium salt/L was well tolerated in the growing pig for the first 3 weeks of life. No adverse effects were noted in any parameters tested.

3'SL sodium salt at any of the doses tested had no effect on the small or large intestinal length, small intestine weight, or organ weights. Ascending and descending colon and cecum pH content were measured on day 22 immediately after sampling. Administration of 3'SL sodium salt at any of the doses tested did not affected the ascending and descending colon content pH. However, cecum content pH in the HIGH (500 mg 3'-SL sodium salt/L or 483 mg 3'-SL/L) was significantly higher relative to the LOW and MOD levels, but it did not differ from CON.

A Piglet Study by Jacobi et al. (2016)

Jacobi et al. (2016) evaluated the safety and efficacy of different isomers of SL related to brain sialic acid concentrations and microbiome in developing neonatal piglets. In this study, a day-old pigs were randomly allocated to 6 diets (control, 2 or 4 g 3'-SL/L, 2 or 4 g 6'-SL/L, or 2 g polydextrose/L + 2 g galacto-oligosaccharides/L; n = 9) and fed 3 times/day for 21 days. The safety parameters (growth and gastrointestinal tolerance) were measured. There were no differences ($P > 0.05$) in initial body weight, weight gain, feed intake, feed:gain ratios, fecal consistency, and diarrhea scores across the treatment groups, indicating that the oligosaccharide diets were well tolerated by the pigs. In addition, dietary SL did not adversely affect colonic microbiome and ganglioside-bound sialic acid in the corpus callosum of pigs fed 3'-SL or 6'-SL. The authors proposed two potential routes by which sialyllactose may positively affect the neonate: serving as a source of sialic acid for neurologic development and promoting beneficial microbiota. Overall, 3'-SL at doses up to 4,000 mg/L was well tolerated in neonatal piglets.

Of note, an unpublished study by Donovan (2017) tested lower doses of 3'-SL in piglets than those used in the prior published report by Jacobi et al. (2016) (up to 483 mg/L vs 4,000 mg/L). Its outcomes confirmed those of the earlier study reporting that 3'-SL was well tolerated in piglets. Thus, the unpublished status of the 2017 Donovan study has no impact on the overall conclusion of this GRAS determination if qualified experts do not have access to such data and information.

6.E.5. Subchronic Toxicity Study of GeneChem's 3'-SL Sodium Salt in Rats

The oral toxicity of 3'-SL sodium salt has been evaluated in a 90-day subchronic toxicity study in rats conducted in accordance with the Organization for Economic Cooperation and Development (OECD) Test Guidelines (Kim et al., 2018). 3'-SL sodium salt was administered once daily to six-week old Sprague-Dawley rats by oral gavage for 13 weeks at dose levels of 0, 500, 1,000, and 2,000 mg/kg bw. Each group consisted of 10 males and 10 females. Parameters evaluated included: clinical signs, body weights, food consumption, ophthalmic examination, urinalysis, hematology, clinical chemistry, gross post mortem examinations, organ weights, and histopathological evaluations of selected tissues. No treatment-related effects were noted in clinical signs, body weight, food consumption, ophthalmic examination, urinalysis, hematology, clinical chemistry, absolute and relative organ weights, and gross post mortem examinations. Histopathological examinations also revealed no treatment-related abnormalities.

In this study, the NOAEL for 3'-SL sodium salt was determined to be higher than 2,000 mg/kg bw/day, the highest dose tested. 3'-SL sodium salt was well-tolerated at doses of up to 2,000 mg/kg bw/day for 13 weeks.

Conclusions from Animal Toxicity Studies

Based on these studies, for purposes of this evaluation, the NOAEL of 2,000 mg/kg bw/day, the highest dose tested, was chosen for 3'-SL sodium salt in rats. 3'-SL sodium salt, like other oligosaccharides, belongs to the group which has the lowest toxicity rating (Kim et al., 2018). Additionally, the addition of 3'-SL at concentrations of up to 4,000 mg/L was well tolerated and supported normal growth patterns in neonatal piglets with no adverse effects (Jacobi et al., 2016).

Of note, an unpublished study by Donovan (2017) tested doses of up to 500 mg/L 3'-SL sodium salt in piglets (corresponding to 483 mg 3'-SL/L), lower doses than those used in previous reports by Jacobi et al. (2016), which employed doses up to 4,000 mg/L. The results of this unpublished study confirmed those of an earlier published study reporting that 3'-SL was well tolerated in neonatal piglets. Thus, the unpublished status of the study has no impact on the overall conclusion of this GRAS determination if qualified experts do not have access to such data and information.

6.E.4. Animal Efficacy Studies

Several animal studies evaluated the effects of 3'-SL sodium salt on microbiota, growth, and cognition parameters (Table 23). Any studies using modified genes or chemically induced disease were not included in this review since the data from chemically induced disease conditions and/or modified genes in animals may not be relevant when evaluating the safety of 3'-SL or 3'-SL sodium salt. None of these studies summarized in Table 23 reported adverse effects on measured outcomes. The 3'-SL at supplemented at up to 5% of diet was well tolerated with no side effects.

It is noteworthy that the study by Hamilton et al. (2017) used bovine milk derived oligosaccharides (BMOS; source-whey; Hilmar Ingredients) containing 35% SLs, of which the proportion of 3'-SL and 6'-SL was 4:1.

Studies Evaluating the Effects of 3'-SL on Microbiota

Tarr et al. (2015) tested whether 3'-SL (5% of diet) and 6'-SL (5% of diet) would prevent stressor-induced alterations in gut microbial community composition and attenuate stressor-induced anxiety-like behavior. Mice were fed standard laboratory diet, or laboratory diet containing 3'-SL or 6'-SL for 2 weeks prior to being exposed to either a social disruption stressor or a non-stressed control condition. Stressor exposure significantly changed the structure of the colonic mucosa-associated microbiota in control mice, as indicated by changes in beta diversity. These effects were not evident in mice fed milk oligosaccharides; stressor exposure did not significantly change microbial community structure in mice fed 3'-SL or 6'-SL, indicating that milk oligosaccharides support normal microbial communities and behavioral responses during stressor exposure, potentially through effects on the gut microbiota-brain axis. No adverse effects of 3'-SL were reported.

Hamilton et al. (2017) examined the effect of prebiotics BMOS (containing 35% SL of which the ratio of 3'-SL and 6'-SL was 4:1) in the presence of high fat diet in diet-induced obese mice. C57BL/6 mice were fed a control diet (low fat), high fat (40% fat/kcal), or high fat + prebiotic (6% BMO or inulin) for 1, 3, or 6 weeks. Gut microbiota and intestinal permeability were assessed in the ileum, cecum, and colon. Addition of BMOS to the high fat diet significantly attenuated high fat diet-induced weight gain, decreased adiposity, and decreased caloric intake. No adverse effects were reported.

Boudry et al. (2017) demonstrated the effects of BMOS (7% wt/wt) and *Bifidobacterium longum* ssp. *infantis* (*B. infantis*) on restoring diet-induced obesity intestinal microbiota and barrier function defects in mice. Male C57/BL6 mice were fed a Western diet (40% fat/kcal) or normal chow (C, 14% fat/kcal) for 7 weeks. During the final 2 weeks of the study, the diet of a subgroup of Western diet -fed mice was supplemented with BMOS. Weekly gavage of *B. infantis* was performed in all mice starting at week 3. Supplementation of the Western diet with BMOS had no adverse effects on measured outcomes.

Rasmussen et al. (2017) evaluated the clinical affection, feces, hydration, and necrotizing enterocolitis (NEC) lesions in preterm pigs fed either 5% of 4 HMO blends

containing 10.3% 6'-SL or 7% 25 HMO blends containing 3.6% 6'-SL and 3.7% 3'-SL. The final daily doses of HMOs were 0.8 g/kg bw for 4 HMO blends and 0.84 g/kg bw for 25 HMO blends, providing 3'-SL at doses of 0 or 31.1 mg/kg bw/day, respectively. Infant formula supplemented with the mixture of 4 HMOs or 25 HMOs blends in the first 5-11 days did not have adverse effects on measured outcomes.

Studies Evaluating the Effects of 3'-SL on Cognition

In a study by Sakai et al. (2006), the learning behavior of adult Sprague-Dawley rats was evaluated using a water-filled multiple T-maze apparatus and a Morris swimming-maze after feeding lactose, galactosyllactose (GL), *N*-acetylneuraminic acid (Neu5Ac), sialyllactose (SL: a combination of 87% 3'-SL and 13% 6'-SL), galactosylated *N*-acetylneuraminic acid, or a control diet for 2 weeks. Concentration of each test ingredient was 1% of diet. No adverse effects of 3'-SL were reported.

Conclusions from Animal Efficacy Studies

These animal efficacy studies mentioned above tested the efficacy and the safety of 3'-SL doses up to 1.2-1.4% of the diet for 6-7 weeks in mice (Boudry et al., 2017; Hamilton et al., 2017) or 5% of the diet for 2 weeks in mice (Tarr et al., 2015). No adverse effects of 3'-SL were noted. The 3'-SL at supplemented at up to 5% of diet was well tolerated with no side effects in adult mice.

Table 23. Summary of Animal Efficacy Studies

Objective	Animal	Dose	Duration	Measurements	Reference
To test whether SL could impact stressor-induced anxiety-like behavior, impact the effects of stressor exposure on brain cell proliferation and stability, and could prevent stressor-induced effect	Male mice, C57/BL6 (6-8 wk old, 9 per group)	3 Groups: 1) control diet (AIN-93G); 2) AIN-93G diet + 3'-SL (5% of diet); or 3) AIN-93G diet + 6'-SL (5% of diet)	2 wk	Body and spleen mass; serum concentrations of corticosterone and IL-6; fecal microbiota; brain cell proliferation and immature neuronal assessment and analyses	Tarr et al., 2015
To examine the effects of prebiotic BMO in presence of high fat diet in diet-induced obesity	Male C57BL/6 mice (4-week-old; 6 per group)	4 Groups: 1) control diet; 2) high fat (HF); 3) HF + 6% inulin; 4) HF + 6% BMOS (source-whey, containing ~35% SL; 80% 3'-SL and 20% 6'-SL); Amount of daily intake, not specified	1, 3, or 6 wk	Fat pad analysis; plasma lipopolysaccharide-binding protein; histology analyses; luminal contents of cecum; fecal microbiota DNA and sequencing; microbiota bioinformatics analysis	Hamilton et al., 2017
To demonstrate the effects of BMO and *B. infantis* on restoring diet-induced obesity intestinal microbiota and barrier function defects in mice	16 Male C57/BL6 (3 week-old)	3 Groups: 1) control diet, 7 wk; 2) Western diet, 7 wk; 3) Western diet 7 wk + 7% BMOS-last 2 wk (3'-SL or 6'-SL content not defined); *B. infantis* once a wk. Amount of daily intake, not specified	7 wk; BMO-last 2 wk only	Microbiota analysis; quantitative PCR for TNF- α on colon tissues; plasma biochemical analyses (leptin and lipopolysaccharide-binding protein levels)	Boudry et al., 2017
To investigate the effect of SL on swimming learning behavior and brain	Male SD rats (8 wk old; 6 per group)	6 Groups: 1) Control; 2) 1% lactose; 3) 1% galactosyllactose; 4) 1% N-acetylneuraminic acid;	2 wk	Sialic acid content in serum; lipid content of brain; ganglioside content of brain	Sakai et al., 2006

lipid composition of adult rats		5) 1% SL; 6) 1% galactosylated N-acetylneruaminic acid			
To investigate HMO effects on intestinal function, bacterial colonization and necrotizing enterocolitis (NEC) resistance immediately after preterm birth	Preterm Pigs (gesta-tion day 105-106)	2 Groups: 1) standard formula with 0.84 g/kg/d 25-HMO (providing 31.1 mg/kg/d 3'-SL and 30.2 mg/kg/d 6'-SL); 2) standard formula with maltodextrin	5 d	Clinical affection, feces, and hydration scores; NEC; organ weight; intestinal enzyme activities; colonic bacterial microbiota composition; inflammatory cytokines in middle small intestine and colon; plasma citrulline concentration	Rasmussen et al., 2017

AST=aspartate aminotransferase; ALP= alkaline phosphatase; *B. infantis* = *Bifidobacterium longum* ssp. *infantis*; BMO = bovine milk oligosaccharides; bw = body weight; d = days; FFU = focus-forming unit; FL = 2'-fucosyllactose; h = hours; HMO = human milk oligosaccharides; IL = interleukin; SD = Sprague Dawley; SL = sialyllactose; TNF = tumor necrosis factor; wk = weeks; wt = weight.

6.F. Human Intervention Studies of 3'-SL

In addition to the human clinical study conducted by GeneChem (unpublished), several human clinical studies in infants and adults were identified from the literature (Table 24). Most of these studies focused on the safety and the tolerance of 3'-SL. Some studies included microbiota or the efficacy of 3'-SL in suppressing *H. pylori* activities as measurement endpoints.

6.F.1. Intervention Studies in Infants

Studies by Cooper et al. (2017), Radke et al. (2017), Simeoni et al. (2016), and Meli et al. (2014) were sponsored by one company, Nestle. Cooper et al. (2016), Radke et al. (2017), Simeoni et al. (2016) evaluated the effect of a formula supplemented with a prebiotic, a mixture of bovine milk-derived oligosaccharides (BMOS) generated from whey permeate and the probiotic *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) on the safety and efficacy in various infant populations. These papers (Cooper et al., 2016; Radke et al., 2017; Simeoni et al., 2016) described the test substance as a mixture of BMOS generated from whey permeate (containing galactooligosaccharides [GOS] and milk oligosaccharides such as 3'-SL and 6'-SL) at a total oligosaccharide concentration of 5.8 ± 1.0% of powder formula (or 8 g/L in the reconstituted formula) and a probiotic *B. lactis* (10^7 cfu/g of powder formula). Although these studies indicated that BMOS contained 3'-SL and 6'-SL, a quantitative composition was not specified.

It is noteworthy that the study by Hamilton et al. (2017) also used BMOS containing 35% SLs, of which the proportion of 3'-SL and 6'-SL was 4:1. Assuming the BMOS used in the studies of Cooper et al. (2017), Radke et al. (2017), Simeoni et al. (2016), and Meli et al. (2014) had a similar composition to that described in Hamilton et al. (2017), it is reasonable to assume that these infant formula studies mentioned above tested the efficacy and safety of BMOS containing 2.03% SLs (1.62% 3'-SL and 0.41% 6'-SL) in dry powder or 2.8 g/L (2.24 g/L 3'-SL and 0.56 g/L 6'-SL) in reconstituted formula.

Cooper et al. (2017) tested the effect of a formula supplemented with a prebiotic, a mixture of BMOS generated from whey permeate containing GOS, 3'-SL, and 6'-SL (individual oligosaccharides quantities, not specified), and the probiotic *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) strain CNCM I-3446 on the safety and the bifidobacterial counts in the guts of infants born in HIV positive mothers. A total of 430 healthy, full-term infants born to HIV-positive mothers who had elected to feed their child beginning from birth (≤3 days old) exclusively with formula were randomized into four parallel groups. A total of 421 infants who had study formula intake were included in the fully analysis set. The first two groups consisted of cesarean-delivered infants assigned to the test formula (n=92), a starter infant formula containing BMOS at a total oligosaccharide concentration of 5.8±1.0 g/100 g of powder formula (8 g/L in the reconstituted formula + $1x10^7$ cfu/g of *B. lactis*) or a control infant formula (n= 101). The second two groups consisted of vaginally delivered infants randomized to the same test (n= 115) or control (n= 113) formulas from the time of enrollment to 6 months. The primary safety outcome was daily weight gain (g/day) between 10 days and 4 months and the primary efficacy outcome was fecal bifidobacterial count at 10 days. The supplemented infant formula was well tolerated,

lowered the fecal pH, and improved the fecal microbiota in both normal and cesarean-delivered infants.

Radke et al. (2017) evaluated the efficacy and safety of an infant formula containing BMOS containing 3'-SL and 6'-SL (8 g/L reconstituted formula or 5.8 g/100 g powder; amount of 3'-SL, not specified) plus *B. lactis* (CNCM I-3446; 1×10^7 cfu/g powder) on the safety and efficacy (incidence of diarrhea and febrile infections) during the first year of life. Full-term infants receiving test with BMOS and *B. lactis*, or control formula were enrolled in a multicenter, randomized, controlled, and double-blind trial with a reference breastfeeding group. 413 infants were assigned between test (n= 206) and control (n= 207) formula. There was no significant difference in diarrhea and febrile infection incidence between the groups at 6 months (test vs. control: 25 vs 15%, P=0.096; 38 vs 38%, NS, respectively) and 12 months (43 vs 31%, P=0.119; 81 vs 80%, P=1, respectively). Test formula was well tolerated. Anthropometrics parameters were not significantly different between the groups, and aligned with WHO growth standards up to 12 months. The test group and breastfed infants had comparable gut microbiota pattern, fecal IgA (test vs. breastfed: 3 mo, 57.2 vs 74.2 mg/L; 6 mo, 31.2 vs 53.7 mg/L, P=0.0002; respectively), and stool pH (3 mo: 5.74 vs 5.48, P=0.078; 6 mo: 6.03 vs. 5.79, P= 0.083; respectively). An infant formula enriched with BMOS and *B. lactis* supports normal infant growth and was well tolerated.

Simeoni et al. (2016) tested the effect of feeding a formula supplemented with a mixture of BMOS generated from whey permeate, containing 3'-SL and 6'-SL (total oligosaccharides, 8.0 g/L reconstituted formula or 5.7 g/100 g powder; amount of 6'-SL, not specified) plus *B. animalis* subsp. *lactis* (*B. lactis*) strain CNCM I-3446 (1×10^7 cfu/g powder). Breastfed infants served as reference group. Compared with a non-supplemented control formula, the test formula showed a similar tolerability and supported a similar growth in healthy newborns followed for 12 weeks. In the test group the probiotic *B. lactis* increased by 100-fold in the stool and was detected in all supplemented infants. BMOS containing 3'-SL had no adverse effects on fecal microbiota. The test formula was well tolerated and supported a healthy growth.

Meli et al. (2014) evaluated the growth and safety in infants fed formula supplemented with a mixture of BMOS. Healthy term infants, ≤ 14 days old, were randomly assigned to standard formula (control; n=84), standard formula with BMOS (IF-BMOS; n=99), or standard formula with BMOS and probiotics (*Bifidobacterium longum*, *Lactobacillus rhamnosus*) (IF-BMOS + Pro; n=98). A breastfed reference group was also enrolled (n=30). The primary outcome was mean weight gain/day from enrollment to age 4 months. No significant differences were observed between the control and BMOS groups in caregivers' reports of flatulence, vomiting, spitting up, crying, fussing, and colic. Infants in the bovine milk-derived oligosaccharides groups had more frequent ($p < 0.0001$) and less hard ($p = 0.0003$) stools.

Fanaro et al. (2005) investigated the effect of acidic oligosaccharides derived from pectin hydrolysis and GOS/fructo-oligosaccharides (FOS)/acidic oligosaccharides on intestinal flora and stool characteristics as well as acceptance and tolerance. Human milk

contains 75% to 85% neutral and 15% to 25% acidic oligosaccharides. In this prospective, randomized, double blind study, a mixture of 80% neutral oligosaccharides (GOS and FOS) with 20% acidic oligosaccharides, derived from pectin analysis, was investigated. Forty-six term infants were fed a standard formula supplemented with either maltodextrin control (n= 15), 0.2 g acidic oligosaccharides (n= 16), or the latter plus 0.6 g neutral oligosaccharides (mixture of GOS and FOS; n= 15). Stool characteristics and possible side effects (crying, vomiting, and regurgitation) were recorded as the primary safety measures. There was no difference in growth, crying, vomiting, and regurgitation patterns between the groups. In summary, acidic oligosaccharides from pectin hydrolysate were well tolerated in infants and did not affect intestinal microecology as measured as changes in fecal bifidobacterial counts.

Conclusions from Human Clinical Studies in Infants

In summary, infant formula studies of Cooper et al. (2017), Radke et al. (2017), Simeoni et al. (2016), and Meli et al. (2014) tested the safety and efficacy of infant formulas supplemented with BMOS with probiotics for up to 12 months. These studies mentioned that BMOS contained 3'-SL and 6'-SL without specifying the content of those SLs. The study by Hamilton et al. (2017) also used BMOS containing 35% SLs, of which the proportion of 3'-SL and 6'-SL was 4:1. Assuming the BMOS used in the infant formula studies mentioned above had a similar composition to that described in Hamilton et al. (2017), it is reasonable to assume that BMOS used in these infant formula studies contained 2.03% SLs (1.62% 3'-SL and 0.41% 6'-SL) in dry powder or 2.8 g/L (2.24 g/L 3'-SL and 0.56 g/L 6'-SL) in reconstituted formula. Thus, it is reasonable to conclude that the intended use level, 3'-SL supplemented at concentrations of 230 mg/L in infant formula (ready-to-drink or reconstituted), is safe. The proposed use level of 3'-SL in term infant formula appears to be approximately one tenth of the level tested in those infant formula studies.

6.F.2. Studies in Adults

Unpublished Study by Gurung et al. (2017)

In an unpublished study conducted by Gurung et al. (2017), 48 *H. pylori* positive subjects with chronic gastritis were given either GeneChem's 3'-SL sodium salt (12 g/day divided in 3 doses) or placebo for 4 weeks. The primary endpoint of this study was the safety (gastrointestinal symptoms and clinical chemistry), and the secondary endpoint was the efficacy in *H. pylori* infection control. Clinical chemistry parameters included serum activities or concentrations of asparate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), high density lipoprotein-cholesterol (HDL-C), total bilirubin (T bil), total cholesterol (TC), triglycerides (TG), albumin, creatine, and glucose. No significant differences in compliance of subjects, clinical laboratory tests, vital signs, physical examination results, the number of adverse events, and the efficacy (delta ^{13}C-urea breath test values, the maximum decreases of ^{13}C-urea breath test value) were noted between the groups. 3'-SL sodium salt was considered safe. Details of methods used in this study is described below.

Study Design

A double-blind, controlled, and randomized design was used. It consisted of one 4-week treatment period with 3'-SL sodium salt or placebo powder administered on day 0. Compliance was evaluated by unused investigational products returned to the clinical trials center pharmacy on day 28.

Participants

Males and females (aged 19-70 years) were screened by an in-person medical interview, physical examination, and clinical laboratory tests. Study subjects were screed for *H. pylori* infection using ^{13}C-urea breath test, in which ≥ 2.6 per mil was considered *H. pylori* positive. No biopsies were conducted to confirm *H. pylori* infection. Subjects were randomly assigned to one of two groups: 4 g of 3'-SL sodium salt three times per day after breakfast, lunch, and dinner or placebo powder. Vital signs, physical examinations, and clinical laboratory tests were measured at screening/week 0 and the final visit on week 4. Compliance was assessed at week 4. Adverse events were monitored throughout the study.

Measurement Endpoints

The primary endpoint was safety including clinical chemistry and gastrointestinal tolerance of 3'-SL sodium salt. The secondary endpoint was delta ^{13}C-urea breath test (UBT values) at week 4 compared with baseline.

Clinical Chemistry as a Safety Measure

The serum biochemical parameters were measured using by automatic analyzer (HITACHI 7080 Chemistry Analyzer, Hitachi, Japan): asparate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), blood urea nitrogen (BUN), high density lipoprotein-cholesterol (HDL-C), total bilirubin (T bil), total cholesterol (TC), triglycerides (TG), albumin, creatine, and glucose.

Gastrointestinal (GI) Symptoms

A modified version of the GI symptom rating scale (GSRS) was used to evaluate changes in perceived GI symptoms between baseline and Week 4. The modified GSRS included 5 questions pertaining to abdominal discomfort: stomach grumbling, bloating, belching, and flatulence. These were scored on a scale of 0 to 3, with 0 being 'no symptoms' and 3 being 'extreme symptoms'. One question each related to consistency and frequency of bowel movements scored on a scale of 0 to 4, with 0 and 4 indicated opposite extremes and 2 indicated normal frequency and consistency.

In summary, oral administration of 4 g of 3'-SL sodium salt after breakfast, lunch, and dinner (total 12 g/day) for 28 days in adults with *H. pylori* positive was well tolerated and was proven safe. Of note, the unpublished study by Gurung et al. (2017) tested a lower dose of 3'-SL (12 g/day) in humans than those used in prior published reports by Parente et al. (2003) and by Rasko et al. (2000), which employed daily doses up to 20 g. Details of published studies are described below. The outcomes of a study by Gurung et al. (2017) confirmed those of the earlier published studies reporting that 3'-SL was well tolerated in humans. Thus, the unpublished status of the 2017 Gurung study has no

impact on the overall conclusion of this GRAS determination even if qualified experts do not have access to such data and information.

Published Studies

Parente et al. (2003) tested whether 3'-SL sodium salt can suppress or cure *H. pylori* colonization *in vivo* and determined its safety in humans. Seventy-one consecutive dyspeptic patients with *H. pylori* infection documented by histology and ^{13}C-Urea Breath Test (UBT) were initially recruited to this study. Patients with urea breath test values <15 were excluded, thus reducing the enrollment to 65 patients. They were given two different dosages of 3'-SL sodium salt (10 g or 20 g/day) in three daily administrations before meals or placebo for 4 weeks, according to a randomized double-blind protocol. A standardized ^{13}C-urea breath test (using 100 mg of ^{13}C labelled urea) was repeated in all patients at fixed intervals during treatment (at the end of weeks 1, 2 and 4) and 4 weeks after treatment withdrawal. Patients' compliance and side-effects were evaluated at each weekly visit. 61 patients correctly completed the study (17, 3'-SL sodium salt 10 g/day; 22, 3'-SL sodium salt 20 g/day; and 21, placebo). No serious adverse events were observed during therapy in any of the three groups. The authors concluded that 3'-SL sodium salt was safe and well tolerated but did not suppress or cure *H. pylori* colonization in humans.

In the study by Rasko et al. (2000), 3'-SL was administered to *H. pylori*-positive asymptomatic subjects to evaluate safety, tolerance, and efficacy of 3'-SL (measured by the expression of Lewis antigens by the gastric pathogen *H. pylori*). In this study, a total of 26 asymptomatic subjects were given various doses of 3'-SL for up to 56 days (4 or 8 g/day for 56 days; 20 g/day for 28 days). Gastric biopsies were performed during the dosing period, as well as 30 days after dosing, which provided 127 *H. pylori* isolates that were examined by use of ELISA and immunoblot. Oral supplementation with 3'-SL (doses of 4, 8, or 20 g/day) for several weeks did not change Lewis antigen expression of *H. pylori* strains isolated from human gastric mucosa. 3'-SL supplementation had no adverse effects on the safety and the tolerance.

In a study with a small number of adult human subjects infected with *H. pylori*, one-day oral treatment with 3'-SL was well tolerated but was ineffective in reducing the number of *H. pylori* (Opekun et al., 1999). In this study, healthy *H. pylori* infected volunteers received one of three treatments: hyperimmune bovine colostral immune globulins, 3'-SL, or recombinant human lactoferrin. Outcome was assessed by urea breath test or histological assessment of the number of *H. pylori* present. A total of 10 g 3'-SL was administered in a day to each subject after a standardized meal or snack. Oral administration of 3'-SL did not have measurable effects on the density of the *H. pylori* present, nor on the severity of the inflammatory response. There were no significant changes in serum liver transaminase tests after 3'-SL therapy (data not shown). There were no adverse reactions or adverse events.

Smilowitz et al. (2017) determined the safety and tolerability of BMOS consumption by 12 healthy human participants and its effects on fecal microbiota and microbial metabolism. Participants consumed three supplements (placebo-control and low- and

high BMOS) for 11 consecutive days. After a 2-week washout period, they consumed low- and high-BMO doses (25% and 35%, respectively) of each person's daily fiber intake. The low dose corresponded to 6.3-9.8 g of HMOs, and the high dose to 8.8-13.7 g of HMOs. SL accounts for 45.7% of BMOS, with a 4:1 ratio of 3'-SL to 6'-SL (personal written communication with Dr. Barile, the coauthor of this paper, University of California-Davis), resulting in an estimated daily doses of 5.0 g for 3'-SL and 1.25 g for 6'-SL. Safety and tolerability were measured using standardized questionnaires on gut and stomach discomfort and stool consistency. Fecal extracts were profiled for bacterial populations by next-generation sequencing (NGS) and bifidobacteria presence was confirmed using quantitative PCR. Urine was analysed for changes in microbial metabolism using nuclear magnetic resonance spectroscopy (^{1}H-NMR). Consumption of both the low and high BMOS doses was well tolerated and did not change stool consistency from baseline. Multivariate analysis of the NGS results demonstrated no change in fecal microbiota phyla among the placebo-control and BMOS supplement groups. The authors concluded that BMOS supplementation was well tolerated in healthy adults with no side effects.

Conclusions from Human Clinical Studies in Adults

In adults, daily doses up to 20 g of 3'-SL (sodium salt) were well tolerated with no adverse effects. Of note, an unpublished study by Gurung et al. (2017) tested a dose of 12 g/day of 3'-SL in humans, a lower dose than those used in previous reports by Parente et al. (2003) and Rasko et al. (2000), which employed doses up to 20 g/day. The results of this study confirmed those of an earlier study reporting that 3'-SL was well tolerated in humans. Thus, the unpublished status of the 2017 Gurung study has no impact on the overall conclusion of this GRAS determination if qualified experts do not have access to such data and information.

Table 24. Summary of Human Clinical Studies of 3'-SL

Subject	Dose	Duration	Results	Reference
Studies with Infants – Published Studies				
430 healthy, full-term infants born to HIV-positive mothers	BMOS (total oligosaccharide concentration of 5.8% of powder formula [or 8.0 g/L in the reconstituted formula]) + *B. lactis* (1×10^7 cfu/g); or control formula	6 mo	No adverse effects on mean daily weight gain and growth parameters and fecal pH and bifidobacteria counts	Cooper et al, 2017
413 infants	BMOS (total oligosaccharide concentration of 5.8% of powder formula [or 8.0 g/L in the reconstituted formula]) + 1×10^7 cfu/g *B. lactis*; test control; or breast milk	12 mo	Test formula was well tolerated. No difference in anthropometrics parameters between groups and aligned with WHO growth standards and in diarrhea and febrile infection incidence between groups.	Radke et al, 2017
115 healthy full-term infants	BMOS (5.7% in powder or 8.0 g/L in reconstituted formula) + *B. lactis* CNCM I-3446 (10^7 cfu/g); control; breast milk	12 wk	The test formula was well tolerated and supported a healthy growth.	Simeoli et al, 2016
245 healthy term infants ≤14 days old	Standard formula (control); standard formula with BMO; or standard formula with BMO and probiotics*; and a breast fed reference group	3.5 - 4 months	No significant differences were observed between the control and BMOS groups in caregivers' reports of flatulence, vomiting, spitting up, crying, fussing, and colic. Infants in the bovine milk-derived oligosaccharides groups had more frequent ($p < 0.0001$) and less hard ($p = 0.0003$) stools	Meli et al., 2014
46 term infants	0.2 g acidic oligosaccharides; 0.2 g	6 weeks	There was no difference in growth, crying, vomiting, and regurgitation patterns and	Fanaro et al., 2005

	acidic oligosaccharides plus 0.6 g neutral oligosaccharides (mixture GOS/FOS); or maltodextrin control		bifidobacterial counts between control and SL groups.	
Studies with Adults - Unpublished				
48 *H.pylori* positive subjects, aged 19 to 70 years	0 or 12 g of GeneChem's 3'-SL sodium salt (divided in 3 doses)	4 weeks	No significant differences in compliance of subjects, clinical laboratory tests, vital signs, physical examination results, and the number of adverse events were noted between the groups. 3'-SL sodium salt was considered safe.	Gurung et al., 2017
Studies with Adults - Published studies				
26 *H. pylori* positive asymptomatic subjects	0, 4, 8, or 20 g	Up to 56 days (4 or 8 g/day for 56 d; 20 g/day for 28 d)	3'-SL was safe and well tolerated but was not protective against *H. pylori* infection.	Rasko et al., 2000
71 consecutive dyspeptic patients with *H. pylori* infection	0, 10, or 20 g 3'-SL sodium salt	4 weeks intervention with 8 follow up at 8 weeks	3'-SL sodium salt was safe and well tolerated but did not suppress or cure *H. pylori* colonization as measured by 13C-Urea Breath Test	Parente et al., 2003
Healthy *H. pylori* infected volunteers	0 or 10 g	1 day	There were no significant changes in *H. pylori* and serum liver transaminase levels after 3'-SL therapy. There were no adverse reactions.	Opekun et al., 1999
12 healthy adults	BMO providing up to 5 g 3-SL plus 1.25 g 6'-SL	11 days	BMO was well tolerated.	Smilowitz et al., 2017

BMO=bovine milk-derived oligosaccharides; *probiotics, *Bifidobacterium longum and Lactobacillus rhamnosus.*

6.G. Safety of Enzymes

3'-SL sodium salt is synthesized using enzymes cytidylate kinase (CMK), acetate kinase (ACK), CMP-NeuAc synthetase (NEU), *N*-acetyl-D-glucosamine-2-epimerase (NANE), NeuAc aldolase (NAN), and α2,3-sialyltranferase (PST2,3st) obtained from a strain of beta-D-galactosidase deficient *Escherichia coli* BW25113 (Baba et al., 2006), originated from non-pathogenic *E.coli* K-12. The comprehensive safety assessments of *E.coli* K-12 and its derivatives have been conducted by the U.S. Environmental Protection Agency (U.S. EPA) (U.S.EPA, 1997). The enzymes are produced under cGMP using master and working cell banks and safe food production procedures. The enzyme preparation meets the general purity specifications for enzyme preparations as described in the monograph "Enzyme Preparations" of the current edition of Food Chemicals Codex (FCC, 2016).

In addition to general Basic Local Alignment Search Tool (BLAST) searches, an allergenicity search was conducted using the Allergen Online database (http://www.allergenonline.org), the database maintained by the Food Allergy Research and Resource Program of the University of Nebraska. The Allergen online database version 16 (updated January 27, 2016) was used to conduct a preliminary screen of the complete enzyme protein sequence for relevant matches against putative allergens. A FASTA3 overall search of Allergen Online was conducted using default settings (E cutoff = 1 and maximum alignments of 20). No sequences with E value <1.0 were identified. An 80 amino acid sliding window (segments 1-80, 2-81, 3-82, etc.) also was used to scan the amino acid sequence of the protein against the allergen database using FASTA3 to search for matches of 35% identity or more. This 35% identity for 80 amino acid segments is a suggested guideline proposed by Codex for evaluating proteins in genetically modified crops (Codex, 2003; Goodman et al., 2008).

The results of the FASTA3 alignments of all possible 80 amino acid segments of the enzymes against all putative allergen sequences in the database were all less than the 35% threshold over 80 amino acids. Based on the information demonstrating the widespread history of exposure to the enzyme from *Enterobacteriaceae* sp. residing in the gastrointestinal tract of all humans combined with the findings from the bioinformatics assessment, it can be concluded that all enzymes used for the synthesis of 3'SL sodium salt do not have an allergenic or toxicity risk. More importantly, the enzymes are effectively removed during manufacturing using sequential filtrations. Enzyme protein and DNA residues are absent from finished product as verified using enzyme specific enzyme linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (qPCR).

6.H. Safety Determination

The following safety evaluation fully considers the composition, intake, and microbiological, and toxicological properties of 3'-SL sodium salt, as well as appropriate corroborative data.

1. Analytical data from multiple lots indicate that 3'-SL sodium salt powders comply reliably with the established food-grade product specifications and meet all applicable purity standards.

2. GeneChem's 3'-SL sodium salt is intended for use in non-exempt term infant formulas (milk-, soy-, amino acid-, and hydrolyzed protein-based). The maximum use level is 230 mg/L for 3'-SL or 238 mg/L for 3'-SL sodium salt in ready-to-drink or reconstituted formula.

3. This maximum use level of 3'-SL sodium salt in term infant formulas is based on providing a similar level of 3'-SL as in mature human breast milk, which ranges from 42-840 mg/L. Typical infant formula is estimated to contain 17-19 mg/L of 3'-SL. The addition of 3'-SL sodium salt to term infant formulas is consistent with efforts to produce infant formula that closely match the nutrient composition of human milk.

4. From the use of 3'-SL in infant formula only, in all-user infants aged 0 to 11.9 months old, the estimated mean and 90th percentile intakes of 3'-SL were determined to be 187 and 278 mg/person/day, respectively. On a body weight basis, these intakes were determined to be 25.9 and 43.1 mg/kg bw/day, respectively. In all formula-fed infants aged 0 to 11.9 months old, the estimated mean and 90th percentile intakes of 3'-SL from the use in infant formula and other foods and beverages were determined to be 232 to 326 mg/person/day (or 31.1 and 43.9 mg/kg bw/day), respectively.

5. In all-users aged 1 year and above, the estimated mean and 90th percentile intakes of 3'-SL from the use in foods and beverages were determined to be at 72.5 and 129.3 mg/person/day, respectively. On a body weight basis, these intakes were determined to be 1.8 and 3.6 mg/kg bw/day, respectively. From the use of 3'-SL in foods and beverages, the all-user estimated mean and 90th percentile intakes of 3'-SL were greatest in toddlers at 75 and 139 mg/person/day, respectively. On a body weight basis, these intakes correspond to 5.7 and 11.5 mg 3'-SL/kg bw/day, respectively. These EDIs are within safe intake levels.

6. The LD_{50} of 3'-SL was determined to be higher than 20 g/kg bw, the highest dose tested (Kim et al., 2018). A subchronic oral toxicity study indicates that the NOAEL for GeneChem's 3'-SL sodium salt was greater than 2,000 mg/kg bw/day, the highest dose tested, in rats.

7. The addition of GeneChem's 3'-SL sodium salt at the dose of 140, 200, or 500 mg/L was well tolerated and supported normal growth patterns in neonatal piglets (Donovan, 2017; unpublished). A published study by Jacobi et al. (2016) also reported no adverse effects of 3'-SL at concentrations of up to 4,000 mg/L in

8. Infant formula studies of Cooper et al. (2017), Radke et al. (2017), Simeoni et al. (2016), and Meli et al. (2014) tested the safety and the efficacy of infant formulas supplemented with BMOS with probiotics for up to 12 months. These studies mentioned that BMOS contained 3'-SL and 6'-SL without specifying the content of those SLs. The study by Hamilton et al. (2017) also used BMOS containing 35% SLs, of which the proportion of 3'-SL and 6'-SL was 4:1. Assuming the BMOS used in the infant formula studies mentioned above had a similar composition to that described in Hamilton et al. (2017), it is reasonable to assume that BMOS used in these infant formula studies contained 2.03% SLs (1.62% 3'-SL and 0.41% 6'-SL) in dry powder or 2.8 g/L (2.24 g/L 3'-SL and 0.56 g/L 6'-SL) in reconstituted formula. Thus, it is reasonable to conclude that 3'-SL supplemented at concentrations of 230 mg/L in infant formula (ready-to-drink or reconstituted) is safe.

9. Human clinical studies found that 3′-SL sodium salt was well tolerated with no side effects at daily doses of up to 20 g in adults (Parente et al., 2003; Rasko et al., 2000).

6.I. Conclusions and General Recognition on the Safety of 3'-SL Sodium Salt

3′-SL is a naturally occurring trisaccharide found in human milk, and is therefore typically referred to as a human milk oligosaccharide (HMO). The presence of HMOs in breast milk has been associated with a variety of nutritional effects including the establishment and maintenance of healthy intestinal bacterial microflora. Typical infant formula is estimated to contain 17-19 mg/L of 3′-SL. The addition of 3′-SL to term infant formulas is consistent with efforts to produce infant formula that closely matches the nutrient composition of human milk. The 3′-SL part of GeneChem's 3'-SL sodium salt is chemically and structurally identical to the 3′-SL which is found in human milk, and therefore, the safety of GeneChem's 3′-SL sodium salt for all intended uses is supported by the known consumption of 3′-SL from human breast milk in infants. Additionally, in all the studies summarized in these GRAS determinations, there were no significant adverse effects/events or tolerance issues attributable to 3'-SL in both adults and infants. Because this safety evaluation was based on generally available and widely accepted data and information, it satisfies the so-called "common knowledge" element of a GRAS determination.

In addition, the intended uses of 3'-SL sodium salt have been determined to be safe though scientific procedures as set forth in 21 CFR 170.3(b), thus satisfying the so-called "technical" element of the GRAS determination. The 3'-SL sodium salt that is the subject of this GRAS determination is produced by enzymes isolated from genetically engineered, non-toxigenic *E. coli* K12, and its purity is over 98%. The 3'-SL sodium salt is manufactured consistent with cGMP for food (21 CFR Part 110 and Part 117 Subpart B). The raw materials and processing aids used in the manufacturing process are food

grade and/or commonly used in food manufacturing processes. No toxicants have been detected from GeneChem's 3'-SL sodium salt ingredient.

Literature search did not identify safety or toxicity concerns related to 3'-SL. Toxicity studies of GeneChem's 3'-SL include acute and subchronic toxicity in rats, subacute toxicity in dogs, and a battery of mutagenicity and genotoxicity studies. The LD_{50} of 3'-SL was determined to be higher than 20 g/kg bw, the highest dose tested. A compound which has a LD_{50} value of >15 g/kg bw as 'relatively harmless.' Thus, 3'-SL, like other non-digestible oligosaccharides or carbohydrates, belongs to the group which has the lowest toxicity rating. The addition of 3'-SL or its sodium salt at the dose of up to 4,000 mg/L was well tolerated and supported normal growth patterns in neonatal piglets. The literature also contains a wealth of publicly available studies on the safety of 3'-SL in infants and other human age groups. This evidence is sufficient to support the safety and GRAS status of the proposed use of 3'-SL sodium salt in these infants and other human populations.

We have concluded that GeneChem's 3'-SL sodium salt is GRAS under the intended conditions of use on the basis of scientific procedures, and other experts qualified to assess the safety of food ingredients would concur with these conclusions. Therefore, it is excluded from the definition of a food additive and may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

PART 7. REFERENCES

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7.B. Data and Information That Are Not Generally Available (The report/manuscript are attached as Appendices C and D)

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Appendix A. Batch data for GeneChem's 3'-SL sodium salt

Table A-1. Product analysis for 5 non-consecutive batches of 3'-SL sodium salt.

Tests	Unit	Batch # 150622-01	Batch # 160509-01	Batch # 160510-01	Batch # 160515-01	Batch # 160601-01
Appearance		Complied	Complied	Complied	Complied	Complied
Solubility		Complied	Complied	Complied	Complied	Complied
^{1}H NMR SPECTRUM		Complied	Complied	Complied	Complied	Complied
Mass SPECTRUM		Complied	Complied	Complied	Complied	Complied
Purity (HPLC Area)	%	98.8	99.08	99.25	99.05	98.68
Moisture	%	1.83	3.21	2.72	2.95	2.65
Ash	%	7.59	7.14	7.01	7.14	7.43
Fat	g/100g	0.26	0.25	0.25	0.25	0.25
Protein	g/100g	0.0	0.0	0.0	0.0	0.0
Sodium	%	1.79	1.71	2.47	2.12	2.45
Arsenic	ppm	ND	ND	ND	ND	ND
Cadmium	ppm	ND	ND	ND	ND	ND
Lead	ppm	ND	ND	ND	ND	ND
Mercury	ppm	<0.01	<0.01	<0.01	<0.01	<0.01
Gene residue		Negative	Negative	Negative	Negative	Negative
Endotoxins	EU/g	<50	<50	<50	59.9	<50
Enzyme residue		Negative	Negative	Negative	Negative	Negative
Total Colony Counts	CFU/g	180	0.0	5.0	5.0	0.0
Coliform	CFU/g	Negative	Negative	Negative	Negative	Negative
Salmonella	CFU/g	Negative	Negative	Negative	Negative	Negative
Yeasts and Molds	CFU/g	Negative	Negative	Negative	Negative	Negative
Listeria monocytogenes		ND	ND	ND	ND	ND
Enterobacter sakazakii (*Cronobacter* spp.)		ND	ND	ND	ND	ND

ND= Not Detected; EU = endotoxin unit; CFU = colony forming units; Limit Of Detection: Lead 0.004 ppm, Arsenic 0.002 ppm, Cadmium 0.004 ppm, Mercury 0.0002 ppm, Gene residue 0.007 ng/g, Protein residue 20 ng/g.

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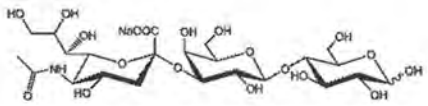
CERTIFICATE of ANALYSIS

Product Name	**3'-Sialyllactose sodium salt**
Product Structure	
Product Number	GCBO0007
Batch Number	150622-01
CAS Number	128596-80-5
Molecular Formula	$C_{23}H_{36}NO_{19}Na$
Molecular Weight	655.5
Storage Temperature	Room temperature

TESTS	SPECIFICATION	RESULTS
Appearance	White powder	Complied
Solubility	Clear colorless solution	Complied
^{1}NMR SPECTRUM	Consistent with structure	Complied
Mass Spectrum	Confirms molecular weight	Complied
Assay (HPLC)	≥ 98%	98.8
Moisture Contents	≤ 8.5%	1.83
Ash Contents	≤ 6%	7.59
Fat	≤ 0.5	0.26
Protein	≤ 0.1	0.0
Sodium	≤ 3.5%	1.79
Lead	≤ 0.02ppm	ND
Arsenic	≤ 0.5ppm	ND
Cadmium	≤ 0.1ppm	ND
Mercury	≤ 0.5ppm	< 0.01
Gene Residue	Negative	Negative
Endotoxins	≤ 300EU/g	< 50
Enzyme residue	Negative	Negative
Total Colony Counts	≤ 200 CFU/g	180
Coliform	Negative	Negative
Salmonella	Negative	Negative
Yeasts & Molds	≤ 200 CFU/g	0 x 10^1
Listeria monocytogenes	Negative	Negative
Enterobacter sakazakii	Negative	Negative

ND = Not detected.

REMARKS : "PASSED"

Manager S. Y. Kang
Q.C. Department

GeneChem Inc.

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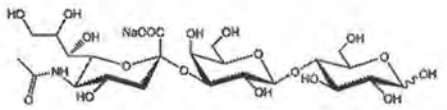
CERTIFICATE of ANALYSIS

Product Name	**3'-Sialyllactose sodium salt**
Product Structure	
Product Number	GCBO0007
Batch Number	160509-01
CAS Number	128596-80-5
Molecular Formula	$C_{23}H_{38}NO_{19}Na$
Molecular Weight	655.5
Storage Temperature	Room temperature

TESTS	SPECIFICATION	RESULTS
Appearance	White powder	Complied
Solubility	Clear colorless solution	Complied
^{1}NMR SPECTRUM	Consistent with structure	Complied
Mass Spectrum	Confirms molecular weight	Complied
Assay (HPLC)	≥ 98%	99.08
Moisture Contents	≤ 8.5%	3.21
Ash Contents	≤ 6%	7.14
Fat	≤ 0.5	0.25
Protein	≤ 0.1	0.0
Sodium	≤ 3.5%	1.71
Lead	≤ 0.02ppm	ND
Arsenic	≤ 0.5ppm	ND
Cadmium	≤ 0.1ppm	ND
Mercury	≤ 0.5ppm	< 0.01
Gene Residue	Negative	Negative
Endotoxins	≤ 300EU/g	< 50
Enzyme residue	Negative	Negative
Total Colony Counts	≤ 200 CFU/g	0.0
Coliform	Negative	Negative
Salmonella	Negative	Negative
Yeasts & Molds	≤ 200 CFU/g	0 x 10^1
Listeria monocytogenes	Negative	Negative
Enterobacter sakazakii	Negative	Negative

ND = Not detected.

REMARKS : "PASSED"

Manager S. Y. Kang
Q.C. Department

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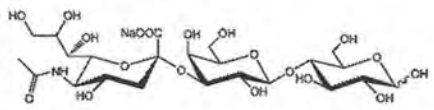
CERTIFICATE of ANALYSIS

Product Name	**3'-Sialyllactose sodium salt**
Product Structure	
Product Number	GCBO0007
Batch Number	160510-01
CAS Number	128596-80-5
Molecular Formula	$C_{23}H_{38}NO_{19}Na$
Molecular Weight	655.5
Storage Temperature	Room temperature

TESTS	SPECIFICATION	RESULTS
Appearance	White powder	Complied
Solubility	Clear colorless solution	Complied
^{1}NMR SPECTRUM	Consistent with structure	Complied
Mass Spectrum	Confirms molecular weight	Complied
Assay (HPLC)	≥ 98%	99.25
Moisture Contents	≤ 8.5%	2.72
Ash Contents	≤ 6%	7.01
Fat	≤ 0.5	0.25
Protein	≤ 0.1	0.0
Sodium	≤ 3.5%	2.47
Lead	≤ 0.02ppm	ND
Arsenic	≤ 0.5ppm	ND
Cadmium	≤ 0.1ppm	ND
Mercury	≤ 0.5ppm	< 0.01
Gene Residue	Negative	Negative
Endotoxins	≤ 300EU/g	< 50
Enzyme residue	Negative	Negative
Total Colony Counts	≤ 200 CFU/g	5.0
Coliform	Negative	Negative
Salmonella	Negative	Negative
Yeasts & Molds	≤ 200 CFU/g	0 x 10^1
Listeria monocytogenes	Negative	Negative
Enterobacter sakazakii	Negative	Negative

ND = Not detected.

REMARKS : "PASSED"

Manager S. Y. Kang
Q.C. Department

GeneChem Inc.

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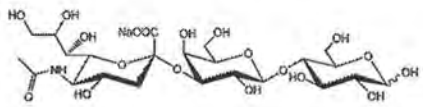
Product Structure	
Product Number	GCBO0007
Batch Number	160515-01
CAS Number	128596-80-5
Molecular Formula	$C_{23}H_{38}NO_{19}Na$
Molecular Weight	655.5
Storage Temperature	Room temperature

TESTS	SPECIFICATION	RESULTS
Appearance	White powder	Complied
Solubility	Clear colorless solution	Complied
^{1}NMR SPECTRUM	Consistent with structure	Complied
Mass Spectrum	Confirms molecular weight	Complied
Assay (HPLC)	≥ 98%	99.05
Moisture Contents	≤ 8.5%	2.95
Ash Contents	≤ 6%	7.14
Fat	≤ 0.5	0.25
Protein	≤ 0.1	0.0
Sodium	≤ 3.5%	2.12
Lead	≤ 0.02ppm	ND
Arsenic	≤ 0.5ppm	ND
Cadmium	≤ 0.1ppm	ND
Mercury	≤ 0.5ppm	< 0.01
Gene Residue	Negative	Negative
Endotoxins	≤ 300EU/g	59.9
Enzyme residue	Negative	Negative
Total Colony Counts	≤ 200 CFU/g	5.0
Coliform	Negative	Negative
Salmonella	Negative	Negative
Yeasts & Molds	≤ 200 CFU/g	0 x 10^1
Listeria monocytogenes	Negative	Negative
Enterobacter sakazakii	Negative	Negative

ND = Not detected.

REMARKS : "PASSED"

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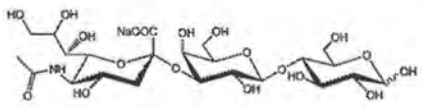
CERTIFICATE of ANALYSIS

Product Name	**3'-Sialyllactose sodium salt**
Product Structure	
Product Number	GCBO0007
Batch Number	160601-01
CAS Number	128596-80-5
Molecular Formula	$C_{23}H_{38}NO_{19}Na$
Molecular Weight	655.5
Storage Temperature	Room temperature

TESTS	SPECIFICATION	RESULTS
Appearance	White powder	Complied
Solubility	Clear colorless solution	Complied
^{1}NMR SPECTRUM	Consistent with structure	Complied
Mass Spectrum	Confirms molecular weight	Complied
Assay (HPLC)	≥ 98%	98.68
Moisture Contents	≤ 8.5%	2.65
Ash Contents	≤ 6%	7.43
Fat	≤ 0.5	0.25
Protein	≤ 0.1	0.0
Sodium	≤ 3.5%	2.45
Lead	≤ 0.02ppm	ND
Arsenic	≤ 0.5ppm	ND
Cadmium	≤ 0.1ppm	ND
Mercury	≤ 0.5ppm	< 0.01
Gene Residue	Negative	Negative
Endotoxins	≤ 300EU/g	< 50
Enzyme residue	Negative	Negative
Total Colony Counts	≤ 200 CFU/g	0.0
Coliform	Negative	Negative
Salmonella	Negative	Negative
Yeasts & Molds	≤ 200 CFU/g	0 x 10^1
Listeria monocytogenes	Negative	Negative
Enterobacter sakazakii	Negative	Negative

ND = Not detected.

REMARKS : "PASSED"

Manager S. Y. Kang
Q.C. Department

Appendix B. Methods of Analysis

The table below shows Korean Food Standards methods of analysis which are equivalent to AOAC or ISO methods.

Contents	Methods	Reference
Ash	Korean Food Standards Codex 7/1/1.1/1.1.2	AOAC Official Method 900.02
Moisture	Korean Food Standards Codex 7/1/1.1/1.1.1/1.1.1.1	AOAC Official Method 941.14
Arsenic (As)	Korean Food Standards Codex 7/7/7.1/7.1.2/7.1.2.3 (ICP)	AOAC Official Method 2013.06
Cadmium (Cd)	Korean Food Standards Codex 7/7/7.1/7.1.2/7.1.2.2 (ICP)	AOAC Official Method 2013.06
Lead (Pb)	Korean Food Standards Codex 7/7/7.1/7.1.2/7.1.2.1 (ICP)	AOAC Official Method 2013.06
Mercury (Hg)	Korean Food Standards Codex 7/7/7.1/7.1.2/7.1.2.4 (Mercury Analyzer)	AOAC Official Method 971.21
Sodium (Na)	Korean Food Standards Codex 7/1/1.2/1.2.1/1.2.1.6 (ICP)	AOAC Official Method 2013.06
Salmonella	Korean Food Standards Codex 7/3/3.11	AOAC Official Method 989.14
Total Colony Counts (The number of bacteria)	Korean Food Standards Codex 7/3/3.5/3.5.1.	AOAC Official Method 986.33
Coliform group	Korean Food Standards Codex 7/3/3.7.1	AOAC Official Method 991.14
Fat	Korean Food Standards Codex 7/1/1.1/1.1.5/1.1.5.1	AOAC Official Method 996.06
Protein	Korean Food Standards Codex 7/1/1.1/1.1.3/1.1.3.3	AOAC Official Method 945.23
Yeasts and Molds	Korean Food Standards Codex 7/3/3.10	AOAC Official Method 2002.11
Listeria monocytogenes	Korean Food Standards Codex 7/3/3.15	AOAC Official Method 996.14
Enterobacter sakazakii	Korean Food Standards Codex 7/3/3.21	ISO/TS 22964:2006

From:	Susan S Cho
To:	Bewry, Nadine
Cc:	Gurung Rit B.
Subject:	GRN 000766 Answers to FDA Questions and Comments
Date:	Friday, June 8, 2018 9:44:28 AM
Attachments:	GRN 000766 Answers to FDA questions 6-8-2018 sent to FDA.docx Dr. Barile"s SL quantification of BMO 1236 used in Hamilton et al 2017.xlsx

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Dear Dr. Bewry,

In response to FDA questions and comments regarding GRN 000766 (3'-SL sodium salt), we have prepared our answers and responses as shown in the attached documents. We would appreciate your kind attention to our responses to FDA questions. Have a nice weekend!

Sincerely,
Susan
Susan Cho, Ph.D.
NutraSource, Inc. 6309 Morning Dew Ct Clarksville, MD 21029 +1-410-531-3336 (O) +1-301-875-6454 (C)

June 8, 2018

To: Dr. Nadine Bewry

Subject: GRN 000766 (3'-Sialyllactose (3'-SL)): Answers to FDA Questions and Comments dated May 31, 2018

From: Susan Cho at Nutrasource, Agent for GeneChem, Inc., the notifier

Dear Dr. Bewry,

Please find out answers and responses to FDa questions and comments as follows.

Toxicology Questions

1) On page 47 section 6.A., GeneChem states that "Several sources of HMOs" (human milk oligosaccharides) "have been evaluated by the FDA and other global regulatory agencies over the past 5 years for incorporation of HMO products in infant formulas".

a) Please specify what the "other global regulatory agencies" are, provide references, and a summary of their conclusions.

Answer:

US FDA

2'-fucosyllactose (2'-FL) -US FDA GRAS
GRN546 – Glycom (Chemical method)
GRN571 – Jennewein (Fermentation using *E. coli*)
GRN650 – Glycom (Fermentation)
GRN749 – DuPont Nutrition & Health (Fermentation, *E coli* K-12, MG1655 INB3051)
GRN735 – Glycosyn & FrieslandCampina (Fermentation using *E. coli* GI724)

Lacto-*N*-neotetraose (LNnT) - US FDA GRAS
GRN547 – Glycom (Chemical method)
GRN659 – Glycom (Fermentation, *E. coli* K-12)

Europe

The European Commission (EC) has approved the following HMOs as Novel Food.
2'-fucosyllactose (2'-FL), synthetic
2'-fucosyllactose (2'-FL), microbial source
lacto-N-neotetraose (LNnT), synthetic
lacto-N-neotetraose (LNnT), microbial source

In 2015, the EC requested that the European Food Safety Authority (EFSA) carry out a risk assessment on the ingredient to determine if it could qualify as a Novel Food. The EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) concluded that 2'-FL is safe for infants up to

one year of age when added to infant and follow-on formulas and when added to other foods at the uses and use levels proposed by the applicant.

The Panel also concluded that LNnT is safe for infants (up to one year of age) when added to infant and follow-on formulae, in combination with 2'-FL, at concentrations up to 0.6 g/L of LNnT and up to 1.2 g/L of 2'-FL, at a ratio of 1:2 in the reconstituted formulae; is safe for young children (older than one year of age) when added to follow-on and young-child formulae, at concentrations up to 0.6 g/L of LNnT (alone or in combination with 2'-FL, at concentrations up to 1.2 g/L, at a ratio of 1:2). The Panel also concludes that LNnT is safe when added to other foods at the uses and use levels proposed by the applicant.

References

EUR-Lex: Access to European Union Law. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32017R2470

EFSA, 2015a. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). Scientific opinion on the safety of 2'-O-fucosyllactose as a novel food ingredient pursuant to Regulation (EC) No 258/97. EFSA J. 13(7),4184, 32 pp. doi:10.2903/j.efsa.2015.4184.

EFSA, 2015b. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). Scientific opinion on the safety of lacto-*N*-neotetraose as a novel food ingredient pursuant to Regulation (EC) No 258/97. EFSA J. 13(7),4183, 32 pp. doi:10.2903/j.efsa.2015.4183.

EFSA, 2015c. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). Statement on the safety of lacto-*N*-neotetraose and 2'-O-fucosyllactose as novel food ingredients in food supplements for children. EFSA J. 13(11), 4299, 11 pp. doi:10.2903/j.efsa.2015.4299.

https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4184

The Food Safety Authority of Ireland (FSAI)

The FSAI decided that 2'-FL produced by fermentation is substantially equivalent to the chemically synthesized comparator which was authorized for the EU market to Glycom A/S (a pdf report available). In addition, FSAI did not identify any safety concerns associated with the consumption of 2'-FL in the proposed food groups and at the intended use levels and therefore considered that it met the criteria for novel food set out in Article 3.1 of the novel food Regulation (EC) No 258/97.

Reference:

https://www.fsai.ie/uploadedFiles/Science_and_Health/Novel_Foods/Notifications/2016%20Glycom%20Fermented%202'FL.pdf (October 2014)
https://www.fsai.ie/uploadedFiles/Science_and_Health/Novel_Foods/Applications/2'FL.pdf

FSAI also agreed on the fact that Lacto-*N*-neotetraose (LNnT) produced by Glycom A/S using microbial fermentation is substantially equivalent to the synthetic counterpart authorized for the EU market. Furthermore, FSAI also granted the status of novel food to LNnT, manufactured by Glycom A/S.

Reference:
https://www.fsai.ie/uploadedFiles/Science_and_Health/Novel_Foods/Notifications/SE%20opinion%20LNnt.pdf (June 2014)

https://www.fsai.ie/uploadedFiles/Science_and_Health/Novel_Foods/Applications/LNnT%20Assessment.pdf

Food Standards Australia and New Zealand (FSANZ)
FSANZ is in the process of evaluating 2'-FL and lacto-N-neotetraose (LNnT) as novel foods in infant formula and other products. The submission of Administrative Assessment Report – Application A1155 2'-FL and LNnT as novel foods in infant formula and other products (Jan 12, 2018) is being evaluated.

Reference
http://www.foodstandards.gov.au/code/applications/Documents/A1155%20-%20AAR.pdf

b) While the notifier sates that several HMOs have been evaluated, it only provides two examples: 2'O-fucosyllactose and lacto-N-neotetraose. Please provide additional examples and explain why and how the GRAS determinations of these two substances support the safety of 3'-SL (i.e. provide the scientific basis why the safety of these above substances support the safety determination of 3'-SL).

Answer:
We agree with FDA that only 2 HMOs have been evaluated: 2'-FL (GRN 749, 735, 650, 571, and 546) and lacto-N-neotetraose (LNnT) (GRN 659 and 547). However, we realize that the manufacturing processes of 2'-FL and LNnT are varied (e.g., synthetic or fermentation). These HMOs are used in consistent with the concentrations present in human milk, and human infants have consumed these HMOs with no side effects. No literature reported adverse effect of these HMOs. Likewise, 3'-SL will also be used in consistent with the concentration naturally present in human milk. Human infants have consumed 3'-SL with no side effects. No literature reported adverse effects of 3'-SL.

2) On page 81 in the References section, GeneChem states that the safety "data and information that are not generally available (the report/manuscript are attached as Appendices C and D)" for the studies by Donovan (2017) and Gurung et al. (2016), respectively. Please note that these sections were not included in the notice. Please provide us with appendices C and D.

Answer:
We apologize for not attaching the appendices to the original submission. Please find the two studies in the attachment (Appendices C and D). Professor Donovan's 2017 piglet study manuscript has been submitted to 'Food & Chemical Toxicology' (Monaco et al. 2018). Recently, Dr. Gurung's 2016 human study has been submitted to 'Nutrients.' These manuscripts are being reviewed by the journals.

3) On page 53 of the notice, GeneChem states that in the unpublished study by Donovan (2017), "the addition of 3'-SL sodium salt at the dose of 140, 200, or 500 mg/L (or up to 483 mg 3'-SL/L) was well tolerated and supported normal growth patterns". On page 51 the notifier states that piglets were provided either "formula+140 mg 3'SL sodium salt/kg body weight (BW), formula+200 mg 3'SL sodium salt/kg BW or formula+ 500 mg 3'SL sodium salt/kg BW." Please clarify if the dose levels were 140, 200, or 500 mg/L of formula (and if so provide what the corresponding levels are in mg 3'-SL/kg bw/day) or if the dose levels were 140, 200, or 500 mg/kg bw/day.

Answer:
The doses on page 51 were mistakenly reported as mg/kg bw/day. The correct doses were 140, 200, or 500 mg 3'-SL sodium salt/L (or 135.3, 193.3, or 483.2 mg 3'-SL/L). These levels correspond to 46.6, 66.7, or 167.2 mg 3'-SL/kg bw/day, respectively, in piglets. Professor Donovan, who conducted the study mentioned above at the University of Illinois-Urbana, provided the following summary table.

Table 1 for Question 3. Concentration of **3'SL** sodium salt used in the studies and final dose based on body weight per day

Study	dose (mg/L formula)	mg /kg BW/day*
Monaco et al., 2018	140	48.27 (46.65)
	200	69.04 (66.73)
	500	172.98 (167.17)
Jacobi et al., 2016**	2000	600
	4000	1200

*Dose of 3'SL sodium salt; numbers in parenthesis- concentration of 3'SL.
**as reported by authors.
Monaco et al. manuscript (2018) = Donovan et al. (2017) in the GRAS document.

4) On page 56 of the notice, GeneChem briefly discusses a published study by Jacobi et al. (2016) in piglets that were administered 0, 2,000, or 4,000 mg 3'-SL/L of milk for 21 days. Please provide what these levels correspond to in mg 3'-SL/kg bw/day to allow comparison to EDIs (see page 201 of the article).

Answer:
Jacobi et al. (2016) stated that 2,000 or 4,000 mg 3'-SL/L correspond to 600 and 1,200 mg 3'-SL/kg bw/day in piglets, respectively.

The highest dose level tested in piglets is 27 times higher than the 90 percentile EDIs of 3'-SL (43.9 mg/kg bw/day) in all users aged 0 - 11.9 month old of both infant formula and other foods

and beverages. This level is also 300 times higher than the EDIs at 90th percentile of 3'-SL (3.6 mg 3'-SL/kg bw/day) from the use of other foods and beverages in all users aged 1 year and above.

Please note:
Through an e mail communication, Dr. Maciej Chichlowski (please see Appendix B), a co-author of the 2016 Jacobi study and a senior scientist at Mead Johnson, confirmed that the study by Jacobi et al. (2016) used GeneChem's 3'-SL sodium salt, although the method section of the paper did not specify the source of 3'-SL. GeneChem sold 3'-SL sodium salt and 6'-SL sodium salt to Mead Johnson, a sponsor of the study, which provided GeneChem's SL to this research lab for this piglet study.

5) On page 59 of the notice, GeneChem states that "These animal efficacy studies mentioned above tested the efficacy and the safety of 3'-SL doses up to 1.2-1.4% of the diet for 6-7 weeks in mice (Boudry et al., 2017; Hamilton et al., 2017) or 5% of the diet for 2 weeks in mice (Tarr et al., 2015)."

a) The above statement implies that the dose of 3'-SL administered to mice in Boudry et al. (2017) is known (i.e. 1.2%). On page 60 the notifier states that the 3'-SLor 6'-SL content of the diets was not defined and amount of daily intake is not specified. If the intake of 3'-SL in this study is not known, this study cannot support the safety of 3'-SL. Moreover, the reference of Boudry et al., 2017 does not support the notifier's statement of "These animal efficacy studies mentioned above tested the efficacy and the safety of 3'-SL doses up to 1.2-1.4% of the diet for 6-7 weeks in mice (Boudry et al., 2017; Hamilton et al., 2017) or 5% of the diet for 2 weeks in mice (Tarr et al., 2015)." Please confirm that you agree and if you disagree, explain why you disagree.

Answer:
We agree that studies by Hamilton et al. (2017) and Boudry et al. (2017) did not define the daily intake of 3'-SL. However, we believe that these studies can be used to support the safety of 3'-SL since we were able to estimate the 3'-SL intake levels based on the information on 3'-SL concentrations in bovine milk oligosaccharides (BMOS) provided by Dr. Daniella Barile.

Dr. Barile, a food chemistry professor at the University of California-Davis (UC-Davis), is a co-author of the 2017 studies by Hamilton et al. and Boudry et al. and a patent owner of BMOS assigned to UC-Davis. She also served as an expert panel member for the GRAS determination of 3'-SL sodium salt and 6'-SL sodium salt manufactured by GeneChem.

b) On page 60 of the notice, GeneChem states that in the Hamilton et al. (2017) study the animals were administered high fat diet with 6% BMOS (bovine milk oligosaccharides) containing 35% SL, of which 80% is 3'-SL and 20% is 6'-SL. That implies that the total 3'-SL in the diet is 1.7% (6x0.35xA0.80) and not 1.4% as stated by the notifier above. Furthermore, please point out where exactly in the article it is stated that the BMOS contains 35% SL, of which 80% is 3'-SL and 20% is 6'-SL. Based on FDA's understanding of the data in Table 2 of the article,

the BMOS contains a total of 31.83% SL (3'-SL and 6'-SL). To our understanding, the exact amount of 3'-SL in this diet is unknown. If you disagree, please explain why and calculate the correct amount of 3'-SL in the diet both in % and then in mg/kg bw/day.

Answer:
We agree with the FDA that the concentration of total SL was 31.83% of BMOS in the 2017 paper by Hamilton et al. and that there were calculation errors.

Recently, Dr. Barile provided the exact amounts of 3'-SL and 6'-SL present in BMOS which was employed in the studies by Hamilton et al. (2017) and Boudry et al. (2017) (personal written communication, June 3-4, 2018; Appendix A-1). She has clarified that the absolute concentrations of 3'-SL and 6'-SL in BMOS were 3.88% and 0.82%, respectively (please see an attached excel file provided by Dr. Barile).

Our initial statement on the SL composition was based on Dr. Daniella Barile's general summary that a typical BMOS consists of 35-50% SL of which 80% was 3'SL and 20% was 6'SL (personal written communication, July 19, 2017; Appendix A-2). We used the lower end of the total SL concentration, i.e., 35%, in our calculations.

We find that the studies by Hamilton et al. (2017) and Boudry et al. (2017) did not fully describe the absolute concentrations of SL and other HMOs. The 2017 Hamilton paper referred to Table 2 for the composition of the BMO obtained by nano-chip HPLC QToF, reporting the relative abundance of SL in BMO as 31.83%. The Boudry paper also presented similar information in a figure. These two papers misled the readers to interpret that BMOS was comprised of 31.83% SL, although the absolute amount of total SL was approximately 6.3%. The missing information in both papers and Dr. Barile's initial e-mail response (July 19, 2017; Appendix A-1) was that BMOS preparation contained many unidentified oligosaccharides and that the relative abundance of SL in identified oligosaccharides only was 31.83%. Regardless, Dr. Barile clarified that the absolute concentration of 3'-SL in BMOS was 3.88%.

In another e-mail on June 4, 2018, she wrote that the absolute amount of SL in another BMOS preparation was indeed 36.2%, indicating that the concentration of SL in BMOS used in the studies by Hamilton (2017) and Boudry (2017) was really low compared to other BMOS preparations.

Using the amended 3'-SL level (3.88% in BMOS) provided by Dr. Barile, a co-author responsible for the chemistry of these two papers, we calculated the 3'-SL concentrations as 0.233 - 0.272% of the diet in the studies of Boudry et al. (2017) and Hamilton et al. (2017). According to a conversion factor proposed by Lehman, these levels may correspond to 350 - 408 mg 3'-SL/kg bw/day.

We have obtained the values using the following calculation method:

3'-SL % in the diet = HMOs % in the diet x 0.0388 (3'-SL concentration in the BMO, g/g)

3'-SL intake (mg/kg bw/day) = 0.15 (a conversion factor) x 3'-SL, mg/kg diet

	Hamilton (2017)	Boudry (2017)
BMOS %	6.0%	7.0%
3'-SL % in diet	0.233%	0.272%
3'-SL in diet, mg/kg diet	2,330	2,720
3'-SL intake, mg/kg bw/day	349.5	408.0

References

Barile D. June3-4, 2018. Personal written communications (Please see e-mail correspondences in the Appendix and an attached excel file).
Barile D. July 19, 2017. Personal written communications (Please see e-mail correspondences the Appendix).
Traas and van Leeuwen, 2007. Chapter 7. Ecotoxicological effects. In: Risk assessment of chemicals (van Leeuwen CJ and Vermeire TG, Eds), 2nd edition. Springer, Dordrecht, The Netherlands. Pages 281-356.
Lehman AJ. Untitled. 1954. Assoc. Food Drug Off Quart Bull 18:66. [file not available]

c) For the Tarr et al. (2015) study, please provide what the 5% dietary level corresponds to in mg 3'-SL/kg bw/day to allow comparison to EDIs.

Answer:
According to a conversion factor proposed by Lehman, 5% dietary level (50,000 mg/kg diet) may correspond to 7,500 mg 3'-SL/kg bw/day in mice. The conversion factor of 0.15 was used to convert 'mg/kg diet' to 'mg/kg bw/day.'

Please note:
Through e mail communications, Dr. Maciej Chichlowski (Appendix B), a co-author of the 2015 Tarr et al. study and a senior scientist at Mead Johnson, confirmed that the study by Tarr et al. (2015) used GeneChem's 3'-SL sodium salt, although the method section of the paper specified a diet formulation company only without specifying the source of 3'-SL. GeneChem sold 3'-SL sodium salt and 6'-SL sodium salt to Mead Johnson, a sponsor of the study, which provided GeneChem's both SL to this research lab for this mice study.

Reference

Traas and van Leeuwen, 2007. Chapter 7. Ecotoxicological effects. In: Risk assessment of chemicals (van Leeuwen CJ and Vermeire TG, Eds), 2nd edition. Springer, Dordrecht, The Netherlands. Pages 281-356.

d) Based on the corrections in 5a) and 5b), please correct your statement of "These animal efficacy studies mentioned above tested the efficacy and the safety of 3'-SL doses up to 1.2-1.4% of the diet for 6-7 weeks in mice (Boudry et al., 2017; Hamilton et al., 2017) or 5% of the diet for 2 weeks in mice (Tarr et al., 2015)."

Answer:
We have revised the statement as the following:
“These animal efficacy studies mentioned above tested the efficacy and the safety of 3'-SL doses up to 0.233-0.272% of the diet (which may correspond to 350 - 408 mg/kg bw/day) for 6-7 weeks (Boudry et al., 2017; Hamilton et al., 2017) and up to 5% of the diet (or 7,500 mg/kg bw/day) for 2 weeks in mice (Tarr et al., 2015).”

We also have revised Table 23 as shown below by removing ‘Amount of daily intake, not specified’ from the summary of studies by Hamilton et al. (2017) and Boudry et al. (2017). We also added the exact concentrations of 3’-SL and 6’-SL to the summaries of these two studies. In addition, we added a 3’-SL dose in mg/kg bw/d to the 2015 Tarr et al. study. The changes or additions are highlighted in yellow. No changes have been made to other studies.

Table 23. Summary of Animal Efficacy Studies (revised)

Objective	Animal	Dose	Duration	Measurements	Reference
To test whether SL could impact stressor-induced anxiety-like behavior, impact the effects of stressor exposure on brain cell proliferation and stability, and could prevent stressor-induced effect	Male mice, C57/BL6 (6-8 wk old, 9 per group)	3 Groups: 1) control diet (AIN-93G); 2) AIN-93G diet + 3'-SL (5% of diet or ~7,500 mg/kg bw/d); or 3) AIN-93G diet + 6'-SL (5% of diet)	2 wk	Body and spleen mass; serum concentrations of corticosterone and IL-6; fecal microbiota; brain cell proliferation and immature neuronal assessment and analyses	Tarr et al., 2015
To examine the effects of prebiotic BMO in presence of high fat diet in diet-induced obesity	Male C57BL/6 mice (4-week-old; 6 per group)	4 Groups: 1) control diet; 2) high fat (HF); 3) HF + 6% inulin; 4) HF + 6% BMO~~S~~ (source-whey, BMO~~S~~ contained 3.88% 3'-SL and 0.82% 6'-SL; or 3'-SL dose of ~350 mg/kg bw/d)	1, 3, or 6 wk	Fat pad analysis; plasma lipopolysaccharide-binding protein; histology analyses; luminal contents of cecum; fecal microbiota DNA and sequencing; microbiota bioinformatics analysis	Hamilton et al., 2017*
To demonstrate the effects of BMO and *B. infantis* on restoring diet-induced obesity intestinal microbiota and barrier function defects in mice	16 Male C57/BL6 (3 week-old)	3 Groups: 1) control diet, 7 wk; 2) Western diet, 7 wk; 3) Western diet 7 wk + 7% BMO~~S~~-last 2 wk (BMO~~S~~ contained 3.88% 3'-SL and 0.82% 6'-SL; 3'-SL dose ~ 408 mg/kg bw/d); *B. infantis* once a wk.	7 wk; BMO-last 2 wk only	Microbiota analysis; quantitative PCR for TNF- α on colon tissues; plasma biochemical analyses (leptin and lipopolysaccharide-binding protein levels)	Boudry et al., 2017*

To investigate the effect of SL on swimming learning behavior and brain lipid composition of adult rats	Male SD rats (8 wk old; 6 per group)	6 Groups: 1) Control; 2) 1% lactose; 3) 1% galactosyllactose; 4) 1% N-acetylneuraminic acid; 5) 1% SL; 6) 1% galactosylated N-acetylneruaminic acid	2 wk	Sialic acid content in serum; lipid content of brain; ganglioside content of brain	Sakai et al., 2006
To investigate HMO effects on intestinal function, bacterial colonization and necrotizing enterocolitis (NEC) resistance immediately after preterm birth	Preterm Pigs (gestation day 105-106)	2 Groups: 1) standard formula with 0.84 g/kg/d 25-HMO (providing 31.1 mg/kg/d 3'-SL and 30.2 mg/kg/d 6'-SL); 2) standard formula with maltodextrin	5 d	Clinical affection, feces, and hydration scores; NEC; organ weight; intestinal enzyme activities; colonic bacterial microbiota composition; inflammatory cytokines in middle small intestine and colon; plasma citrulline concentration	Rasmussen et al., 2017

*Estimated 3'-SL intakes were based on personal written communications with Professor Daniella Barile who indicated that the absolute amount of 3'-SL in BMOS was 3.88%. *B. infantis* = *Bifidobacterium longum* ssp. *infantis*; BMO = bovine milk oligosaccharides; bw = body weight; d = days; HMO = human milk oligosaccharides; IL = interleukin; SD = Sprague Dawley; SL = sialyllactose; TNF = tumor necrosis factor; wk = weeks; wt = weight. [red highlighted texts are irrelevant]

6) Pease provide the intake levels in the human infant formula studies in mg 3'-SL/kg bw/day to allow comparison to EDIs.

Answer: To answer this question, we have made the following approaches:

1) Using the 2013-2014 National Health and Nutrition Examination Survey (NHANES) dataset, we calculated the EDIs of infant formula, excluding amino acid- and hydrolyzed protein-based formulas, in all users of infant formula at various ages.
2) We considered the BMOS concentration of 8 g/L in reconstituted formula as specified in the studies by Cooper et al. (2017), Radke et al. (2017), and Simeoni et al. (2016).
3) We assumed that the 3'-SL concentration of BMOS was 3.88%, as clarified by Dr. Barile, for the studies by Hamilton et al. (2017) and Boudry et al. (2017).
4) We calculated the EDIs of 3'-SL in mg/kg bw/day based on the EDIs of infant formula expressed in g/kg bw/day, the BMOS concentration in reconstituted infant formula, and the SL concentration in BMOS.

For example, we estimated the EDI of the Cooper et al. (2017) study using the following formula:

1) The mean and 90th percentile EDIs of formula for infants aged 0-6 months in all users are 120,110 and 189,340 mg/kg bw/day, respectively.
2) The BMOS concentration is 8 g/L=8 g/1000 g.
3) The 3'SL concentration in BMOS is 3.88%.

The mean EDI of 3'-SL can be calculated as 120,110 mg/kg bw/d x 8 g/1000 g x 0.0388 = 37.28 mg/kg bw/day; the 90th percentile EDI of 3'-SL is 189,340 mg/kg bw/d x 8 g/1000 g x 0.0388 =58.77 mg/kg bw/day.

- 3'-SL consumption (mg/kg bw/day) = Food consumption x HMOs percentage in the diet x 3'-SL percentage in the BMOS by Dr. Barile

	Mean EDI	90th percentile EDI
Infant formula intake, mg/kg bw/day	120,110	189,340
BMOS in Reconstituted formula	8 g/1000 g	8 g/1000 g
3'SL concentration in BMOS	0.0388 g/g	0.0388 g/g
3'SL consumption, mg/kg bw/day	37.28	58.77

* 3'SL percentage in the BMOS by Dr Barile = 3.88%

Based on this calculation method, it is estimated that the 3'-SL doses in average consumers ranged from 15 to 43 mg/kg bw/day in the studies by Cooper et al. (2017), Radke et al. (2017), and Simeoli et al. (2016) (Table 1 for Question 6). In heavy consumers, 3'-SL intakes are estimated to be in the range of 43 - 69 mg/kg bw/day. The mean EDIs of 3'-SL from the proposed use in both infant formula and other foods and beverages in all infant formula users (30.1 - 40.1 mg/kg bw/day; Part 3, Table 16-2) are comparable to the estimated daily doses employed in these studies. The 90th percentile EDIs of 3'-SL from the proposed combined use in both infant formula and other foods and beverages in all infant formula users (30.1 - 56.6 mg/kg bw/day; Part 3) are comparable to or less than the estimated daily doses employed in these studies.

Table 1 for Question 6. EDIs of 3′-SL from the Proposed Use in Infant Formula Only in All-Infant Formula Users

Population group	EDI					
	Infant formula		BMOS		3'SL	
	Mean	90th Percentile	Mean	90th Percentile	Mean	90th Percentile
g/person/day						
0 - 3 months	759.68	1097.70	6.08	8.78	0.24	0.34
0 - 6 months	780.23	1209.57	6.24	9.68	0.24	0.38
0 - 12 months	761.57	1115.27	2.93	7.87	0.11	0.31
mg/kg bw/day						
0 - 3 months	136,530	221,450	1,092	1,772	42.37	68.74
0 - 6 months	120,110	189,340	960	1,510	37.28	58.77
0 - 12 months	50,130	140,550	400	1,120	15.52	43.46

Based on the 2013-2014 National Health and Nutrition Examination Survey (NHANES) dataset; bw = body weight.

Table 2 for Question 6. Summary of Estimated 3'-SL Intakes in Human Clinical Studies

Doses in the Test Group	Infant formula intake, mg/kg bw/d	Mean intake of 3'-SL	90th percentile intake of 3'-SL	Reference
Studies with Infants – Published Studies				
BMOS (total oligosaccharide concentration of 5.8% of powder formula [or 8.0 g/L in the reconstituted formula]) + *B. lactis* (1×10^7 cfu/g)	0-6 months; Mean – 120,110 90th percentile intake – 189,340	3'-SL ~37.2 mg/kg bw/d	3'-SL ~58.6 mg/kg bw/d	Cooper et al, 2017
BMOS (total oligosaccharide concentration of 5.8% of powder formula [or 8.0 g/L in the reconstituted formula]) + 1×10^7 cfu/g *B. lactis*; test	0-12 months; Mean – 50,130; 90th percentile intake -140,550	3'-SL~15.5 mg/kg bw/d	3'-SL ~43.5 mg/kg bw/d	Radke et al, 2017
BMOS (5.7% in powder or 8.0 g/L in reconstituted formula) + *B. lactis* CNCM I-3446 (10^7 cfu/g)	0-12 weeks or 0-3 months; Mean-136,530; 90th percentile-221,450	3'-SL ~42.3 mg/kg bw/d	3'-SL~68.7 mg/kg bw/d	Simeoni et al, 2016

Since the concentration of 3'-SL cannot be estimated in the study by Meli et al. (2014) and Fanaro et al., 2005, we did not include those studies in the calculation of estimated 3'-SL intakes.

In addition, we have revised No. 8 in the 6.H. Safety Determination, Part 6, as follows (changes are highlighted in yellow):

8. Infant formula studies of Cooper et al. (2017), Radke et al. (2017), and Simeoni et al. (2016) tested the safety and the efficacy of infant formulas supplemented with BMOS with probiotics for up to 12 months. These studies mentioned that the BMOS concentration in the reconstituted formula was 8 g/L and that BMOS contained 3'-SL and 6'-SL without specifying the content of these SLs. The study by Hamilton et al. (2017) also used BMOS containing 3.88% 3'-SL. Assuming the BMOS used in the infant formula studies mentioned above had a similar composition to that described in Hamilton et al. (2017), it is reasonable to assume that the infant formulas used in these infant studies contained 310 mg 3'-SL/L in reconstituted formula. Thus, it is reasonable to conclude that 3'-SL supplemented at concentrations of 230 mg/L in infant formula (ready-to-drink or reconstituted) is safe.

Chemistry Questions

7) The notice states that 3'-SL is intended for use in beverages. Please provide the stability data for the ingredient in water.

Answer:

Please see the report summarized below.

Stability of 3'-sialyllactose sodium salt in water

This study was designed to determine the stability of 3'-sialyllactose sodium salt in deionized (DI) water under condition of each temperature such as 4℃ (39.2°F), 25℃ (77°F), and 40℃ (104°F). The microcentrifuge tubes containing 3'-SL sodium salt in distilled water were closed tight with paraffin film but were not vacuum-sealed.

Results

The results show that 3'-SL sodium salt is very stable at 4℃ (39.2°F) and 25℃ (77°F) for 10 months. At an accelerated storage condition (40℃), the stability was reduced to 70% of the original concentration at the 5-month test point. It is expected that the shelf lives would be extended if the liquid would be vacuum-sealed, typical for commercial water-based products.

Table 1 for Question 7. Stability of 3′-Sialyllactose Sodium Salt in DI Water at Low Temperature (Storage condition: 4℃ or 39.2°F)

	Specification	Initial value	10day	1month	3month	5month	10month
Content (mg/L)	480~720	600.0	591.81	590.4	600.0	589.8	591.0
Appearance	Clear, Colorlessness	Complied	Complied	Complied	Complied	Complied	Complied
Oder	Odorless	Complied	Complied	Complied	Complied	Complied	Complied

Table 2 for Question 7. Stability of 3′-Sialyllactose Sodium Salt in DI Water at Room Temperature (Storage condition: 25℃ or 77°F)

	Specification	Initial value	10day	1month	3month	5month	10month
	480~720	600.0	593.94	596.28	577.11	562.59	558.03
	Clear, Colorlessness	Complied	Complied	Complied	Complied	Complied	Complied
	Odorless	Complied	Complied	Complied	Complied	Complied	Complied

Table 3 for Question 7. Stability of 3′-Sialyllactose Sodium Salt in DI Water at Accelerated Storage Condition (Storage condition: 40℃ or 104°F)

	Specification	Initial value	10day	1month	3month	5month	10month
Content (mg/L)	480~720	600.0	595.56	592.62	551.94	422.91	242.52
Appearance	Clear, Colorlessness	Complied	Complied	Complied	Complied	Complied	Complied
Oder	Odorless	Complied	Complied	Complied	Complied	Complied	Complied

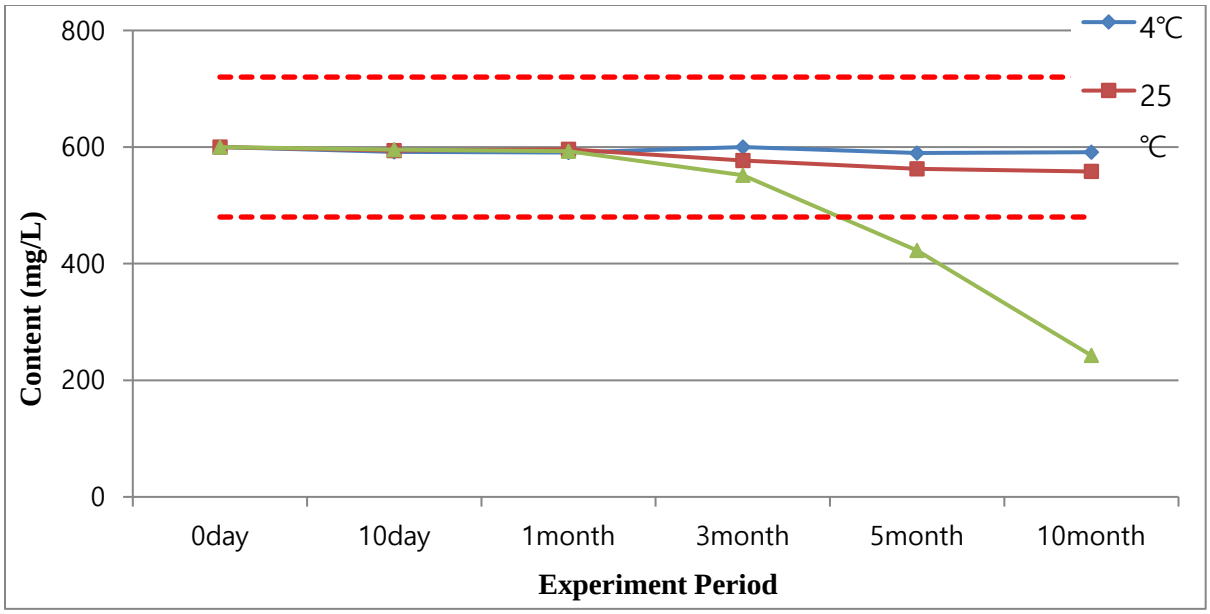


Figure 1. Stability Result for 3'-Sialyllactose Sodium Salt in Water at 4℃, 25℃, and 40℃.

Procedure

1. Dissolve 600 mg of dried 3′-sialyllactose sodium salt into 800 mL of DI water, and add DI water to make 1000 mL, making the final concentration 600 mg/L (600 ppm).
2. Prepare sterilized microcentrifuge tubes (2mL), add 1mL of 3′-sialyllactose sodium salt solution in each, and seal them with parafilm.
3. Place the microcentrifuge tubes containing 3′-sialyllactose sodium salt solution in each chamber of temperatures - 4℃, 25℃, and 40℃.
4. Three samples in 4℃, 25℃, or 40℃ chambers are periodically analyzed for 3′-sialyllactose sodium salt using High-Performance Anion-Exchange Chromatography.

Chromatography conditions

Metrohm 817 Bioscan system;
Column: Metrosep carB1 column (1190005S) with guard (1071.0015)
Flow: 1ml/min
Run time: 40min
Injection volume: 20µl
Detection: Pulsed Amperometric Detector (gold, 35146)
Eluent: 100mM NaOH / 50mM NaOAc

8) On page 6 of the notice, GeneChem states that "… 3'-SL sodium salt is intended for use in non-exempt infant formulas for term infants (milk-, soy, amino acid-, and hydrolyzed protein-based)." Amino acid and extensively hydrolyzed protein-based infant formulas are exempt infant formulas. Exempt infant formulas require a medical rationale for the composition of the infant formula. Please clarify whether the ingredient is intended for use in exempt infant formulas or non-exempt infant formulas for term infants

Answer:
The ingredient is intended for use in non-exempt infant formulas for term infants only.

In addition, we want to add the excel file showing Concentrations of SLs in BMOS, provided by professor Barile, to **7.B. Data and Information That Are Not Generally Available**.

We appreciate your kind attention to our responses to FDA questions. If you have further questions, please contact me.

Sincerely,

Susan

Susan Cho, Ph.D.
Nutrasource, Inc.
(301) 875-6454
Susanscho1@yahoo.com or
contact@nutrasource.center

Appendix A-1.

Email correspondences between Dr. Daniella Barile and Susan Cho, June 3-4, 2018

Re: conc. of 3SL and 6 SL in BMOS used in Hamilton 2017 and Boudry 2017 papers please3
Yahoo/Inbox
Susan Cho
Dear Daniella, We would appreciate it if you could dig out the exact conc of 3SL and 6SL in BMOS used in the above mentioned studies. Sorry to bother you. have a nice day!
Sincerely,
Susan
Susan Cho, Ph.D. NutraSource, Inc. 6309 Morning Dew Ct. Clarksville, MD 21029, USA +1-410-531-3336 (O) +1-301-875-6454 (C)
Jun 3 at 10:12 AM

Daniela Barile <dbarile@ucdavis.edu>
To: Susan Cho
Jun 3 at 9:25 PM
Hi Susan,

Sorry, I wasn't on my computer today - will look into your request right now and will have an answer hopefully by tonight.
Daniela
Show original message

Daniela Barile <dbarile@ucdavis.edu>
To: Susan Cho
Jun 3 at 11:59 PM
Dear Susan,
Attached is the excel file with the data you requested (concentration of 3SL and 6 SL in BMO used in Hamilton 2017 and Boudry 2017 papers).
I am also including our published paper that presents the methods we used to calculate the concentration of 3SL and 6 SL in BMO used in Hamilton 2017 and Boudry 2017 papers.

I don't know if, besides the absolute concentration, you are also interested in the ratio: so I want to point out that in our experience (see also paper attached), usually in enriched products 3SL is 5 to 9 times more concentrated than 6SL.

Hope this helps.
Let me know if you need more info and just remember that I am in California, so please expect some delay in my response as you are 3 hours ahead

Daniela Barile,
Professor of Food Chemistry,
University of California, Davis
Show original message
Download all attachments as a zip file

Quantification method BMO.pdf
354.4kB

Dionex quantification of BMO 1236 used in Hamilton et al 2017.xlsx
16.2kB

Appendix A-2.
Email correspondences between Dr. Daniella Barile and Susan Cho, July 17, 2017
(We also had phone conversations to clarify the email content)
Re: Hamilton 2017 study you coauthored
Yahoo/Inbox

Susan Cho
Do you have a breakdown of 3'-SL and 6'-SL? If not, I can analyze the sample if you can share with me some samples. Is it a commercially available BMOS from Hilamr? Susan
Jul 19, 2017 at 8:22 AM

Daniela Barile <dbarile@ucdavis.edu>
To: Susan Cho
Jul 19, 2017 at 12:04 PM
Susan,
I have those data: being of bovine origin, it's about 80% 3'-SL and 20% 6'-SL (the ratio is inverted compared to human milk) - but the prebiotic activity of the two isomers is the same

---
Hide original message
On Wed, Jul 19, 2017 at 5:22 AM, Susan Cho <sscho397@yahoo.com> wrote:
Do you have a breakdown of 3'-SL and 6'-SL? If not, I can analyze the sample if you can share with me some samples. Is it a commercially available BMOS from Hilamr?

Susan

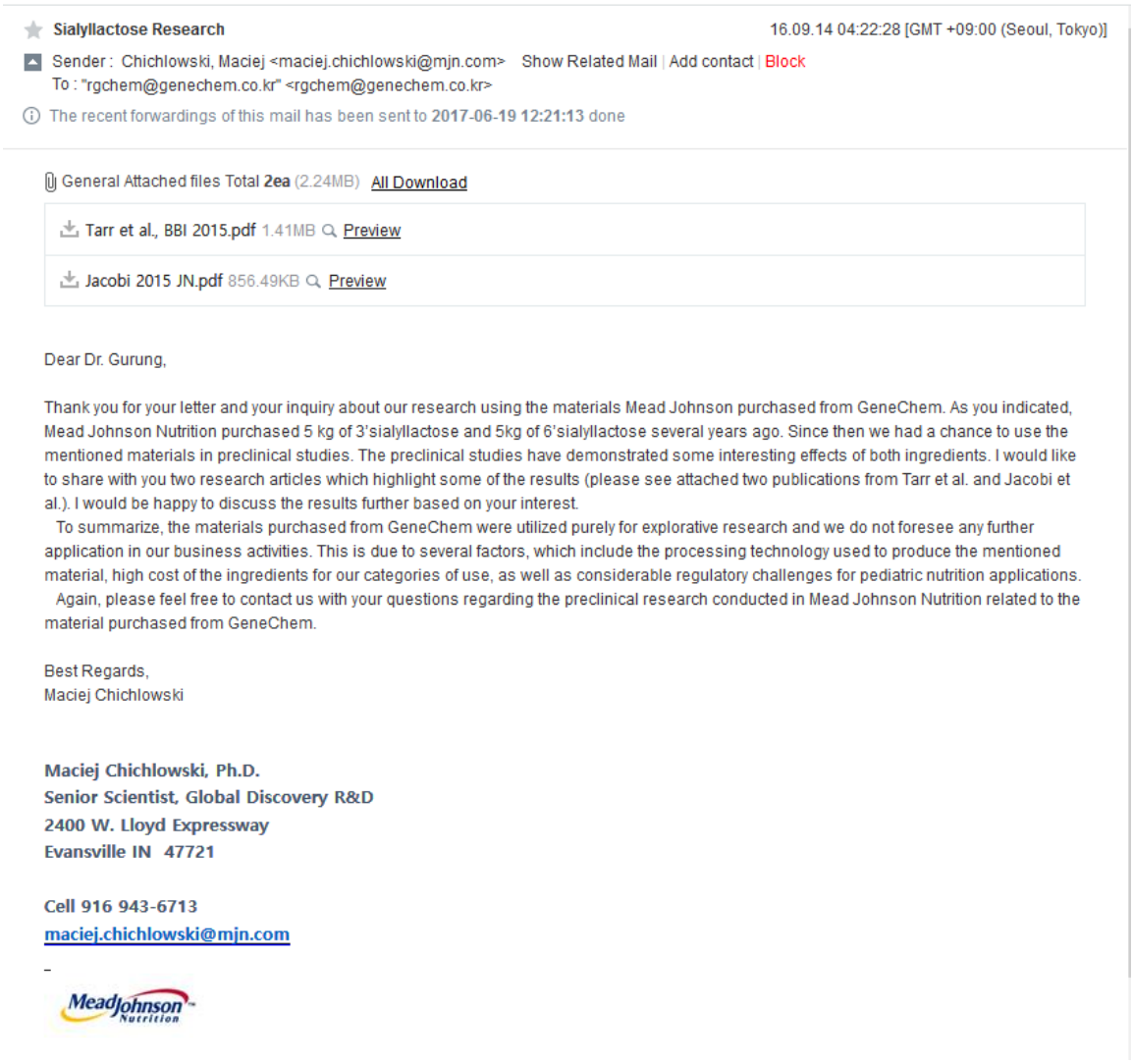
On Wednesday, July 19, 2017, 12:09:43 AM EDT, Daniela Barile <dbarile@ucdavis.edu> wrote:

As I mentioned, our concentrations are between 35% and 50% purity.
Daniela

On Tue, Jul 18, 2017 at 8:19 PM, Susan Cho <sscho397@yahoo.com> wrote:
Method section--Bovine milk oligosaccharides were obtained from purified bovine whey provided from Hilmar Ingredients (California, USA). Do you have the data for 3'-SL and 6'-SL conc?
Table 2 show the composition, but does not show conc of 3'-SL and 6'-SL. There might be some data. Thanks

Susan

Appendix B. E mail of Dr. Chicowski, Mead Johnson, a sponsor of studies, related to the source of 3'-SL for the study by Jacobi et al. (2016) and the study by Tarr et al. (2015)



Sialyllactose Research 16.09.14 04:22:28 [GMT +09:00 (Seoul, Tokyo)]

Sender : Chichlowski, Maciej <maciej.chichlowski@mjn.com> Show Related Mail | Add contact | Block
To : "rgchem@genechem.co.kr" <rgchem@genechem.co.kr>

The recent forwardings of this mail has been sent to **2017-06-19 12:21:13** done

General Attached files Total **2ea** (2.24MB) All Download

Tarr et al., BBI 2015.pdf 1.41MB Preview

Jacobi 2015 JN.pdf 856.49KB Preview

Dear Dr. Gurung,

Thank you for your letter and your inquiry about our research using the materials Mead Johnson purchased from GeneChem. As you indicated, Mead Johnson Nutrition purchased 5 kg of 3'sialyllactose and 5kg of 6'sialyllactose several years ago. Since then we had a chance to use the mentioned materials in preclinical studies. The preclinical studies have demonstrated some interesting effects of both ingredients. I would like to share with you two research articles which highlight some of the results (please see attached two publications from Tarr et al. and Jacobi et al.). I would be happy to discuss the results further based on your interest.

To summarize, the materials purchased from GeneChem were utilized purely for explorative research and we do not foresee any further application in our business activities. This is due to several factors, which include the processing technology used to produce the mentioned material, high cost of the ingredients for our categories of use, as well as considerable regulatory challenges for pediatric nutrition applications.

Again, please feel free to contact us with your questions regarding the preclinical research conducted in Mead Johnson Nutrition related to the material purchased from GeneChem.

Best Regards,
Maciej Chichlowski

Maciej Chichlowski, Ph.D.
Senior Scientist, Global Discovery R&D
2400 W. Lloyd Expressway
Evansville IN 47721

Cell 916 943-6713
maciej.chichlowski@mjn.com

-

Mead Johnson Nutrition

Fifty pages have been removed in accordance with copyright laws. The removed references are:

Monaco, “Safety evaluation of 3'-siallylactose sodium salt supplementation on growth and clinical parameters in neonatal piglets.”, Regul Toxicol Pharmacol. 2019 Feb;101:57-64. doi: 10.1016/j.yrtph.2018.11.008. Epub 2018 Nov 16.

Gurung, “Gastrointestinal tolerance and safety of 3’-sialyllactose in subjects positive with Helicobacter pylori: a pilot study”, EC Nutrition, September 10, 2018.

From:	Susan S Cho
To:	Honigfort, Mical
Subject:	GRN 766-2 emails (with titles in Korean) were forwarded to you
Date:	Friday, September 21, 2018 3:26:51 AM

Dear Dr. Honigfort,

I forwarded you 2 emails which have the titles in Korean. The mail correspondences between GenChem and third party certified analytical labs were written mostly in Korean. But key points were written in English.

The organization (NSF) which analyzed *Cronobacter sakazakii* for GeneChem referenced the FDA BAM Ch 29 and mentioned that the sample size was 60 g for *C.sakazakii*
Another institute (Dongjin Life Sci Research Institute) which analyzed Salmonella for GeneChem referenced the Korean FDA method and mentioned that the sample size was 25 g.
Hope it clarified the sample size issue. Have a nice weekend!

Sincerely,
Susan
Susan Cho, Ph.D.
NutraSource, Inc. 6309 Morning Dew Ct Clarksville, MD 21029 +1-410-531-3336 (O) +1-301-875-6454 (C)

From: Susan S Cho
To: Honigfort, Mical
Subject: Fw: [FW][FW]동진생명연구원 _ 전달
Date: Friday, September 21, 2018 3:09:44 AM
Attachments: Test for Salmonella & Cronobacter sakazakii.docx
0839872001537507542
0839872001537507542

A third party lab which provided Salmonella analysis to GeneChem responded as follows--please see an e mail from js58@nate.com (Dongjin Life Science Research Institute)

'Salmonella assay is analyzed according to Korean Food Standards Codex 7/4 / 4.11 Salmonella, and the amount of test sample is 25g.'

Thank you. Have a nice weekend!

Sincerely,
Susan
Susan Cho, Ph.D. NutraSource, Inc. 6309 Morning Dew Ct Clarksville, MD 21029 +1-410-531-3336 (O) +1-301-875-6454 (C)

----- Forwarded Message -----
From: Gurung Rit B. <rgchem@genechem.co.kr>
To: Dr. Susan S Cho <susanscho1@yahoo.com>
Cc: Dr. Daehee Kim <daeheekim@genechem.co.kr>; Dr Jinsuk Woo <jwoo@genechem.co.kr>
Sent: Friday, September 21, 2018 01:25:54 AM EDT
Subject: [FW][FW]동진생명연구원 _ 전달

Dear Dr. Cho,

We are sending one more email that contains information on *Salmonella* test.

We performed *Salmonella* analysis at Dongjin, and **25 g** sample has been used for analysis which showed absence of *Salmonella* in the test sample.

In addition, attached file also contains detail information of *Salmonella* and *Cronobacter sakazakii* analyses and the official emails from corresponding labs.

Please feel free to contact us if you need any further information.

Thank you very much for your support and cooperation.

Sincerely,

Rit

Rit B. GURUNG, Ph.D.

.....

Director

International Marketing

KS Q ISO 9001:2015/ISO 9001:2015 Certified Company
(Leading Company in *Sialyl-oligosaccharides* Production & *Bioactive Molecule* Glycosylation)

Migun Techno World II, A-201

187 Techno 2-ro, Yongsan-dong,

34025 Yuseong-gu, Daejeon

Republic of Korea.

Tel.: + 82 - 42 - 716 - 0998

Fax: + 82 - 70 - 8280 - 2282

www.genechem.co.kr

Address (Korean): (34025) 대전광역시 유성구 테크노**2**로 **187,** 미건테크노월드 **2**차 **A**동 **201**호

----- Original Message -----
From : 김대희 <daeheekim@genechem.co.kr>
To : "Gurung Rit B." <rgchem@genechem.co.kr>
Cc : "우진석" <jwoo@genechem.co.kr>
Sent : 2018-09-21 14:00:31
Subject : [FW]동진생명연구원 _ 전달

Dr Gurung,

Please find attached the file and check, and than send to Dr cho.

It's mean, We can find analytical methods of the Korea standards codex in KFDA.

However, in order to double-check, we asked the third party lab and got an answer.

Thank you, Have a wanderfull Chuseok.
Dae Hee

Leading Company in Sialylloligosaccharides Production & Bioactive Molecule Glycosylation

Dae Hee Kim, Ph.D.
Managing Director

A-201, 187, Techno 2-ro, Yuseong-Gu, Daejeon 34025
Rep. of Korea
TEL : 82-42-716-0998 (211) FAX : 82-70-8280-2282
H.P : 82-10-4411-4422
E-mail : daeheekim@genechem.co.kr

----- Original Message -----
From : 박희진 <js58@nate.com>
To : <daeheekim@genechem.co.kr>
Cc :
Sent : 2018-09-21 13:24:35
Subject : 동진생명연구원

Salmonella assay is analyzed according to Korean Food Standards Codex 7/4 / 4.11 Salmonella, and the amount of test sample is 25g.

(주)동진생명연구원

TEL:055-293-5440~2(내선 214번)

FAX:055-293-6980

경남 창원시 의창구 차룡로 48번길 61

<Test for Salmonella>

1. Method : Korea Food Standards Codex

- http://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=378

식품의약품안전처 Ministry of Food and Drug Safety 식품 및 식품첨가물공전

식품공전 | 농약잔류 허용기준 | 식품유형별 기준규격 | 식품첨가물공전 | 기구 및 용기포장 공전

▶ 제 7. 일반시험법 ▶ 4. 미생물시험법 ▶ 4.11 살모넬라(Salmonella spp.)

Add 225ml of Buffered Peptone Water to 25g(ml) of the sample

4.11 살모넬라(Salmonella spp.)

가. 증균배양

1) 식품 및 식육: 시료 25 mL(g)에 225 mL의 펩톤식염완충액(Buffered Peptone Water)을 첨가하여 36±1℃에서 18~24시간 배양한 후 이 배양액을 2종류의 증균배지, 즉 10 mL의 Tetrathionate 배지(배지 87)에 1 mL를 첨가함과 동시에 10 mL의 RV 배지(배지 57) 또는 RVS 배지(배지 88)에 0.1 mL를 첨가하여 각각 36±1℃(Tetrathionate 배지) 및 42±0.5℃(RV 배지 또는 RVS 배지)에서 20~24시간 동안 증균배양한다.

2) 소, 돼지도체: 제7. 일반시험법 5. 원유·식육·식용란의 시험법 5.2. 식육 시험법 5.2.3. 세균학적 시험법 가. 시료채취 및 방법 1) 도체 가)소 및 나)돼지의 시료채취 방법에 따라 멸균가아제나 스폰지로 시료를 채취한 후 멸균백에 넣고 50 mL BPW를 넣은 다음 균질화 시킨 후 36±1℃에서 18~24시간 배양한다. 1차 배양액은 1) 축산물가공품 및 식육의 2차 증균과정을 따라 증균시킨다.

3) 닭, 오리도체: 제7. 일반시험법 5. 원유·식육·식용란의 시험법 5.2. 식육 시험법 5.2.3. 세균학적 시험법 가. 시료채취 및 방법 1) 도체 다)닭의 시료 채취방법에 따라 채취한 시료 30 mL를 취하여 30 mL BPW에 넣은 다음 균질화 시키고 36±1℃에서 18~24시간 배양한다. 1차 배양액은 1) 축산물가공품 및 식육의 2차 증균과정을 따라 증균시킨다.

4) 식용란: 식용란 20개를 채취하여 제7. 일반시험법 5. 원유·식육·식용란의 시험법 5.3. 식용란의 시험법 5.3.3. 세균학적 시험법 가. 시료채취 및 조제에 따라 소독 한 후 말린 식용란을 깨서 4L 용량의 멸균비이커 또는 멸균비닐백 등 적정한 용량의 멸균용기에 넣어서 준비한 다음(달걀을 깰 때는 위생장갑을 껴야하며 샘플마다 위생장갑을 바꾸어준다.) 멸균 도구 등을 이용하여 난황과 난백이 섞이도록 균질화를 시킨다. 준비된 시료에 2L의 멸균 TSB를 섞어 35℃에서 24±2시간 동안 증균한다. 1차 배양액은 1) 축산물가공품 및 식육의 2차 증균과정을 따라 증균시킨다.

나. 분리배양

각각의 증균배양액을 XLD Agar(배지 58) 및 BG Sulfa 한천배지(배지 90)[Bismuth Sulfite 한천배지(배지 64), Desoxycholate Citrate 한천배지(배지 31), HE 한천배지(배지 91), XLT4 한천배지(배지 92)]에 도말한 후 36±1℃에서 20~24시간 배양한다. 의심집락은 5개 이상 취하여 확인시험을 실시한다.

다. 확인시험

1) 생화학적 확인시험

의심스러운 집락에 대해 TSI Agar(배지 32) 또는 LIA 사면배지(배지 93)에 천자하여 37±1℃에서 20~24시간 배양한다. TSI 및 LIA 검사결과 살모넬라균으로 추정되는 균에 대해서는 그람 음성의 간균임을 확인하고, Indol(-), MR(+), VP(-), Citrate(+), Urease(-), Lysine(+), KCN(-), malonate(-) 시험등의 생화학적 검사를 실시하여 살모넬라 양성유무를 판정한다.

2) 응집시험

균종 확인이 필요한 경우 살모넬라진단용 항혈청을 사용한 응집반응 결과에 따라 균종을 결정한다. 먼저 살모넬라 O혼합혈청 시험으로서 다가 O항혈청을 사용하여 슬라이드 응집반응검사를 실시한 후 살모넬라 O인자 혈청시험 즉 A, B, C, D, E군 등의 인자 항혈청으로 슬라이드 응집반응을 실시하여 O혈청형을 결정한다. H인자 혈청시험은 편모(H)항혈청 즉 a, b, c, d, e, h, g, k, l, r, y, 1,2, 1,3, 1,5, 1,6 등에 대해 시험관 응집반응을 실시하여 결정한다.

닫기

모든 정보는 반드시 일치하지 않을 수 있으므로 참고자료로 활용하시기 바라며,
정확한 내용 및 시행일자는 식약처 홈페이지(www.mfds.go.kr) 법령자료실에서 확인하시기 바랍니다.

2. Email of Third party Lab : Dongjin Institute of Technology co., LTD

동진생명연구원 18.09.21 13:24:35 [GMT +09:00 (서울, 도쿄)]

보낸사람 : 박희진 <js58@nate.com> 연관 메일 보기

받는사람 : <daeheekim@genechem.co.kr>

최근 이 메일에 대한 답장을 **2018-09-21 13:33:59** 에 하셨습니다.

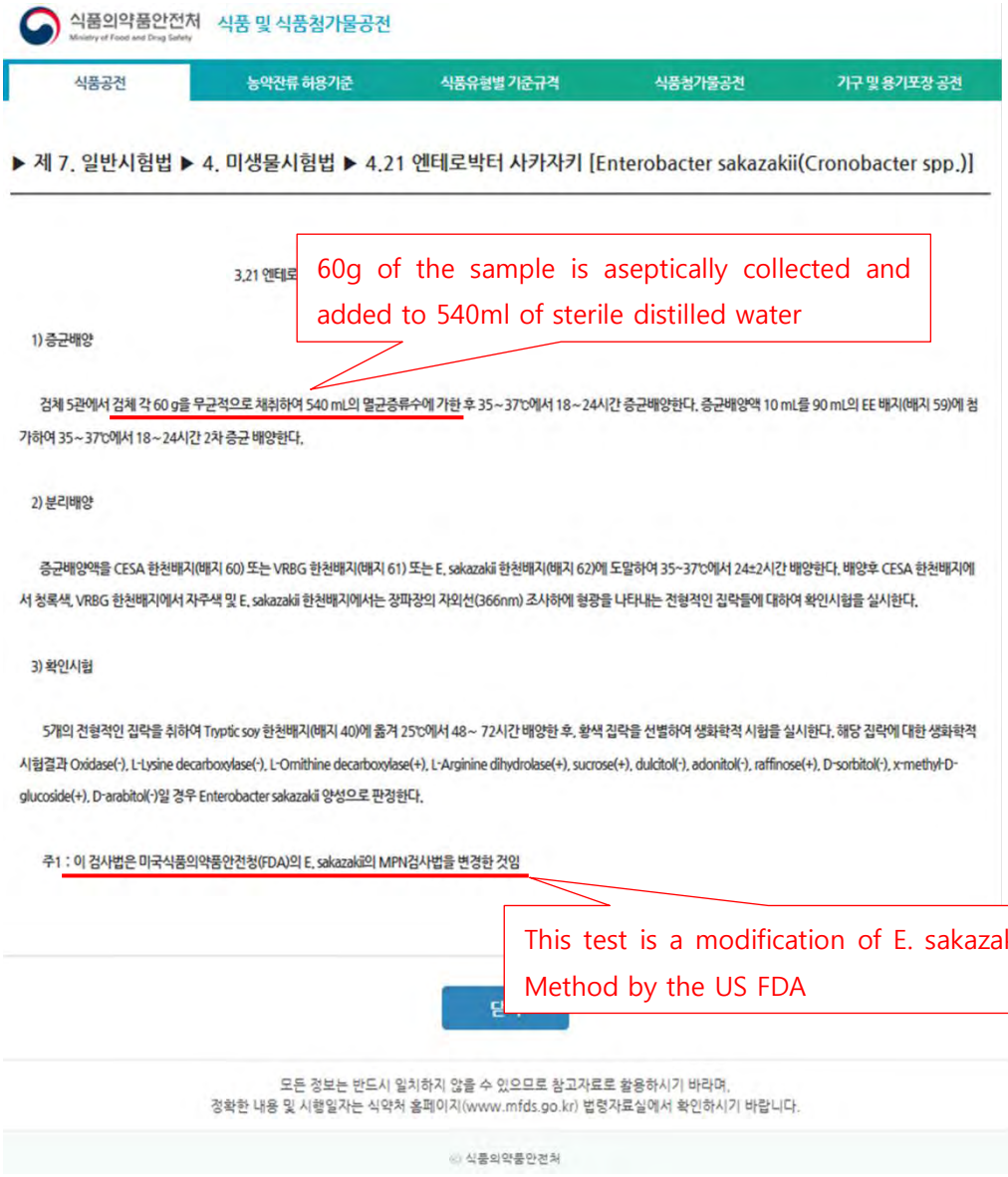
Salmonella assay is analyzed according to Korean Food Standards Codex 7/4 / 4.11 Salmonella, and the amount of test sample is 25g.

(주)동진생명연구원
TEL:055-293-5440~2(내선 214번)
FAX:055-293-6980
경남 창원시 의창구 차룡로 48번길 61

<Test for Cronobacter sakazakii>

1. Method : Korea Food Standards Codex

- http://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=394



- Reference : FDA BAM링크 및 위치: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm289378.htm (Bacteriological Analytical Manual Chapter 29 *Cronobacter,* Method, G. optional: enumeration of *Cronobacter)*

2. Email of Third party Lab : NSF Korea LLC

RE: [FW]분석방법 관련 문의_(주)진켐 18.09.21 11:03:50 [GMT +09:00 (서울, 도쿄)]

보낸사람 : Lee, A-Leum <allee@nsf.org> 연관 메일 보기 | 주소 등록 | 수신차단
받는사람 : "daeheekim@genechem.co.kr" <daeheekim@genechem.co.kr>

최근 이 메일에 대한 답장을 2018-09-21 11:37:33 에 하셨습니다.

일반 첨부파일 총 1건 (828.29KB) 전체 다운로드

ISO22964(2017-04).pdf 828.29KB 미리보기

안녕하세요. 김대희 선생님,

NSF Korea 이아름 입니다.
말씀 주신 내용에 대해 회신 드립니다.
관련 내용은 하기와 같으며, 확인해 보시고 문의사항 있으시면 회신 주세요.

1. 엔테로박터 사카자키 실험에 사용된 검체량? (ex, 외국 업체 제출 서류에는 Absent in 10g or 100g)
식품의 경우 검체 5관 각 60 g, 축산물의 경우 검체 1관 당 60 g 입니다. 저희는 시료량이 부족하여 검체 1관 당 60 g을 사용하였습니다.
2. 실험 방법은 식품공전에 따라서 하신 것으로 알고 있습니다. 유사한 ISO or AOAC method No.를 알수 있을까요?
ISO 시험법 첨부 드렸습니다. 그렇지만 식품공전 시험법과 많이 달라서 레퍼런스로 사용하시기는 어려울 것 같습니다.
3. 두가지 답변을 영어로 회신해주실 수 있으실까요? 간단하게 표기해주시면 될 것 같습니다. (ex, 1. Unit : , 2. Method No. :) 표기 방법이 맞는지 모르겠습니다.
Unit: Negative/60 g (외국업체 제출서류의 표기법과 같이 absent in 60 g 도 무방합니다)
Method: Korea Food Code 7. General Test Method 4. Microbiology Test 7.4.21 Enterobacter sakazakii(Cronobacter spp.) (This test is a quote from MPN test of the FDA E. sakazakii)

4. 참고자료
FDA BAM링크 및 위치: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm289378.htm (Bacteriological Analytical Manual Chapter 29 *Cronobacter*, Method, G. optional: enumeration of *Cronobacter)*

감사합니다.
이아름 드림

A-Leum Lee | Technical Manager, Tech-Testing - Korea | Tel: +82.2.415.8470 | Fax: +82.2.511.8305 | Email: allee@nsf.org
NSF International | CJ food safety Hall B/D 4~5th Fl, Korea University, 145Anam-ro | Seongbuk-gu | Seoul | Korea | www.nsf.org
www.nsfkorea.org

From: Susan S Cho
To: Honigfort, Mical
Subject: Fw: [FW][FW]RE: [FW]분석방법 관련 문의_(주)진켐
Date: Friday, September 21, 2018 3:00:03 AM
Attachments: ISO22964(2017-04).pdf
069406300153749864 8
069406300153749864 8
077774160015374986 48
085670700153749864 8
093598800153749864 8

Dear Dr. Honigfort,

GneChem requested a third party certified lab, NSF, which provided the analysis for GeneChem about the sample size. NSF referenced https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm289378.htm (Bacteriological Analytical Manual Chapter 29 *Cronobacter*, Method, G. optional: enumeration of *Cronobacter)* and mentioned that the sample size was 60 g for *Enterobacter sakazakii*. Please see e mail correspondences--Please see No 4. of the email response from Lee A-Leum <allee@nsf.org>, referencing the FDA website -BAM. (Sorry, most of their e mail correspondences were in Korean).

In summary, GeneChem's 3'-SL was negative for *Enterobacter sakazakii* in 60 g sample. Thank you. Have a nice weekend!

Sincerely,
Susan
Susan Cho, Ph.D. NutraSource, Inc. 6309 Morning Dew Ct Clarksville, MD 21029 +1-410-531-3336 (O) +1-301-875-6454 (C)

----- Forwarded Message -----
From: Gurung Rit B. <rgchem@genechem.co.kr>
To: Dr. Susan S Cho <susanscho1@yahoo.com>
Cc: Dr. Daehee Kim <daeheekim@genechem.co.kr>; Dr Jinsuk Woo <jwoo@genechem.co.kr>
Sent: Thursday, September 20, 2018 10:57:39 PM EDT
Subject: [FW][FW]RE: [FW]분석방법 관련 문의_(주)진켐

Dear Dr. Cho,

NSF Korea responded to our query regarding *Enterobacter sakazakii*.

This analysis was conducted at NSF Korea.

According to them, ***E. sakazakii* is absent in 60 g**.

Please scroll down this email for the official email from NSF Korea.

Should there be any further information required, please feel free to contact us.

Sincerely,

Rit

Rit B. GURUNG, Ph.D.

.....

Director

International Marketing

KS Q ISO 9001:2015/ISO 9001:2015 Certified Company
(Leading Company in *Sialyl-oligosaccharides* Production & *Bioactive Molecule* Glycosylation)

Migun Techno World II, A-201

187 Techno 2-ro, Yongsan-dong,

34025 Yuseong-gu, Daejeon

Republic of Korea.

Tel.: + 82 - 42 - 716 - 0998

Fax: + 82 - 70 - 8280 - 2282

www.genechem.co.kr

Address (Korean): (34025) 대전광역시 유성구 테크노**2**로 **187,** 미건테크노월드 **2**차 **A**동 **201**호

----- Original Message -----
From : 김대희 <daeheekim@genechem.co.kr>
To : "Gurung Rit B." <rgchem@genechem.co.kr>
Cc : "우진석" <jwoo@genechem.co.kr>
Sent : 2018-09-21 11:28:00
Subject : [FW]RE: [FW]분석방법 관련 문의_(주)진켐

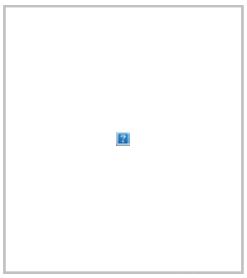
Dr Gurung,

I receved NSF's reply.

Please Check the email below.

Thank you

Dae Hee



Leading Company in Sialylloligosaccharides Production & Bioactive Molecule Glycosylation

Dae Hee Kim, Ph.D.
Managing Director

A-201, 187, Techno 2-ro, Yuseong-Gu, Daejeon 34025
Rep. of Korea
TEL : 82-42-716-0998 (211) FAX : 82-70-8280-2282
H.P : 82-10-4411-4422
E-mail : daeheekim@genechem.co.kr

----- Original Message -----
From : Lee A-Leum <allee@nsf.org>
To : "daeheekim@genechem.co.kr" <daeheekim@genechem.co.kr>
Cc :
Sent : 2018-09-21 11:03:50
Subject : RE: [FW]분석방법 관련 문의_(주)진켐

안녕하세요. 김대희 선생님,

NSF Korea 이아름 입니다.

말씀 주신 내용에 대해 회신 드립니다.

관련 내용은 하기와 같으며, 확인해 보시고 문의사항 있으시면 회신 주세요.

1. 엔테로박터 사카자키 실험에 사용된 검체량? (ex, 외국 업체 제출 서류에는 Absent in 10g or 100g)

식품의 경우 검체 5관 각 60 g, 축산물의 경우 검체 1관 당 60 g 입니다. 저희는 시료량이 부족하여 검체 1관 당 60 g을 사용하였습니다.

2. 실험 방법은 식품공전에 따라서 하신 것으로 알고 있습니다. 유사한 ISO or AOAC method No.를 알수 있을까요?

ISO 시험법 첨부 드렸습니다. 그렇지만 식품공전 시험법과 많이 달라서 레퍼런스로 사용하시기는 어려울 것 같습니다.

3. 두가지 답변을 영어로 회신해주실 수 있으실까요? 간단하게 표기해주시면 될 것 같습니다. (ex, 1. Unit : , 2. Method No. :) 표기 방법이 맞는지 모르겠습니다.

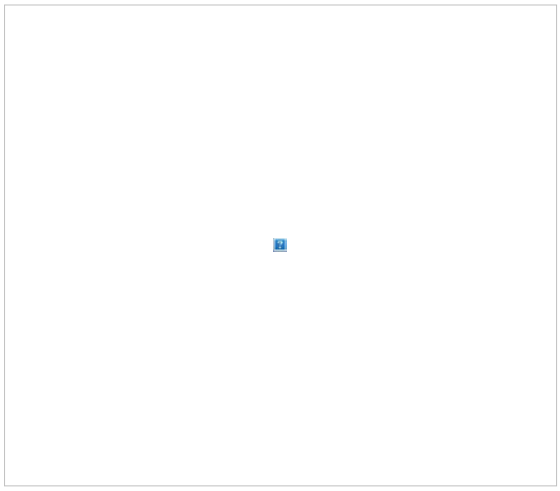
Unit: Negative/60 g (외국업체 제출서류의 표기법과 같이 absent in 60 g 도 무방합니다)

Method: Korea Food Code 7. General Test Method 4. Microbiology Test 7.4.21 Enterobacter sakazakii(Cronobacter spp.) (This test is a quote from MPN test of the FDA E. sakazakii)

4. 참고자료

FDA BAM링크 및 위치: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm289378.htm (Bacteriological Analytical Manual Chapter 29 ***Cronobacter,*** Method, G. optional: enumeration of ***Cronobacter)***

식품공전:



감사합니다.

이아름 드림

A-Leum Lee | Technical Manager, Tech-Testing - Korea | Tel: +82.2.415.8470 | Fax: +82.2.511.8305 | Email: allee@nsf.org

NSF International | CJ food safety Hall B/D 4~5th Fl, Korea University, 145Anam-ro | Seongbuk-gu | Seoul | Korea | www.nsf.org www.nsfkorea.org

From: 김대희 <daeheekim@genechem.co.kr>
Sent: Friday, September 21, 2018 10:08 AM

To: Lee, A-Leum <allee@nsf.org>
Cc: Cha, Seungjin (Jacob) <scha@nsf.org>; Nam, Jonghyun <jnam@nsf.org>
Subject: [FW]분석방법 관련 문의_(주)진켐

이아름 대리님,

안녕하세요. 저는 진켐에 김대희입니다.

저희가 엔테로박터 사카자키 미생물 분석등을 의뢰드렸었는데요.

분석 관련하여 저희가 급하게 문의들일게 있습니다.

미국 FDA에서 질문을 받았습니다.

아래 메일 참고부탁드립니다.

Cronobacter sakazakii(엔테로박터 사카자키) 실험에 사용되는 검체량이 얼마인가요?

저희가 받은 성적서에는 Unit이 없습니다.

급한 사한입니다. 확인부탁드립니다.

급하게 연락드리다보니 정신이 없습니다.

1. 엔테로박서 사카자키 실험에 사용된 검체량? (ex, 외국 업체 제출 서류에는 Absent in 10g or 100g)

2. 실험 방법은 식품공전에 따라서 하신 것으로 알고 있습니다.

   유사한 ISO or AOAC method No.를 알수 있을가요?

3. 두가지 답변을 영어로 회신해주실 수 있으실가요?

   간단하게 표기해주시면 될 것 같습니다. (ex, 1. Unit : , 2. Method No. :) 표기 방법이 맞는지 모르겠습니다.

메일로 회신 부탁드립니다.

감사합니다.

김대희 드림

Leading Company in Sialylloligosaccharides Production & Bioactive Molecule Glycosylation

Dae Hee Kim, Ph.D.

Managing Director

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Rep. of Korea

TEL : 82-42-716-0998 (211) FAX : 82-70-8280-2282

H.P : 82-10-4411-4422

E-mail : daeheekim@genechem.co.kr

----- Original Message -----
From : Susan S Cho <susanscho1@yahoo.com>
To : "Gurung Rit B." <rgchem@genechem.co.kr>, "Dae Hee Kim" <daeheekim@genechem.co.kr>, "Woo Jin Suk" <jwoo@genechem.co.kr>
Cc :
Sent : 2018-09-20 22:41:47
Subject : GRN 766 final q from FDA

Hello Dr. Cho,

I'm assisting with the final stages of GRN 000766 (3'-sialyllactose sodium salt) and the team has one clarifying question for completeness. Could you please confirm the sample sizes for the tests for *Salmonella* and *Cronobacter sakazaki*? If you could provide this information by COB tomorrow (September 21, 2018), we would greatly appreciate it.

Regards,

Mical Honigfort

Mical Honigfort, PhD
Supervisory Consumer Safety Officer

INTERNATIONAL STANDARD

ISO 22964

First edition
2017-04

Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche de Cronobacter *spp.*

Reference number
ISO 22964:2017(E)

Ten pages have been removed in accordance with copyright laws. The removed reference is:

International Organization for Standardization, “Microbiology of the food chain -- Horizontal method for the detection of Cronobacter spp.”, 2017-04, ISO 22964:2017