AIBMR Life Sciences, Inc.

January 19, 2021

Susan Carlson, PhD
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
Department of Health and Human Services
5001 Campus Drive
College Park, MD 20740

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of Phynova Group Limited (the notifier), the undersigned, Timothy Murbach, submits, for FDA review, the enclosed notice that Reducose® 5% is GRAS for use in foods.

Should you have any questions or concerns regarding this notice, please contact me at 253-286-2888 or tim@aibmr.com.

Sincerely,

Timothy Murbach, ND, DABT (agent of the notifier)
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc. ("AIBMR")

Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Reducose® 5% is Generally Recognized as Safe

Submitted by the Notifier:

Phynova Group Limited

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc
1425 Broadway, Suite 458
Seattle WA 98122

January 19, 2021

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Figures and Tables

Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Phynova Group Limited (the notifier) is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that Reducose® 5% (white mulberry leaf extract (*Morus alba* L.) standardized to 5% 1-deoxynojirimycin) is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

1.2 Name and Address of the Notifier and Agent of the Notifier

Notifier

Robert Miller
Chief Executive Officer
Phynova Group Limited
16 Fenlock Court
Blenheim Office Park
Long Hanborough, OX29 8LN
UK

Agent of the Notifier

Timothy Murbach
Senior Scientific & Regulatory Consultant
AIBMR Life Sciences, Inc.
1425 Broadway, Suite 458
Seattle WA 98122
Tel: (253) 286-2888
tim@aibmr.com

1.3 Name of the Substance

White mulberry (*Morus alba* Linn) leaf extract standardized to 5% 1-deoxynojirimycin (DNJ)

Trade name: Reducose® 5%

1.4 Intended Conditions of Use

Reducose® 5% is intended to be used as an ingredient in the food categories and at the addition levels shown in Table 1. Reducose® 5% is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

Table 1. Intended use of Reducose® 5%

NHANES Food Category	NHANES Category Code	Serving Size (g or mL)	Addition level (mg/g)			~Maximum amount per serving (mg)
			Minimum	Median	Maximum	
Bars	537	40	3.1	5	6.2	248
Low sodium crackers	542	30	4.2	6.7	8.4	252
Nonsweet crackers	543	30	4.2	6.7	8.4	252
Salty snacks from grain products	544	30	4.2	6.7	8.4	252
Oat breads	515	50	2.5	4	5	250
Cornbread, corn muffins, tortillas	522	55	2.27	3.6	4.5	248
Flour-milk dumplings, plain	556	30	4.2	6.7	8.4	252
Flour-water patties	555	30	4.2	6.7	8.4	252
Bread, rolls (not further specified)	510	50	2.5	4	5	250
Biscuits	521	55	2.27	3.6	4.5	248
Mixtures, mainly grain, pasta or bread	581 and 582	50	2.5	4	5	250
Multigrain breads, rolls	516	50	2.5	4	5	250
Other breads	518	50	2.5	4	5	250
Wheat, cracked wheat breads, rolls	513	50	2.5	4	5	250
Other quick breads	524	50	2.5	4	5	250
Pastas	561	140	0.9	1.4	1.8	252
Rye bread, rolls	514	50	2.5	4	5	250
White breads, rolls	511	50	2.5	4	5	250
Coffee	921	240	0.5	0.8	1	240
Citrus fruit juices	612	240	0.5	0.8	1	240
Energy drinks	9531	240	0.5	0.8	1	240
Sports drinks	9532	240	0.5	0.8	1	240
Other functional beverages	9534	240	0.5	0.8	1	240
Tea	923	240	0.5	0.8	1	240
Water, bottled, fortified	942	240	0.5	0.8	1	240
Fruit drinks	925	240	0.5	0.8	1	240

Beverage concentrates, dry, not reconstituted	929	5	5	12.5	50	250
Nutrition drinks (or powders to be reconstituted to drinks)	951	240	0.5	0.8	1	240
Cakes	531	140	0.9	1.4	1.8	252
Candies	917	40	3.1	5	6.2	248
Cookies	532	30	4.2	6.7	8.4	252
Cobblers, éclairs, turnovers, other pastries	534	55	2.27	3.6	4.5	248
Other muffins, popovers	523	55	2.27	3.6	4.5	248
Pies (fruit, tart, cream, custard, miscellaneous pies, pie shells)	533	55	2.27	3.6	4.5	248
Sugar and sugar substitute blends	911	4	15	25	30	120
Sweet crackers	541	30	4.2	6.7	8.4	252
Jellies, jams, preserves	914	15	6.7	8.3	10	150
Danish, breakfast pastries, doughnuts	535	55	2.27	3.6	4.5	248
Cereal grains, not cooked	576	55	2.27	3.6	4.5	248
Ready to eat cereals	571–574	55	2.27	3.6	4.5	248
Cooked cereals, rice	562	55	2.27	3.6	4.5	248
Pancakes	551	110	1.14	1.8	2.25	248
Waffles	552	85	1.47	2.35	2.9	247
Flavored milk and milk drinks, fluid	115	240	0.5	0.8	1	240
Yogurt	114	225	0.56	0.89	1.1	248
Puddings, custards, and other milk desserts	132	120	1	1.67	2	240
Tomato sauces	744	30	4.2	6.7	8.4	252
Potato recipes	717	70	1.8	2.9	3.6	252
Potato soups	718	245	0.51	0.82	1.2	294
White potatoes, chips and sticks	712	70	1.8	2.9	3.6	252
Dark-green vegetable soups	723	245	0.51	0.82	1.2	294
Deep-yellow vegetable soups	735	245	0.51	0.82	1.2	294
Frozen plate meals with grain mixture as major ingredient	583	195	0.64	1	1.3	254
Other cooked vegetables, cooked with sauces, batters, casseroles	754	240	0.5	0.8	1	240
Soups with grain product as major ingredient	584	245	0.51	0.82	1.2	294

1.5 Statutory Basis for GRAS Conclusion

The conclusion of GRAS status of Reducose® 5% for its intended conditions of use, stated in Part 1.4 of this notice, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

We have concluded that Reducose® 5% is GRAS for its intended conditions of use, stated in Part 1.4 of this notice, and, therefore, such use of Reducose® 5% is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of Robert Miller at:

Phynova Group Limited
16 Fenlock Court
Blenheim Office Park
Long Hanborough, OX29 8LN
UK

or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

None of the data and information in Parts 2 through 7 of this GRAS notice are considered exempt from disclosure under the Freedom of Information Act (FOIA) as trade secret or commercial or financial information that is privileged or confidential.

1.9 Certification of Completion

We hereby certify that, to the best of our knowledge, this 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 Reducose® 5%.

[redacted]

January 19, 2021

Robert Miller
Chief Executive Officer
Notifier

Date

Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

Reducose® 5% is an iminosugar-rich extract of white mulberry (*Morus alba* L.) leaves that is standardized to a concentration of 5% 1-deoxynojirimycin (DNJ). The major components of Reducose® 5% are listed in Table 2.

Table 2: Composition of Reducose® 5%

Chemical Class	Percent Composition
Total Iminosugars	7–8%
DNJ	4.5–5.5%
Free amino acids/peptides/proteins	25-35%
Minerals/salts	3-5%
Total carbohydrates	30-55%%
Maltodextrin	28–50% (used for standardization)

Morus alba L. (common name white mulberry) is a small deciduous tree belonging to the *Moreae* tribe of the *Moraceae* (common name mulberry) family of flowering plants. The genus *Morus* is comprised of 14 currently accepted species (as well as various hybrids). *M. alba* is native to China, where it is also cultivated, and has become naturalized, as well as being cultivated, throughout the temperate world.

M. alba leaves are rich in carbohydrates and protein, as well as many vitamins and minerals such as beta-carotene, iron, calcium, and zinc.[1] They also possess various polyhydroxy alkaloids, stilbenoids (such as resveratrol and oxyresveratrol), flavonoids (including quercetin and kaempferol), and anthocyanins.[2, 3] The polyhydroxylated alkaloids found in *M. alba*. belong to the chemical class called iminosugars or azasugars and are one of the characteristic identifying compounds found in *Morus* spp. The most predominate iminosugar in *M. alba* is the piperidine alkaloid iminosugar DNJ, a D-glucose analogue with a nitrogen group replacing the oxygen on the pyranose ring (see Table 3 and Figure 1).[4, 5]

Table 3: Attributes of 1-deoxynojirimycin

Chemical Class	Percent Composition
IUPAC Name	(2R, 3R, 4R, 5S)-2-(hydroxymethyl)piperidine-3,4,5-triol
CAS #	19130-9602
Molecular Formula	$C_6H_{13}NO_4$
Molecular Weight	163.17172 g/mol

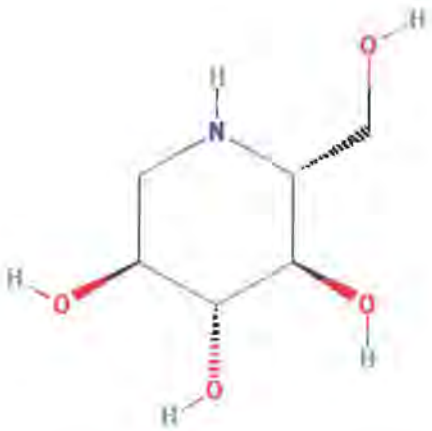

Figure 1. Structural Formula of 1-deoxynojirimycin[6]

M. alba leaves taken from the top of the trees in the summer (exposed to the most sunlight) contain the most DNJ.[7] In one study, 33 different cultivars of dried mulberry spp. leaves contained 1.389–3.483 mg/g DNJ (0.14–0.35%).[3] Others have indicated lower levels of naturally occurring DNJ in *M. alba* leaves (0.10–0.14%) and various levels in commercial *M. alba* products (<0.05–0.48%).[4] The CAS registry number for *M. alba* leaf extracts is 95167-05-2.

2.2 Manufacturing

2.2.1 Manufacturing Narrative

Phynova's manufacturing process produces an aqueous extract of *M. alba* that has a reduced color and scent, making it more desirable for food applications. The unground dried mulberry leaf raw material is extracted with water under controlled temperature and time. The extract is filtered to remove the solids (e.g., proteins, chlorophyll), which are re-extracted. The re-extract is filtered, and the extraction and re-extraction filtrates are combined. The clarified extract solution is loaded into a column filled with a strong acidic cation exchange resin. The column is then washed with distilled water followed by eluting the column with 0.5M ammonia solution. The water and ammonia eluents are combined to maximize recovery and concentrated under vacuum. The concentrate is then subjected to serial filtration. The final filtrate is concentrated under vacuum and then dried to produce a powder. During the drying process, maltodextrin is added to standardize the concentration of the final ingredient. The final product is a free-flowing powder (see Figure 2). Superscript numbers in the figure below indicated quality control points as follows:

1) moisture, appearance, foreign matter, purity; 2) extract medium temperature, record pH; 3) purity; 4) purity, appearance; 5) purity.

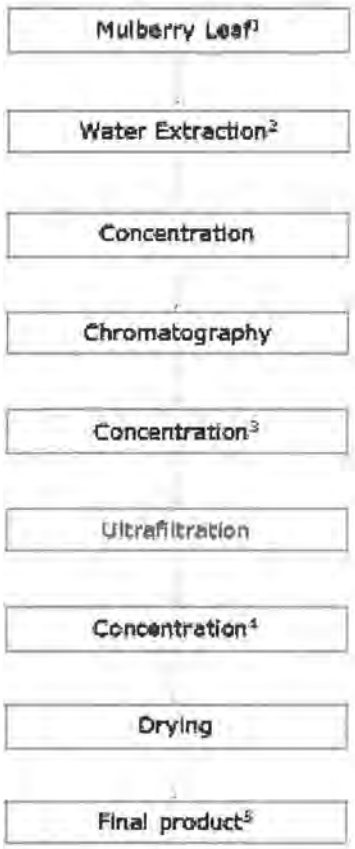

Figure 2. Manufacturing Flowchart

2.2.2 Good Manufacturing Practice

Reducose® 5% from Phynova is produced by Hill Pharmaceuticals Co. Ltd, 128 TaoYuanXi Road, LengShuiTan, Yongzhou, Hunan Province, China, under strict adherence to current Good Manufacturing Practice (cGMP). Hill Pharmaceuticals holds external certifications for a) compliance with GMP Requirements in NSF/ANSI Standard 173, Section 8, which includes FSMA and cGMP requirements of 21 CFR 117 and 21 CFR 111 (issued by NSF); b) compliance with the National Standard of China (GB), GB/T 19001-2016 and ISO 9001:2015 for their Quality Management System (issued by China Quality Certification (CQC)); and c) compliance with GB/T 22000-2006 and ISO 22000:2005 for their Food Safety

Management System, including a Hazard Analysis and Critical Control Point system (issued by CQC).

2.2.3 Raw Materials

Phynova sources the raw leaf material from mulberry farmers according to an internal raw material specification (see below). A voucher specimen is retained at Phynova's subsidiary in China, and the identity of each lot of material purchased is verified by a botanist. The raw material is analyzed for DNJ content, heavy metals, pesticide residues, yeast and molds and is then air-dried by the raw material suppliers.

Other raw materials used in the production of Reducose® 5% are purchased with certification of appropriate food grade. The potable water used in the extraction process is subjected to monthly testing for total plate count (aerobic microbes) and total coliforms, pH, and appearance as well as annual testing of additional parameters as required for drinking water according to GB 5749-20. Purified water (produced in a multiple stage process) is used for downstream processes. Reducose® 5% is non-GMO and not irradiated.

2.3 Specifications

The specifications for the food-grade product Reducose® 5%, along with the specification methods, are listed in Table 4 below.

Table 4. Reducose® 5% Specifications

Tested Parameters	Specification	Method
Chemical Analysis		
Deoxynojirimycin (DNJ)	5.0% ± 0.5%	Internal Method (HPLC-ELSD)
Moisture	<7.0%	GB5009.3-2016
Acid insoluble ash	<2.0%	GB5009.4-2016 or equivalent method
Physical Characteristics		
Color and Appearance	Light brown to brown free flowing powder	GB16740-2014 (Organoleptic)
Taste and odour	Characteristic odour. Malt taste with slight bitterness	GB16740-2014
Solubility	Easily dissolved in - Water - 50% ethanol Not soluble in oil	CP2015/General notices 15.2
Heavy Metals		
Arsenic	<1.5 ppm	BS EN ISO17294-2 2016 mod. (ICP-MS)

Mercury	<0.1 ppm	BS EN ISO17294-2 2016 mod (ICP-MS)
Cadmium	<0.5 ppm	BS EN ISO17294-2 2016 mod (ICP-MS)
Lead	<1 ppm	BS EN ISO17294-2 2016 mod. (ICP-MS)
Microbiological Tests		
Total Aerobic Plate Count	$<10^4$ CFU/g	ISO 4833-1:2013
Yeast & Mold	$<10^3$ CFU/g	ISO 21527:2008
Total coliforms	$<10^2$ CFU/g	AOAC 991.14
Escherichia coli	Absent in 10g	ISO 7251:2005
Salmonella	Absent in 25 g	ISO 6579-1: 2017
Pesticide Residues*		
Panel per USP 561 Table 5	Complies with USP 561 (Table 5)	BS EN 15662:2018; BS EN 12393:2013 or equivalent method
Aflatoxins**		
Aflatoxin B1	<5.0 ppb	DIN EN 14123, mod. or equivalent method
Total aflatoxin (B1, B2, G1, G2)	<20.0 ppb	

Abbreviations: AOAC, Association of Official Analytical Collaboration; BS EN, British Standards European Standards; CFU, colony forming units; CP, Chinese Pharmacopeia; DIN EN, German Institute for Standardization (Deutsches Institut für Normung) European Standards; GB, National Standard of China; HPLC-ELSD, high performance liquid chromatography-evaporative light scattering detector; ICP-MS, inductively coupled plasma-mass spectrometry; ISO, International Organization for Standardization; USP, U.S. Pharmacopoeia; ppb, parts per billion; ppm, parts per million.

*Mulberry leaf raw material tested for pesticides in accordance with EU Regulations 396/2005. Pesticide testing on finished product in accordance with USP 561 according to HACCP plan.

** Skip-lot testing of alfatoxins is conducted for compliance with USP561 according to HACCP plan.

2.3.1 Batch Analysis

Production conformity and consistency of Reducose® 5% are tested in production lots. Batch analyses of eight lots are shown in Tables 5 and 6 below and are reasonably consistent and met the product specifications, except as indicated. Lots made after 2015 contained L-leucine. L-leucine was historically added as a processing aid to facilitate more efficient drying beginning in 2015. L-leucine was removed from use as of the date of this GRAS notice due to a regulatory issue with its use and will not be utilized in any future batches. Data on the lots below produced prior to the introduction of L-leucine (Table 5) were tested according to an older specification and demonstrate the ability to produce food grade lots without the use of L-leucine. The lots containing L-leucine (Table 6) have been included to demonstrate conformity and consistency of the product as a food grade ingredient across the manufacturing change with and without L-leucine and have been produced to the current specification. Removal of L-leucine does not affect the product specification, quality control, or the safety of the ingredient. The changes to the product specifications over the years were made in accordance with data collection and analysis as part of Phynova's ongoing commitment to quality and compliance with regulatory guidance and were unrelated to the inclusion or exclusion of L-leucine as a processing aid.

Table 5. Reducose® 5% Batch Analyses without L-leucine

Tested Parameters	Specification	Lot No./Date of Manufacture			
		ML20110420 2011-04-20	NB6556-1 2015-08	NB6556-2 2015-08	NB6556-3 2015-08
Chemical Analysis					
Deoxynojirimycin (DNJ)	5.0% ± 0.5%	5.08%	5.4%	5.1%	5.3%
Moisture	<7.0%	4.47%	NT[1]	NT[1]	NT[1]
Acid insoluble ash	<2.0%	0.84%	NT[1]	NT[1]	NT[1]
Physical Characteristics					
Color and Appearance	Light brown to brown free flowing powder	Conforms	Conforms	Conforms	Conforms
Taste and odour	Characteristic odour. Malt taste with slight bitterness	Conforms	NT[1]	NT[1]	NT[1]
Solubility	Easily dissolved in water & 50% EtOH; not soluble in oil.	Conforms	NT[1]	NT[1]	NT[1]
Heavy Metals					
Arsenic	<1.5 ppm	0.37 ppm	0.28 ppm	0.22 ppm	0.26 ppm
Mercury	<0.1 ppm	NT[2]	<0.05 ppm	<0.05 ppm	<0.05 ppm
Cadmium	<0.5 ppm	NT[2]	<0.05 ppm	<0.05 ppm	<0.05 ppm
Lead	<1 ppm	0.36 ppm	0.055 ppm	0.097 ppm	0.54 ppm
Microbiological Tests					
Total Aerobic Plate Count	$<10^4$ CFU/g	Conforms*	3800 cfu/g	2400 cfu/g	1100 cfu/g
Yeast & Mold	$<10^3$ CFU/g	Conforms*	10 cfu/g	10 cfu/g	<10 cfu/g
Total coliforms	$<10^2$ CFU/g	NT[2]	ND	ND	ND
Escherichia coli	Absent in 10g	ND	ND	ND	ND
Salmonella	Absent in 25 g	ND	ND	ND	ND
Pesticide Residues					
Panel per USP 561 Table 5	Complies with USP 561 (Table 5)	NT[2]	NT[2]	NT[2]	NT[2]
Aflatoxins**					
Aflatoxin B1	<5.0 ppb	NT[2]	<1	<1	<1
Total aflatoxin (B1, B2, G1, G2)	<20.0 ppb	NT[2]	<1†	<1†	<1†

Abbreviations: CFU, colony forming units; ND, not detected; NT, lot not tested (1) in accordance with skip lot procedure or (2) due to change in specification; ppb, parts per billion; ppm, parts per million.

*Specification reporting was changed from qualitative to quantitative in 2015

**Skip-lot testing of alfatoxins is conducted for compliance with USP561 according to HACCP plan.

†Previous versions of the specification tested Total aflatoxins (B1, B2)

Table 6. Reducose® 5% Batch Analyses with L-leucine

Tested Parameters	Specification	Lot No./Date of Manufacture			
		A1701191 2017-07-15	181102 2018-11-06	20191123 2019-11-23	1912601 2019-12-06
Chemical Analysis					
Deoxynojirimycin (DNJ)	5.0% ± 0.5%	5%	5.3%	5.3%	5.1
Moisture	<7.0%	5.5%	5.4%	5.4%	4.8
Acid insoluble ash	<2.0%	0.3%	0.4%	0.4%	0.5
Physical Characteristics					
Color and Appearance	Light brown to brown free flowing powder	Conforms	Conforms	Conforms	Conforms
Taste and odour	Characteristic odour. Malt taste with slight bitterness	Conforms	Conforms	Conforms	Conforms
Solubility	Easily dissolved in water & 50% EtOH; not soluble in oil.	Conforms	Conforms	Conforms	Conforms
Heavy Metals					
Arsenic	<1.5 ppm	0.970 ppm	0.458 ppm	0.458 ppm	0.460
Mercury	<0.1 ppm	0.026 ppm	0.016 ppm	<0.001 ppm	<0.005
Cadmium	<0.5 ppm	0.158 ppm	0.083 ppm	0.016 ppm	0.010
Lead	<1 ppm	0.003 ppm	<0.001 ppm†	0.083 ppm†	<0.05
Microbiological Tests					
Total Aerobic Plate Count	$<10^4$ CFU/g	7.5×10^2 cfu/g	40 cfu/g	50 cfu/g	45 cfu/g
Yeast & Mold	$<10^3$ CFU/g	Y: <10 cu/g M: <10cfu/g	Y: <10 cfu/g M: <10 cfu/g	20 cfu/g	Y <10 cfu/g M <10 cfu/g
Total coliforms	$<10^2$ CFU/g	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Escherichia coli	Absent in 10g	ND	<10 cfu/g	ND	ND
Salmonella	Absent in 25 g	ND	ND	ND	ND
Pesticide Residues					
Panel per USP 561 Table 5	Complies with USP 561 (Table 5)	Complies	Complies	Complies	Complies
Aflatoxins*					
Aflatoxin B1	<5.0 ppb	NT	ND	NT	ND
Total aflatoxin (B1, B2, G1, G2)	<20.0 ppb	NT	ND	NT	ND

Abbreviations: CFU, colony forming units; ND, not detected/below limit of detection; NT, lot not tested in accordance with skip lot procedure; ppb, parts per billion; ppm, parts per million.
*Skip-lot testing of alfatoxins is conducted for compliance with USP561 according to HACCP plan.

2.4 Physical or Technical Effect

Reducose® 5% is not intended to produce any physical or other technical effects that are relevant to the safety of the ingredient.

Part 3: Dietary Exposure

3.1 Intended Use

For the purpose of this GRAS notice, Phynova's Reducose® 5% manufactured in accordance with current GMP, is intended to be used as an ingredient in the food categories and at the addition levels shown in Table 7 below.

Reducose® 5% is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA.

Table 7. Intended use of Reducose® 5%

NHANES Food Category	NHANES Category Code	Serving Size (g or mL)	Addition level (mg/g)			~Maximum amount per serving (mg)
			Minimum	Median	Maximum	
Bars	537	40	3.1	5	6.2	248
Low sodium crackers	542	30	4.2	6.7	8.4	252
Nonsweet crackers	543	30	4.2	6.7	8.4	252
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Oat breads	515	50	2.5	4	5	250
Cornbread, corn muffins, tortillas	522	55	2.27	3.6	4.5	248
Flour-milk dumplings, plain	556	30	4.2	6.7	8.4	252
Flour-water patties	555	30	4.2	6.7	8.4	252
Bread, rolls (not further specified)	510	50	2.5	4	5	250
Biscuits	521	55	2.27	3.6	4.5	248
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Multigrain breads, rolls	516	50	2.5	4	5	250
Other breads	518	50	2.5	4	5	250
Wheat, cracked wheat breads, rolls	513	50	2.5	4	5	250
Other quick breads	524	50	2.5	4	5	250
Pastas	561	140	0.9	1.4	1.8	252
Rye bread, rolls	514	50	2.5	4	5	250
White breads, rolls	511	50	2.5	4	5	250
Coffee	921	240	0.5	0.8	1	240
Citrus fruit juices	612	240	0.5	0.8	1	240
Energy drinks	9531	240	0.5	0.8	1	240
Sports drinks	9532	240	0.5	0.8	1	240
Other functional beverages	9534	240	0.5	0.8	1	240
Tea	923	240	0.5	0.8	1	240
Water, bottled, fortified	942	240	0.5	0.8	1	240
Fruit drinks	925	240	0.5	0.8	1	240
Beverage concentrates, dry, not reconstituted	929	5	5	12.5	50	250
Nutrition drinks (or powders to be reconstituted to drinks)	951	240	0.5	0.8	1	240

Cakes	531	140	0.9	1.4	1.8	252
Candies	917	40	3.1	5	6.2	248
Cookies	532	30	4.2	6.7	8.4	252
Cobblers, éclairs, turnovers, other pastries	534	55	2.27	3.6	4.5	248
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Pies (fruit, tart, cream, custard, miscellaneous pies, pie shells)	533	55	2.27	3.6	4.5	248
Sugar and sugar substitute blends	911	4	15	25	30	120
Sweet crackers	541	30	4.2	6.7	8.4	252
Jellies, jams, preserves	914	15	6.7	8.3	10	150
Danish, breakfast pastries, doughnuts	535	55	2.27	3.6	4.5	248
Cereal grains, not cooked	576	55	2.27	3.6	4.5	248
Ready to eat cereals	571–574	55	2.27	3.6	4.5	248
Cooked cereals, rice	562	55	2.27	3.6	4.5	248
Pancakes	551	110	1.14	1.8	2.25	248
Waffles	552	85	1.47	2.35	2.9	247
Flavored milk and milk drinks, fluid	115	240	0.5	0.8	1	240
Yogurt	114	225	0.56	0.89	1.1	248
Puddings, custards, and other milk desserts	132	120	1	1.67	2	240
Tomato sauces	744	30	4.2	6.7	8.4	252
Potato recipes	717	70	1.8	2.9	3.6	252
Potato soups	718	245	0.51	0.82	1.2	294
White potatoes, chips and sticks	712	70	1.8	2.9	3.6	252
Dark-green vegetable soups	723	245	0.51	0.82	1.2	294
Deep-yellow vegetable soups	735	245	0.51	0.82	1.2	294
Frozen plate meals with grain mixture as major ingredient	583	195	0.64	1	1.3	254
Other cooked vegetables, cooked with sauces, batters, casseroles	754	240	0.5	0.8	1	240
Soups with grain product as major ingredient	584	245	0.51	0.82	1.2	294

3.2 Exposure Estimates

Exposure to Phynova's Reducose® 5% from the intended use categories was estimated for the U.S. population using food consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). The most recent data available at the time of this writing (2015–2016) were analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). These data were obtained from 7027 individuals who underwent two non-consecutive 24-hour dietary recall interviews (the first was collected in-person, the second by phone 3–10 days later).

WWEIA food codes that were considered most similar to the intended use categories were utilized in the assessment and were assigned the relevant intended use concentrations.

Creme software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual food ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data are shown for "food consumers" (which includes only data from individuals who reported consuming one or more food/beverage categories intended to contain the ingredient over the two-day survey period, as opposed to the whole population). Results are given as both absolute exposure (mg/day), as well as exposure relative to body weight (mg/kg bw/day).

The relative standard error (RSE; calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population (the larger the RSE the less reliable the estimate).[8] RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[8, 9] For the purpose of this safety assessment, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown in the tables below for the 90th percentile values only, as the 90th percentile values are the most pertinent for the exposure estimates.

The Reducose® 5% exposure estimates derived from the Creme assessment based on the intended use categories and concentrations are shown below in Tables 8 and 9.

Table 8. Total (aggregate) absolute exposure to Reducose® 5% by proposed use food consumers using NHANES 2015–16 data using a 100% Presence Probability Factor

Population Group	Age in yrs	N (% of total)	Absolute Reducose 5% consumption Daily Average by Food Consumers (mg/day)				90th% RSE Value
			Mean	Mean std err	90th%	90th% std err	
Children	2–12	1480 (100)	1858	32.2	3002.6	73.9	2.5
Adolescents	13–19	847 (99.8)	2256.6	57.3	3864.0	137.3	3.6
Adults	20+	4203 (100)	2446.8	30.1	4099.6	74.6	1.8
Total Population	2+	6530 (100)	2342.6	25.1	4005.6	49.4	1.2

Creme run #496

Table 9. Total (aggregate) exposure to Reducose® 5% by proposed use food consumers relative to body weight using NHANES 2015–16 data using a 100% Presence Probability Factor

Population Group	Age in yrs	N (% of total)	Reducose 5% consumption relative to body weight Daily Average by Food Consumers (mg/kg bw/day)				90th% RSE Value
			Mean	Mean std err	90th%	90th% std err	
Children	2–12	1480 (100)	71.9	1.4	120.1	2.7	2.3
Adolescents	13–19	847 (99.8)	35.0	0.9	58.1	2.4	4.1
Adults	20+	4203 (100)	30.8	0.4	53.6	1.0	1.8
Total Population	2+	6530 (100)	37.2	0.4	67.8	1.5	2.2

Creme run #496

According to the estimates in the tables above, approximately 100% of the U.S. total population (ages 2 and above) are identified as potential consumers of Reducose® 5% from one or more of the wide number of proposed food uses. The 90th percentile estimated exposure to Reducose® 5% in the total population is 4005.6 mg/day (67.8 mg/kg bw/day). The highest potential consumer population at the 90th percentile on a relative to body weight basis is children (ages 2–12), at an estimated 120.1 mg/kg bw/day, although children also have the lowest *absolute* daily estimated exposure at 3002.6 mg/day.

It should be noted that these estimates are considered extremely conservative, as they assume that 100% of the large number of intended use food products in the market will contain the maximum intended use levels of Reducose® 5%. While food labels will list Reducose® 5% as an ingredient and may even highlight it in marketing, it is assumed that many consumers will not always realize that it is present in a food product. In other words, it may be an "invisible" ingredient to some consumers, which decreases the chance that only food products that contain it will be chosen by consumers. Additionally, there will be cost and market share limitations of adding the ingredient to foods and beverages in general, making it even less likely that an individual would consume them in all of the intended use food groups consumed daily.

In order to calculate a slightly more realistic exposure estimation for Reducose® 5% from the proposed food uses, an additional Creme exposure assessment was performed that assumed a presence probability of 10% Reducose® 5% in all of the proposed food categories. The 10% presence probability factor was intended to represent an approximate 10% market share of the ingredient in foods from each of the intended use categories, which is still considered a highly conservative, yet more realistic, assumption. The maximum addition level for each food category was still utilized. The resulting exposures to Reducose® 5% by food consumers using the 10% presence probability factor are shown in Tables 10 and 11 below.

Table 10. Total (aggregate) absolute exposure to Reducose® 5% by proposed use food consumers using NHANES 2015–16 data using a 10% Presence Probability Factor

Population Group	Age in yrs	N (% of total)	Absolute Reducose 5% consumption Daily Average by Food Consumers (mg/day)				90th% RSE Value
			Mean	Mean std err	90th%	90th% std err	
Children	2–12	990 (67.9)	297.3	14.6	704.6	48.3	6.9
Adolescents	13–19	502 (61.7)	389.2	26.0	927.0	82.7	8.0
Adults	20+	2712 (66.1)	371.4	11.1	856.8	42.8	5.0
Total Population	2+	4204 (66.1)	361.8	8.7	839.0	38.4	4.6

Creme run #498

Table 11. Total (aggregate) exposure to Reducose® 5% by proposed use food consumers relative to body weight using NHANES 2015–16 data using a 10% Presence Probability Factor

Population Group	Age in yrs	N (% of total)	Reducose 5% consumption relative to body weight Daily Average by Food Consumers (mg/kg bw/day)				90th% RSE Value
			Mean	Mean std err	90th%	90th% std err	
Children	2–12	990 (67.9)	11.3	0.6	25.7	1.9	7.4
Adolescents	13–19	502 (61.7)	5.8	0.4	13.8	1.2	8.7
Adults	20+	2712 (66.1)	4.7	0.1	11.0	0.4	3.6
Total Population	2+	4204 (66.1)	5.8	0.1	13.1	0.4	2.9

Creme run #498

According to the estimates using a 10% presence probability factor in the tables above, approximately 66.1% of the U.S. total population (ages 2 and above) are identified as potential consumers of Reducose® 5% from one or more of the wide number of proposed food uses. The 90th percentile estimated exposure to Reducose® 5% in the total population is 839.0 mg/day (13.1 mg/kg bw/day). The highest potential consumer population at the 90th percentile on a relative to body weight basis is children (ages 2–12), at an estimated 25.7 mg/kg bw/day, although again, children also have the lowest *absolute* daily estimated exposure at 704.6 mg/day.

Additionally, because available pharmacokinetic data on *M. alba* leaf preparations is given primarily with respect to their DNJ content, we have also calculated exposure in terms of DNJ content of Reducose® 5% using the upper limit of the product specification of 5.5%. Based on above estimates using 100% and 10% presence probability (PP) factors, the maximum DNJ exposure from the intended use of Reducose® 5% in the total population at the 90th percentile of consumers is calculated as 220.3 mg/day (3.73 mg/kg bw/day) and 46.1 mg/day (0.722 mg/kg bw/day), respectively. The highest potential consumer population at the 90th percentile on a relative to body weight basis is children (ages 2–12), at an estimated 6.60 mg/kg bw/day (100% PP) and 1.41 mg/kg bw/day (10% PP), although again, children also have the lowest *absolute* daily estimated exposure at 165.1 mg/day (100% PP) and 38.6 mg/day (10% PP).

Part 4: Self-limiting Levels of Use

There are no known inherent self-limiting levels of use.

Part 5: Experience Based on Common Use in Food Prior to 1958

The GRAS conclusion for Reducose® 5% is based on scientific procedures, and thus, experience based on common use in food prior to 1958 is not considered pivotal information.

Part 6: Narrative

6.1 Absorption, distribution, metabolism, and excretion (ADME)

6.1.1 Rats

The oral pharmacokinetics of 95% pure DNJ extracted from *M. alba* leaves were investigated by Nakagawa et al. (2007) in rats.[10] Following gavage administration of 110 mg/kg bw DNJ, C_{max} of 15 μg (92 nmol)/mL was observed at T_{max}, 30 minutes. Thereafter, DNJ plasma concentration decreased rapidly and was no longer detected (limit of detection <1 μg (6 nmol)/mL) by the fourth hour post administration; total AUC was 1% of the ingested dose. No DNJ metabolites were detected in plasma, indicating DNJ was absorbed intact. Urine and tissue DNJ levels were assessed 24h following administration, and 2, 7, and 1% of the ingested dose was found intact in the urine, large intestine, small intestine, respectively, while DNJ was not detected in the liver, kidney, pancreas or spleen. Dose-dependent plasma concentrations were observed following administration of 1.1, 11, or 110 mg/kg bw DNJ. These results suggest that orally ingested DNJ is rapidly, but poorly, dose-dependently absorbed and rapidly eliminated in the urine intact.

Kim et al. (2010) compared absorption and excretion of DNJ from an *M. alba* leaf hot water extract (0.35% DNJ as calculated by AIBMR) to 98% pure DNJ using both a rat model and a Caco-2 cell model.[5] In vitro absorption of DNJ was evaluated by incubating Caco-2 monolayers with pure DNJ or *M. alba* leaf extract (MLE) at DNJ concentrations of 0, 10, 20, 50, or 100 µM DNJ. Concentrations of DNJ absorbed were lower following incubation with MLE compared to pure DNJ but increases were concentration-related with both substances.

In order to evaluate plasma DNJ time course changes in vivo, groups of fasted male Sprague-Dawley rats were orally administered 3 or 6 mg/kg bw pure DNJ or 1.7 g (6 mg DNJ equivalent)/kg bw MLE. Blood was collected before and 30 minutes after administration of DNJ and before and at multiple intervals over 6 hours after administration of MLE. Following administration of MLE to rats, C_{max} of 12.01 µmol/L DNJ was observed at T_{max}, 30 minutes, then rapidly declined becoming undetectable by hour 4 (limit of detection, $6x10^{-4}$ µmol/L). Administration of 3 or 6 mg/kg bw pure DNJ resulted in a statistically significant, dose-related increase in plasma DNJ levels. Thirty minutes following administration 6 mg/kg bw pure DNJ plasma levels were 25.66 µmol/L, which was a statistically significant increased compared to the DNJ C_{max} following MLE administration. Plasma levels were approximately 8 µmol/L (as estimated by AIBMR from a bar graph) 30 minutes following administration of 3 mg/kg bw pure DNJ.

For determination of DNJ in plasma (collected 30 minutes following administration), urine (collected in a metabolic cage from 0 to 24h), and feces

(collected from 0 to 48h), fasted animals were orally administered 30 mg/kg bw pure DNJ or 0.85 g (3 mg DNJ equivalent)/kg bw MLE. According to the authors, rats administered pure DNJ ingested about 9.6 mg DNJ/rat and rats administered MLE ingested about 0.98 mg DNJ/rat. Means of 4.08 ± 0.83 and 0.07 ± 0.07 mg intact DNJ were recovered in the urine of rats receiving pure DNJ and MLE, respectively, while 7.22 ± 2.26 and 1.27 ± 0.60 mg intact DNJ were recovered in the feces of rats receiving pure DNJ and MLE, respectively. From this data, it appears the majority of DNJ, regardless of source is excreted in feces with a smaller amount absorbed and excreted intact in the urine although the proportion absorbed appears to be much greater with the pure compound based on absolute urine and feces levels (interestingly, the authors stated plasma measurements were obtained in the second experiment with 30 mg/kg pure DNJ and 0.85 mg/kg MLE but failed to report any results of the plasma analysis). These results also stand in apparent contrast to the results obtained by Nakagawa et al. who observed only 1% of the ingested dose was absorbed based on AUC. This contrast could be explained by sublinearity of absorption kinetics above a threshold dose; however, based on our literature searches, this possibility has not been explored.

Yang et al (2017) examined the plasma pharmacokinetics in rats of an alkaloid fraction of *M. alba* branches (MBE) containing 37.5% DNJ.[11] Groups of rats were administered MBE at doses of 40, 200, and 1000 mg/kg bw orally and 4 mg/kg intravenously (iv) and blood samples were collected at intervals from 0.08–36h post administration. Additionally, 98% pure DNJ was administered at 15 mg/kg bw orally and 1.5 mg/kg bw iv in order to compare the effect other MBE constituents on DNJ plasma pharmacokinetics. Tissue distribution of DNJ was evaluated in groups of rats administered 40 mg/kg MBE and sacrificed at 0.25, 0.5, and 2 hours, and elimination was evaluated in rats kept in metabolic caged for collection of urine a feces before dosing and at various intervals over the following 48h; bile was also collected from cannulated animals. An in situ single-pass infusion study was conducted with both MBE and pure DNJ to further explore the effect of other constituents on absorption of DNJ, and an in vitro study, in which MBE was independently incubated with intestinal homogenate and rat cecal microbiota cultures, was conducted to evaluate biotransformation by gut enzymes and microbes.

DNJ exhibited non-linear pharmacokinetics following administration of MBE. At the lower doses T_{max} occurred at 0.67h and C_{max} was 6.3700 (40 mg/kg) and 10.4822 (200 mg/kg) μg/mL while at 1000 mg/kg bw T_{max} occurred at 0.43h and C_{max} was 25.0905 μg/mL, and absolute bioavailability decreased with dose at 72.41, 38.61, and 33.29%, respectively. Half-lives increased from 1.3h at the low dose to 3.52h at the high dose, and the AUC exhibited a dual peak suggesting saturable absorption as low bile concentrations in the elimination experiment ruled out a significant effect of enterohepatic recycling. As compared to the low-dose MBE (equivalent dose of

DNJ), when pure DNJ was administered, T_{max} (0.67h) and C_{max} (4.8633 µg/mL) were similar, but $t_{1/2}$ was statistically significantly shorter at 0.88h and AUC statistically significantly lower resulting in reduced bioavailability (59.36%).

DNJ was rapidly distributed to the examined tissues (liver, kidney, pancreas, stomach, duodenum, jejunum, ileum, cecum, colon, and the content of the gastrointestinal tract), but did not bioaccumulate and was mostly cleared by hour 2 (with the exception of ilium, cecum, and colon). Highest distributions were found in the gastrointestinal tract and kidney. Consistent with the tissue distribution experiment, major excretory pathways were urine (65.32% of oral dose) and feces (43.97% of oral dose) with only a small amount found in the bile (0.29% of oral dose) and excretion was mostly complete within 24 hours.

In the in situ experiment, absorption of DNJ from MBE statistically significantly exceeded absorption of the equivalent amount of pure DNJ suggesting other components of MBE enhanced the absorption of DNJ. In the in vitro experiments, incubation of MBE with intestinal homogenate did not significantly affect DNJ levels; however, incubation with and rat cecal microbiota culture increased DNJ content by 115.5% suggesting a slight potential of gut microbes to biotransform other MBE constituents to DNJ. Thus, the increased bioavailability of DNJ observed with MBE compared to pure DNJ may have been due to both effects on absorption exerted by extract components and biotransformation of other components of the extract.

Takasu et al. (2018) conducted a mass balance experiment in rats using radiolabeled DNJ produced by *Bacillus amyloliquefaciens* AS385.[12] Following preparation of the test item, ^{15}N labeled DNJ was administered orally at a dose equivalent to 10 mg DNJ/rat and urine and feces were collected over 48h by housing the animals in metabolic cages . Based on the provided graphs, approximately 65% of radioactivity was recovered in urine and approximately 20% was recovered in feces over 48 hours, and the authors concluded that DNJ is rapidly absorbed and rapidly excreted intact. The authors speculated the remaining unaccounted-for percent may have been distributed to organs and tissues.

In a study in mice, Parida et al (2019) investigated tissue distribution of DNJ from a culture broth powder (CBP) derived from *B. amyloliquefaciens* AS385.[13] Groups of mice were administered 0 or 0.8% CBP in the diet for 5 consecutive weeks. As CBP contained 1% DNJ, this resulted in the presence of DNJ at 80 mg/kg diet (0.008%). Following the treatment period, the mice were sacrificed, and the following tissues were prepared for evaluation: liver, kidney, intestine, lung, heart, brain, spleen, pancreas, and epididymal, retroperitoneal, and mesenteric white adipose tissue (WAT). DNJ was quantifiable in most organs evaluated following 5 weeks of dietary supplementation, with the exceptions being the pancreas and retroperitoneal WAT where only trace amounts were found. The highest

concentrations were found organs associated with absorption and excretion: intestines (119.0+/-37.6 ng/g), kidneys (102.7+/-16.7 ng/g), and liver (63.2 +/- 10.6 ng/g). The remaining tissue concentrations were considered moderate, ranging from 7.5 to 17.0 ng/g.

Takasu et al. (2020) investigated absorption and tissue distribution of purified aza-sugars (DNJ isolated from *Bacillus amyloliquefaciens* AS385 and 2-*O*-α-D-galactopyranosyl-1-deoxynojirimycin (GAL-DNJ) and fagomine isolated from mulberry leaves).[14] Transport of azo-sugars was also investigated in a human hepatocellular carcinoma cell line (HepG2). The test items were administered to fasting rats at molecular equivalents (40 μmol/kg bw), Plasma levels of the aza-sugars reached maximums approximately 30 minutes following oral administration and returned nearly to baseline within 240 minutes, and urinary excretion was complete within 8 hours. Two plasma peaks were observed (one for GAL-DNJ and one for DNJ) following administration of GAL-DNJ, suggesting some metabolism of the disaccharide to it monosaccharide constituents before or during absorption. The rank order of C_{max} and AUCs ($_{0\text{-}t}$ and $_{0\text{-}inf}$) was DNJ > fagomine > GAL-DNJ > DNJ from GAL-DNJ. Six hours following administration, DNJ and fagomine were present in all analyzed tissues (liver, kidney, brain, pancreas, and mesenteric and perinephric adipose) with maximum concentrations found in the kidneys, while GAL-DNJ tissue concentrations were very low.

In the second experiment of the study, non-fasting rats were administered the test item and blood was collected followed by a 5-day washout and a second administration of the test item 10 minutes following administration of the sodium-glucose cotransporter (SLGT) inhibitor phlorizin. Non-fasting status did not affect C_{max} of DNJ and fagomine compared to the first experiment, but the $AUC_{0\text{-}t}$ levels were statistically significantly greater in the non-fasted state, likely due to prolonged transit time due to the presence of food. In contrast plasma concentrations of GAL-DNJ were statistically significant decreased compared to the fasting state suggest increased hydrolysis due to the presence of food. Phlorizin inhibited intestinal absorption of DNJ (from both DNJ are GAL-DNJ), but not of fagomine or GAL-DNJ. Thus, DNJ does not appear to be a SLGT substrate, and the GAL-DNJ results suggest GAL-DNJ is partially digested in the intestine prior to absorption,

In the in vitro study, HepG2 cells were incubated with each azo-sugar both alone and in the presence of phlorizin or glucose transporter (GLUT) inhibitor cytochalasin B. In the experiments with azo-sugars alone, HepG2 intracellular concentrations of GLU-DNJ were statistically significantly lower than those of DNJ and fagomine with fagomine having the highest concentration of the three. SLGT inhibition statistically significantly lowered intracellular concentration of DNJ while only slightly affecting uptake of fagomine and GLU-DNJ. GLUT inhibition statistically significantly suppressed both DNJ and fagomine uptake; GLUT inhibition of DNJ uptake was also notably greater than that of SLGT inhibition.

Both inhibitors had a mild, but non-significant effect on GLU-DNJ uptake in HepG2 cells. Overall the results were consistent with that of the in vivo experiment in terms of SLGT inhibition, and future suggested that the same transporters are likely involved in intestinal and hepatic uptake of DNJ. While these experiments suggest that GLUT and SLGT are likely involved in DNJ uptake in humans, it is not worthy that these transporters are upregulated in cancer cell; therefore, care should be taken in making extrapolations. Consideration should also be paid to the differential effects of these inhibitors on transporter isoforms and the relative presence of the isoforms in various tissues.

6.1.2 Humans

Following their study in rats, Nakagawa et al. (2008) validated an analytical method with a 25-fold improved sensitivity in the detection limit in order to investigate pharmacokinetics of DNJ from MLE in humans.[15] Following ingestion of 1.2 g MLE containing 6.3 mg of DNJ by two healthy male subjects, plasma samples were obtained for evaluation at intervals from 0.5 to 48 hours, and two sequential 24-hour urine collections were obtained for evaluation. C_{max} of 520 ng/mL was observed at T_{max} 1.5 hours. 7.0 µg/mL DNJ was detected in the first 24h urine collection with only trace levels detectable in the 24–48h collection. While the authors did not report the mean volume of urine collected, they concluded, in contrast to observations in rats, the majority of the oral dose of DNJ from MLE was absorbed and excreted intact within 24h. This conclusion is consistent when considering the normal range of daily urine output in humans is 800–2000 mL and the ingested dose of DNJ was 6.3 mg (7µg/mL x 800–2000 mL = 5.6–14 mg).

6.2 Toxicology Studies Conducted on Reducose® Ingredients

Various toxicological studies have been conducted in order to evaluate the safety Phynova's Reducose® 1% and 5% products for use in foods. Reducose® 5% was evaluated in an acute oral toxicity study in mice performed by the Drug and Safety Evaluation Centre, Beijing Municipal Institute for Drug Control, Beijing, China, the results of which are reported as part of the findings of a broader research project.[16] Additionally, Phynova sponsored a 28-day repeated-dose oral toxicity study in rats of Reducose® 5% that was performed by Toxi-Coop Zrt, Budapest, Hungary.[17] For purposes of a novel food application, Reducose® 1% was evaluated in a battery of genetic and oral toxicity studies performed by the Chinese Centre for Food Safety Risk Assessment, the results of which have been published.[18] These studies are summarized below. The test item evaluated in the acute study by Liu et al. is identical to the article of commerce that is the subject of this GRAS Notice, and the test items evaluated by Marx et al. and Li et al. are identical to the article of commerce that is subject to this GRAS Notice, except that, due to a manufacturing

process change in order to comply with US regulations governing GRAS substances, L-leucine is no longer used as a processing aid. L-leucine is still present in the ingredient at approximately 1–2% as a naturally occurring constituent of the white mulberry leaf extract.

6.2.1 Acute Oral Toxicity Study in Mice (Reducose® 5%)[16]

Methods: ICR (SPF) mice (10/sex/group) received 5 g/kg bw Reducose® 5% or purified water by gavage (0.4 mL/10 g bw) once and were monitored for general observations, signs of toxicity, and death continuously prior to administration of the test item through the forth hour following administration and daily thereafter for 14 days. Body weight and food intake were recorded on Study Days 1, 4, 7, 11, and 14. Animals were sacrificed on Study Day 14, and organs and tissues were inspected for gross pathological findings at necropsy. Body weight differences were evaluated statistically for normality follow by analysis of variance or a nonparametric rank sum test using SPSS software.

Results: No mortality or abnormal signs or reactions (such as abnormal appearance, activities, response to stimuli, secretions, or excretions) were observed within 4 hours after administration of test item or during the 14-day observation period. Body weight development was normal during the observation period with no statistically significant changes in body weight compared to controls on Study Days 1, 4, 7, 11 and 14. Food intake was also similar in the control and treated groups. No gross pathological changes were observed at necropsy.

Conclusions: The LD_{50} of the test item was >5 g/kg bw.

6.2.2 Twenty-Eight Day Repeated-Dose Oral Toxicity Study (Reducose® 5%)[17]

Methods: The GLP compliant 28-day study was conducted under the permission of the Institutional Animal Care and Use Committee (IACUC) of Toxi-Coop Zrt and in compliance with the National Research Council Guide for Care and Use of Laboratory Animals[19] and the principles of the Hungarian Act 2011 CLVIII (modification of Hungarian Act 1998 XXVIII) regulating animal protection. The study protocol was in accordance with OECD TG 407 (adopted 03 October 2008)[20] and the standard operating procedures of the laboratory.

Four groups of 10 SPF Hsd.Han Wistar rats/sex/group were administered the test item at doses of 0 (vehicle-control), 1000, 2000 and 4000 mg/kg bw/day by gavage for 28 days. The vehicle and negative control were distilled water. All tests and examinations were conducted according to study protocols and in full compliance with above stated guideline. Additionally, ophthalmological examinations were carried out on animals prior to the experimental period and on control and high-dose

group animals prior to study termination. Euthanasia was by exsanguination from the abdominal aorta after induction of narcosis with Isofluran CP® anesthesia. Statistically analyses were conducted on all quantitative data using SPSS PC+ software.

Results: No mortality or test item-related clinical signs were observed in any dose group throughout the study except for slight salivation that occurred transiently in three female rats of the 4000 mg/kg bw/day group shortly after administration of the test item. No abnormalities were observed during the functional observation battery. No toxicologically relevant effects on body weight, body weight gain, food consumption, or feed efficiency occurred. Some transient changes observed with respect to controls were small in magnitude and did not affect overall body weight development. No eye alterations were observed in ophthalmoscopic examinations

Slight, statistically significant changes compared to controls were noted in some clinical pathology parameters but remained within the historical control range of the laboratory and were not accompanied by correlating histopathological findings (see Tables 12 and 13). Similarly, some slight but statistically significant differences compared to controls were observed in absolute and relative organ weights, but all remained well within historical control ranges and were without correlating histopathology (see Tables 14–16).

At the gross and histopathological examinations one-sided renal pelvic dilatation of slight degree was observed as an individual finding in a single male high-dose animal and histologically was without medullar or cortical atrophy, inflammatory infiltrates, hemorrhage, hemosiderin, or degenerative or fibrotic lesion. Furthermore, there was no histological evidence in the investigated organs of this animal in correlation with the elevated number of granulocytes or decreased number of lymphocytes observed in the clinical chemistry evaluation. All other gross and histopathological findings occurred with similar incidence among the examined dose groups. All observed findings were of a nature commonly observed in experimental rats (see Tables 17 and 18).[21-27]

Table 12. Summary of Hematology—28-day Study, Reducose 5%

Group (mg/kg bw/d)	WBC $\times 10^9$/L	NEU %	LYM %	MONO %	EOS %	BASO %	RBC $\times 10^{12}$/L	HGB g/L	HCT L/L	MCV fL	MCH pg	MCHC g/L	PLT $\times 10^9$/L	RET %	PT sec	APTT sec
Male (n=10/group)																
Control	11.26 ± 2.46	10.24 ± 2.80	86.79 ± 3.54	2.26 ± 0.80	0.64 ± 0.37	0.08 ± 0.04	8.97 ± 0.24	167.90 ± 4.82	0.457 ±0.015	51.00 ± 1.64	18.74 ± 0.64	367.30 ± 3.02	922.0 ± 96.92	4.03 ± 0.53	22.11 ± 1.15	18.52 ± 2.85
1000	9.63 ± 1.87	13.13 ± 3.04	84.04 ± 3.15	1.96 ± 0.44	0.81 ± 0.32	0.06 ± 0.05	8.98 ± 0.49	169.40 ± 4.20	0.459 ± 0.011	51.75 ± 2.64	19.11 ± 0.92	369.20 ± 2.49	984.50 ± 45.56	4.21 ± 0.65	21.48 ± 1.41	19.14 ± 1.83
2000	9.84 ± 1.45	12.44 ± 5.06	84.98 ± 5.39	1.83 ± 0.48	0.69 ± 0.32	0.06 ± 0.05	8.97 ± 0.25	170.60 ± 5.78	0.467 ± 0.013	52.14 ± 1.68	19.04 ± 0.57	365.10 ± 5.20	970.00 ± 127.94	4.03 ± 0.54	21.80 ± 1.61	20.47 ± 2.27
4000	9.50 ± 1.91	**15.11 ± 4.93***	**81.79 ± 5.02***	2.15 ± 0.46	0.89 ± 0.59	0.07 ± 0.05	8.81 ± 0.44	170.50 ± 6.06	0.464 ± 0.015	52.77 ± 1.64	19.37 ± 0.51	367.30 ± 3.53	966.60 ± 99.33	**3.94 ± 0.83***	21.44 ± 1.19	20.59 ± 2.07
Historical Ranges	6.59–18.27	3.4–30.3	66.9–95.7	0.5–4.9	0.0–1.1	0.0–0.4	7.4–9.9	142–184	0.39–0.52	47.8–57.6	17.8–20.3	353–375	478–1119	3.52–7.97	18.9–25.8	14.2–22.2

Group (mg/kg bw/d)	WBC $\times 10^9$/L	NEU %	LYM %	MONO %	EOS %	BASO %	RBC $\times 10^{12}$/L	HGB g/L	HCT L/L	MCV fL	MCH pg	MCHC g/L	PLT $\times 10^9$/L	RET %	PT sec	APTT sec
Female (n=10/group)																
Control	7.55 ± 1.25	9.22 ± 4.76	87.75 ± 4.90	2.06 ± 0.46	0.94 ± 0.28	0.03 ± 0.07	8.66 ± 0.42	160.40 ± 7.81	0.455 ± 0.021	52.52 ± 1.37	18.54 ± 0.60	352.90 ± 4.48	805.00 ± 53.89	4.08 ± 0.90	20.33 ± 0.82	17.93 ± 0.99
1000	**6.11 ± 1.39***	**12.21 ± 2.64***	84.91 ± 2.94	1.81 ± 0.42	1.04 ± 0.40	0.03 ± 0.07	8.31 ± 0.77	156.80 ± 14.16	0.439 ± 0.037	52.95 ± 2.02	18.89 ± 0.55	356.80 ± 6.09	785.70 ± 86.51	**4.06 ± 0.43***	19.81 ± 1.17	18.79 ± 2.24
2000	6.34 ± 1.43	**15.04 ± 4.91****	**81.72 ± 5.22****	2.12 ± 0.31	1.12 ± 0.37	0.00 ± 0.00	8.43 ± 0.44	156.70 ± 6.48	0.442 ± 0.014	52.50 ± 1.31	18.61 ± 0.40	354.30 ± 4.14	778.10 ± 56.54	4.56 ± 0.79	20.83 ± 0.95	**20.82 ± 1.82****
4000	7.33 ± 1.14	**13.97 ± 2.12****	**83.05 ± 2.32***	1.87 ± 0.59	1.09 ± 0.24	0.02 ± 0.06	8.46 ± 0.33	161.00 ± 5.19	0.451 ± 0.017	53.30 ± 2.43	19.06 ± 0.66	357.60 ± 6.04	793.50 ± 88.42	4.28 ± 0.41	20.28 ± 1.11	**22.13 ± 3.42****
Historical Ranges	3.54–12.73	4.9–44.2	47.7–93.9	0.5–7.3	0.3–1.9	0.0–0.2	5.0–9.1	98–169	0.27–0.48	48.9–59.1	18.2–20.6	346–376	509–1096	3.33–6.10	15.3–23.9	14.8–22.8

Data represent the mean values and the standard deviation.

*P ≤ 0.05 and **P < 0.01

Minimum and maximum levels reported as the range of historical control values

APTT: activated partial thromboplastin time; BASO, basophils; EOS, eosinophils; GLUC, glucose; HCT, hematocrit; HGB, hemoglobin; LYM, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocytes/RBU, neutrophils; PLT, platelet; PT, prothrombin time; RBC, red blood cells; RET, reticulocyte; TP, total erythrocytes; WBC, white blood cell.

Table 13. Summary of Clinical Chemistry—28-day Study, Reducose 5%

Group (mg/kg bw/d)	ALT U/L	AST U/L	GGT U/L	ALP U/L	TBIL µmol/L	CREA µmol/L	UREA mmol/L	GLUC mmol/L	CHOL mmol/L	Pi mmol/L	Ca^{++} mmol/L	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	ALB g/L	TPROT g/L	A/G
Males (n=10/group)																	
Control	39.54 ± 6.08	95.20 ± 12.16	–	143.3 ± 28.4	2.08 ± 0.23	23.13 ± 2.14	7.57 ± 0.92	6.03 ± 0.68	1.93 ± 0.25	2.65 ± 0.14	2.86 ± 0.05	139.8 ± 0.9	4.12 ± 0.19	102.53 ± 0.69	33.49 ± 1.08	57.91 ± 1.40	1.37 ± 0.08
1000	43.51 ± 5.00	92.53 ± 6.92	–	159.7 ± 26.1	1.73 ± 0.41	22.53 ± 2.41	7.76 ± 0.99	6.16 ± 0.63	1.76 ± 0.15	2.71 ± 0.24	2.72 ± 0.08	139.3 ± 0.7	**4.37 ± 0.29***	102.64 ± 0.92	34.61 ± 1.40	**61.25 ± 3.07****	1.31 ± 0.10
2000	44.42 ± 9.07	94.77 ± 12.07	–	166.7 ± 26.4	**1.51 ± 0.45****	**20.97 ± 1.32***	8.25 ± 1.28	**5.50 ± 0.91***	1.87 ± 0.37	2.52 ± 0.20	2.71 ± 0.08	**138.3 ± 0.5****	**4.49 ± 0.18****	102.34 ± 0.87	34.18 ± 0.76	58.58 ± 1.89	1.42 ± 0.08
4000	**50.96 ± 8.29****	**108.26 ± 12.26***	–	146.8 ± 17.5	1.85 ± 0.32	**20.79 ± 1.31***	8.02 ± 1.30	5.91 ± 0.47	1.72 ± 0.25	2.71 ± 0.24	2.68 ± 0.04	**138.5 ± 1.4***	4.26 ± 0.22	102.64 ± 0.84	34.13 ± 0.58	58.94 ± 3.01	1.39 ± 0.16
Historical Ranges	42.4–76.7	68.3–144.8	0.1–1.9	112–321	0.64–2.76	17.7–30.3	5.27–11.12	4.66–7.69	1.32–2.74	2.11–3.23	2.49–2.89	132.0–143.0	3.56–4.94	95.1–102.2	31.5–35.8	51.4–65.4	1.1–1.8

Group (mg/kg bw/d)	ALT U/L	AST U/L	GGT U/L	ALP U/L	TBIL µmol/L	CREA µmol/L	UREA mmol/L	GLUC mmol/L	CHOL mmol/L	Pi mmol/L	Ca^{++} mmol/L	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	ALB g/L	TPROT g/L	A/G
Female (n= 10/group)																	
Control	45.42 ± 5.77	97.78 ± 10.30	–	97.40 ± 27.62	1.91 ± 0.39	26.12 ± 1.57	7.31 ± 1.00	5.59 ± 0.68	1.95 ± 0.35	1.99 ± 0.34	2.62 ± 0.06	140.40 ± 0.97	3.90 ± 0.21	104.10 ± 1.19	34.45 ± 1.34	57.22 ± 2.76	1.53 ± 0.09
1000	45.16 ± 5.22	92.90 ± 8.10	–	105.30 ± 24.53	1.70 ± 0.33	24.54 ± 2.03	7.23 ± 1.30	5.35 ± 0.94	2.03 ± 0.30	1.72 ± 0.23	2.55 ± 0.06	**139.10 ± 1.29***	4.02 ± 0.27	104.42 ± 1.05	34.29 ± 0.70	56.83 ± 2.52	1.53 ± 0.13
2000	44.41 ± 8.51	95.59 ± 8.31	–	109.40 ± 13.74	1.57 ± 0.45	24.71 ± 2.21	7.71 ± 0.85	5.87 ± 0.64	2.00 ± 0.32	1.90 ± 0.39	**2.34 ± 0.07***	139.70 ± 1.06	3.99 ± 0.30	104.58 ± 0.89	34.00 ± 1.09	56.56 ± 1.80	1.53 ± 0.09
4000	46.13 ± 9.65	93.95 ± 8.20	–	107.90 ± 21.06	1.72 ± 0.23	24.54 ± 1.29	7.79 ± 1.40	5.54 ± 1.09	1.72 ± 0.18	1.99 ± 0.20	**2.58 ± 0.04***	**138.20 ± 1.03****	4.07 ± 0.28	104.03 ± 1.04	34.21 ± 0.80	56.95 ± 1.36	1.51 ± 0.07
Historical Ranges	38.8–86.4	75.8–272.1	0.1–2.5	58–192	0.59–2.86	18.3–31.1	4.67–10.94	3.40–7.68	1.03–2.57	1.73–2.89	2.36–2.87	136.0–149.0	3.04–5.36	95.8–103.9	32.3–38.4	53.2–65.2	1.2–1.7

Data represent the mean values and the standard deviation.

*P ≤ 0.05 and **P < 0.01

Minimum and maximum levels reported as the range of historical control values

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; Ca^{++}, calcium; CHOL, cholesterol; Cr, chloride; CREA, creatinine; GGT, gamma glutamyl transferase; K^+, potassium; Na+, sodium; Pi, inorganic phosphate; TBIL, total bilirubin; TPROT, total protein

*As no data the quantitation limit of GGT: ? U/L

Table 14. Summary of Organ Weights—28-day Study, Reducose 5%

Group (mg/kg bw/d)	Body Weight	Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididymides	Adrenals
Males (n=10/group)										
Control	273.7 ± 20.07	1.95 ± 0.12	7.83 ± 0.74	1.90 ± 0.15	0.89 ± 0.09	0.58 ± [illegible]	0.58 ± 0.09	3.04 ± 0.23	1.10 ± 0.11	0.080 ± 0.013
1000	273.5 ± 23.02	2.03 ± 0.07	8.50 ± 1.10	1.92 ± 0.20	0.91 ± 0.09	0.52 ± 0.08	0.58 ± 0.06	3.11 ± 0.14	1.11 ± 0.15	0.079 ± 0.007
2000	273.6 ± 12.15	1.90 ± 0.09	8.66 ± 0.76	1.90 ± 0.10	0.88 ± 0.08	0.54 ± 0.08	**0.50 ± 0.07***	3.06 ± 0.37	**0.99 ± 0.11***	0.080 ± 0.011
4000	260.7 ± 17.38	2.00 ± 0.06	8.58 ± 0.73	1.98 ± 0.16	0.83 ± 0.11	**0.42 ± 0.07****	**0.49 ± 0.05****	3.00 ± 0.13	**0.95 ± 0.07****	0.075 ± 0.008
Historical Range[a]	241–348	1.80–2.18	6.11–11.34	1.44–2.50	0.75–1.22	0.25–0.80	0.46–0.99	2.29–3.72	0.56–1.47	0.053–0.100
Females (n=10/group)								Uterus	Ovaries	
Control	177.3 ± 8.15	1.92 ± 0.06	5.33 ± 0.53	1.31 ± 0.12	0.67 ± 0.05	0.47 ± 0.09	0.46 ± 0.06	0.63 ± 0.24	0.146 ± 0.018	0.081 ± 0.012
1000	173.0 ± 7.57	1.84 ± 0.08	5.35 ± 0.49	1.28 ± 0.12	0.62 ± 0.07	0.43 ± 0.07	**0.39 ± 0.04***	0.51 ± 0.15	**0.123 ± 0.016***	0.078 ± 0.008
2000	174.4 ± 10.76	**1.83 ± 0.10***	5.66 ± 0.71	1.31 ± 0.11	0.63 ± 0.07	0.46 ± 0.07	0.42 ± 0.06	0.56 ± 0.14	**0.120 ± 0.033***	0.080 ± 0.013
4000	174.1 ± 5.40	**1.81 ± 0.10***	5.51 ± 0.31	1.31 ± 0.09	0.62 ± 0.05	0.40 ± 0.08	**0.39 ± 0.06***	0.63 ± 0.27	**0.107 ± 0.019****	0.080 ± 0013
Historical Range[a]	155.0–203.0	1.67–2.07	4.69–6.76	1.08–1.52	0.52–0.82	0.26–0.54	0.34–0.75	0.26–1.09	0.058–0.180	0.055–0.116

Data represent the mean values and the standard deviation.

*$p < 0.05$ and **$p < 0.01$

[a]minimum and maximum levels reported as the range of historical control values

Table 15. Summary of Organ Weights Relative to Body Weight—28-day Study, Reducose 5%

Group (mg/kg bw/d)	Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididymides	Adrenals
Males (n=10/group)									
Control	0.713 ±	2.859 ± 0.143	0.694 ± 0.026	0.324 ± 0.025	0.212 ± 0.019	0.209 ± 0.018	1.110 ± 0.064	0.404 ± 0.045	0.029 ± 0.004
1000	0.745 ± 0.072	**3.098 ± 0.159****	0.701 ± 0.045	0.333 ± 0.026	**0.190 ± 0.018***	0.214 ± 0.022	1.143 ± 0.113	0.407 ± 0.063	0.029 ± 0.003
2000	0.695 ± 0.035	**3.163 ± 0.168****	0.697 ± 0.041	0.320 ± 0.022	0.196 ± 0.026	**0.184 ± 0.024***	1.118 ± 0.134	0.363 ± 0.033	0.029 ± 0.004
4000	**0.769 ± 0.045***	**3.290 ± 0.130****	**0.759 ± 0.052****	0.320 ± 0.038	**0.161 ± 0.023****	**0.187 ± 0.019***	1.158 ± 0.109	0.367 ± 0.043	0.029 ± 0.003
Historical Range[a]	0.600–0.851	2.314–3.481	0.545–0.788	0.263–0.399	0.095–0.306	0.171–0.355	0.722–1.227	0.224–0.473	0.0190–0.0357
Females (n=10/group)							Uterus	Ovaries	
Control	1.082 ± 0.049	3.003 ± 0.211	0.741 ± 0.063	0.377 ± 0.031	0.262 ± 0.045	0.256 ± 0.032	0.357 ± 0.137	0.0824 ± 0.0092	0.0456 ± 0.0050
1000	1.068 ± 0.071	3.089 ± 0.177	0.740 ± 0.057	0.360 ± 0.037	0.247 ± 0.036	**0.225 ± 0.016***	0.295 ± 0.096	0.0708 ± 0.0088	0.0448 ± 0.0041
2000	1.049 ± 0.046	**3.239 ± 0.239***	0.749 ± 0.056	0.361 ± 0.040	0.266 ± 0.043	0.243 ± 0.031	0.320 ± 0.082	**0.0689 ±0.0190***	0.0458 ± 0.0063
4000	1.039 ± 0.055	3.168 ± 0.216	0.750 ± 0.046	0.355 ± 0.034	0.232 ± 0.048	**0.226 ± 0.033***	0.360 ± 0.154	**0.0614 ± 0.0119****	0.0460 ± 0.0072
Historical Range[a]	0.865–1.174	2.672–3.406	0.590–0.843	0.306–0.437	0.141–0.308	0.191–0.426	0.142–0.661	0.034–0.102	0.031–0.074

Data represent the mean values and the standard deviation.

*$p < 0.05$ and **$p < 0.01$

[a]minimum and maximum levels reported as the range of historical control values

Table 16. Summary of Organ Weights Relative to Brain Weight—28-day Study, Reducose 5%

Group (mg/kg bw/d)	Body Weight	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididymides	Adrenals
Males (n=10/group)									
Control	14095.6 ± 987.66	403.44 ± 39.55	97.82 ± 7.12	45.70 ± 4.78	29.82 ± 3.15	29.53 ± 3.69	156.28 ± 10.90	56.78 ± 5.60	4.15 ± 0.75
1000	13523.2 ± 1258.46	420.24 ± 55.87	94.69 ± 9.53	44.88 ± 4.08	**25.75 ± 4.10***	28.80 ± 2.87	153.48 ± 7.27	54.60 ± 7.05	3.90 ± 0.33
2000	14423.2 ± 666.83	**456.59 ± 37.73***	100.51 ± 7.94	46.12 ± 3.44	28.26 ± 4.39	26.57 ± 4.22	161.27 ± 21.65	52.38 ± 5.50	4.19 ± 0.46
4000	**13039.5 ± 751.36***	429.14 ± 33.25	98.87 ± 6.46	41.63 ± 4.85	**21.02 ± 3.30****	**24.34 ± 2.38****	150.34 ± 8.00	**47.60 ± 3.77****	3.75 ± 0.34
Historical Range‡	11756.1–16666.7	316.6–532.4	80.0–124.4	38.7–61.7	13.0–38.3	24.1–46.6	113.4–178.2	28.9–68.1	2.69–4.78
Females (n=10/group)							Uterus	Ovaries	
Control	9261.4 ± 399.47	278.33 ± 25.25	68.60 ± 6.38	34.87 ± 2.91	24.29 ± 4.63	23.72 ± 2.95	33.06 ± 12.66	7.62 ± 0.86	4.23 ± 0.52
1000	9400.7 ± 629.26	290.89 ± 31.17	69.45 ± 5.93	33.85 ± 3.79	23.29 ± 3.97	21.21 ± 2.58	27.39 ± 7.88	**6.63 ± 0.67***	4.22 ± 0.59
2000	9550.7 ± 421.90	**309.41 ± 27.12***	71.45 ± 4.46	34.37 ± 3.37	25.44 ± 4.52	23.25 ± 3.38	30.68 ± 8.57	6.58 ± 1.80	4.37 ± 0.60
4000	9645.9 ± 510.57	**305.12 ± 19.86***	72.24 ± 3.94	34.17 ± 3.12	22.40 ± 4.84	21.71 ± 2.40	34.99 ± 15.90	**5.90 ± 1.04****	4.42 ± 0.52
Historical Range‡	8516.5–11556.9	248.9–361.5	56.5–84.0	28.6–45.6	13.8–30.9	18.7–39.3	14.8–62.6	3.3–9.8	3.0–6.5

Data represent the mean values and the standard deviation.

*$P < 0.05$ and **$P < 0.01$

‡ minimum and maximum levels reported as the range of historical control values

Table 17. Summary of Gross Pathology—28-day Study, Reducose 5%

Organs	Observations	Control	1000 mg/kg bw/day	2000 mg/kg bw/day	4000 mg/kg bw/day
Male	No macroscopic findings	10/10	10/10	9/10	9/10
Skin (on the neck)	Alopecia, scar	0/10	0/10	1/10	0/10
Kidneys	Pyelectasia - one side	0/10	0/10	0/10	1/10
Female	No macroscopic findings	9/10	7/10	8/10	8/10
Uterus	Hydrometra	1/10	3/10	2/10	2/10
Remark:	Frequency of observations: = Number of animals with findings / Number of animals observed				

Table 18. Summary of Histopathology—28-day Study, Reducose 5%

Organs	Observations	Incidence of observations per group	
		Control	4000
Male			
Kidneys	Pyelectasia	0/10	1/10
Lungs	Alveolar emphysema	2/10	2/10
	Hyperplasia of BALT	1/10	1/10
Female			
Lungs	Alveolar emphysema	2/10	1/10
Uterus	Dilatation	1/10	2/10

Abbreviations: /, not examined; BALT, bronchus associated lymphoid tissue.
Data represent the number of animals with observation per number of animals observed.
Organs without lesions in 10/10 control or high-dose animals not shown.

Conclusions: Repeated administration by gavage of 1000, 2000 or 4000 mg/kg bw/day of Reducose® 5% for 28 days did not cause adverse effects or signs of toxicity in male or female SPF Hsd.Han Wistar rats; the NOAEL was determined to be 4000 mg/kg bw/day; the highest dose tested.

6.2.3 Bacterial Reverse Mutation Test (Reducose® 1%)[18]

Methods: Four strains of *Salmonella typhimurium* (TA97, TA98, TA100 and TA102) were tested in the presence and absence of rat liver S9 metabolic activation in two independent tests conducted in triplicate. Based on the results of a preliminary cytotoxicity test, concentrations of the test item were: 0, 62, 185, 556, 1667 and 5000 µg/plate, and concurrent negative (untreated and vehicle (distilled

water)) and strain specific positive ($C_6H_7N_3O_2$ (TA97 and TA98; -S9), sodium azide (TA100; -S9), 2-AF (TA97, TA98, TA100; +S9), Mitomycin C (TA102; -S9), $C_{14}H_8O_4$ (TA102; +S9)) controls were also run. A result was considered positive if revertant colonies numbers were greater than 2-fold that of the vehicle control with a dose-response.

Results: Spontaneous revertant colony numbers of the vehicle control agreed with historical control data, and positive controls induced the expected responses. No biologically relevant increases were seen in revertant colony numbers of any of the four bacterial strains upon treatment with the test item at any of the concentration levels either in the presence or absence of an S9 activation system. All results were unequivocally negative according to the study criteria for both positive and biologically relevant responses.

Conclusions: Under the experimental conditions applied, Reducose® 1% failed to induce gene mutations by base pair changes or frameshifts in the genome of the strains used at concentrations up to the maximum recommended test concentration of 5000 µg/plate.

6.2.4 In vivo Mammalian Micronucleus Test (Reducose® 1%)[18]

Methods: Reducose® 1% was administered twice, at an interval of 24 hours, by gavage to male and female (5/sex/group) Kunming SPF mice at doses of 0 (vehicle-control), 2.5, 5.0, and 10.0 g/kg bw. The negative control/vehicle was distilled water. Cyclophosphamide was used as the positive control at 40 mg/kg bw. All treatments were administered at a uniform volume of 20 mL/kg bw. The mice were sacrificed by cervical dislocation six hours after the final treatment and sternum bone marrow was collected and diluted with calf serum for the smears. The ratio of polychromatic erythrocytes (PCE) to total erythrocytes was calculated by counting 200 erythrocytes per animal, and 1000 PCEs per animal were scored for frequency of micronuclei; a Poisson distribution analysis was carried out.

Results: The ratio of PCEs to total erythrocytes was similar among negative controls and treated groups and was within 20% of the negative controls in the positive control group, indicating no significant cytotoxicity. No significant differences in the micronucleus incidence between the test groups and the negative control group were found while the positive control induced the expected statistically significant increases in micronucleus incidence compared to the negative control.

Conclusions: Reducose® 1%, at concentrations up to 10.0 g/kg bw, was negative for producing chromosomal damage in the bone marrow of mice under the experimental conditions applied.

6.2.5 In vivo Mammalian Sperm Deformity Test (Reducose® 1%)[18]

Methods: Thirty-five adult male Kunming SPF mice were randomly divided into five groups of seven animals. The test item was administered at 0 (vehicle-control), 2.5, 5.0 and 10.0 g/kg bw/day by gavage for 5 days. Cyclophosphamide (40 mg/kg bw) was used as a positive control and distilled water was used as a negative control and vehicle. Thirty days after the final administration, five mice were randomly chosen from each group and sacrificed by cervical dislocation. The bilateral epididymides were recovered from each animal and processed to obtain sperm for preparation of microscope slides. The sperm deformity rate was calculated but counting 1000 sperm per mouse, and a chi-square test was performed for statistical analysis.

Results: No statistically significant difference in sperm deformity rate between the test item-treated groups and the negative control group was observed. The positive control caused a statistically significant increase in sperm deformity rate compared to the negative control and test item-treated groups.

Conclusions: Reducose® 1% did not cause sperm deformities in mice under the applied conditions test.

6.2.6 Acute Oral Toxicity Study in Rats (Reducose® 1%)[18]

Methods: Ten male and 10 female SPF Sprague-Dawley (SD) rats were administered 15.0 mL/kg of a 0.5 g/mL test solution twice in one day, resulting in a dose of 15.0 g/kg bw of the test item. The test solution was prepared by mixing 37.5 g of Reducose® 1% with 75 mL of distilled water. The animals were observed daily for mortality and general behavior for 14 days after treatment.

Results: No mortality or signs of toxicity were observed.

Conclusions: Following oral administration of Reducose® 1% to male and female SD rats, the LD_{50} was considered >15 g/kg bw/day.

6.2.7 Thirty-Day Repeated-Dose Oral Toxicity Study in Rats (Reducose® 1%)[18]

Methods: Eighty SPF SD rats (10/sex/group) were administered Reducose® 1% in the diet at concentrations formulated to provide target doses of 0, 1.88, 3.75 and 7.5 g/kg/bw for 30 days. Animals were observed daily for mortality and clinical signs daily. Body weight and food consumption were measured weekly. Prior to sacrifice, and following 16–18h food deprivation, blood was collected under anesthesia from the inner canthus vein for hematological and clinical chemistry evaluations. Gross pathological examinations were conducted on all animals at necropsy, absolute and relative to body weight organ weights were determined, and tissues were processed

for histological examination. Histological examinations were conducted on liver, spleen, kidneys, stomach, duodenum, and testes or ovaries of control and high-dose animals as well as any gross lesions observed in any animals.

Results: No mortality or clinical signs of toxicity were observed in any animals. No effects on body weight gain compared to controls were observed in the treated groups, and a statistically significant decrease in food consumption observed in mid-dose females was within the historical control range of the laboratory. Alanine aminotransferase activity was statistically significantly decreased in high-dose females compared to controls but remained within the historical control range of the laboratory, was without correlating histopathology, and was without a dose-response. No other statistically significant alterations were observed in the clinical chemistry or hematological parameters. A few statistically significant alterations compared to controls were observed for absolute and relative organ weights as follows: kidney weights (absolute and relative to body weight) were increased in high-dose females, liver weight to body weight ratio was increased in mid- and high-dose males, and spleen weights (absolute and relative to body weight) were decreased in all male dose groups. All changes in absolute and relative organ weights remained within the historical control range of the laboratory and were without correlating histopathology. No gross pathological lesions were observed. Histopathological changes observed were low in incidence, were common lesions observed in untreated laboratory rats, and either occurred in controls only or occurred with the same incidence in controls and high-dose animals (see Table 19).

Table 19. Summary of Histopathology—30-day Study, Reducose 1%

Histopathologic Findings		Control	7.5 g/kg
Organs	**Observations**	**N=20**	**N=20**
Kidneys	Renal tubular calcium deposits	1/20	0/20
Liver	Spotty necrosis of liver cells	1/20	1/20
	Focal necrosis of liver cells	2/20	0/20
Spleen	Slight dilation and congestion of sinus	INR	INR

Abbreviations: INR, incidence not reported.
Data represent the number of animals with observation per number of animals observed.
Organs without lesions in 10/10 control or high-dose animals not shown.

Conclusions: The NOAEL was determined to be 7.5 g/kg bw/day Reducose® 1%, the highest dose tested, in male and female Sprague-Dawley rats.

6.3 Toxicology Studies Conducted on Related Substances

In additional to the above studies conducted using the article of commerce, several studies on other mulberry extracts or other DNJ containing substances have been published and are discussed below.

6.3.1 Other *Morus alba* Leaf Preparations

Genetic toxicity studies on various *M. alba* leaf preparations located are summarized in tabular format below. The study by Kim et al., was conducted as part of a larger assessment related to efficacy of the test item while the study by Chichioco-Hernandez et al. was conducted as part of a larger evaluation of a number of plants traditionally consumed in the Philippines. The study by de Oliveria et al. was part of a test battery conducted to evaluate both toxicity and efficacy of an ethanolic *M. alba* leaf extract. The studies by Wu et al., were conducted as part of a toxicological test battery on a test item that was a mixture of ingredients and included an undescribed *M. alba* leaf extract as one component. No evidence of genetic toxicity was observed in any of the reported studies.

Table 20. Genetic Toxicity Tests—Other *Morus alba* Leaf Preparations

Author	Test Item	Study Type	Design	Results
Kim et al. (2007) [28]	*M. alba* leaf methanol extracted phenolic-rich ethyl acetate fraction	BMRT	*S. typhimurium* strains TA98 and TA100 with and without S9 activation. Concentrations of 0, 0.5, 1, 2 and 4 mg per plate.	All concentrations demonstrated a mutation frequency below 2.0x the solvent control value and no dose-response was noted. The extract was determined non-mutagenic.
Chichioco-Hernandez et al. (2011)[29]	*M. alba* leaf methanolic extract	Vitotox® assay*	Two GE TA104 *S. typhimurium* strains TA104 with and without S9. 1/100 to 1/12,800 serial dilutions of 1 mg/mL stock solution. Light emission was recorded every 5 min. over 4h after the addition of extract concentrations.	*M. alba* leaf extract displayed no genotoxic or cytotoxic activity at any concentration.

de Oliveria et al. (2016)[30]	*M. alba* leaf ethanolic extract	MT	Male Swiss mice (5 per dose). Doses were 0, 75, 150 and 300 mg/kg bw. An additional group was administered the positive control, cyclophosphamide, by IP injection. Animals were sacrificed 48 hours after administration and peripheral blood was prepared for evaluation of MPCE/2000 PCE.	Observations during the 48h between dosing and sacrifice not reported. No SS increases in MPCE observed at any dose. Positive control induced statistically significant increase in MPCE.
Wu et al. (2017)[31]	*M. alba* leaf extract as 0.2% of a mixture	BMRT	*S. typhimurium* TA1535, TA100, TA98, TA1537, and TA102. Concentrations were 5.0, 2.5, 1.25, 0.6, and 0.3 mg/plate with and without S9. Concurrent positive (strain and ±S9 specific) and negative controls were run. All experiments conducted in triplicate.	No SS increase in mean revertants per plate in any strain at any concentration in the presence or absence of S9 (note, maximum concentration of the *M. alba* leaf extract was equivalent to 10 µg/plate). SS positive responses induced by all positive controls.
		CAT	Chinese hamster ovary cell cultures. Concentrations were 5.0, 2.5, 1.25, 0.6, and 0.3 mg/mL. Treatment/sampling times were 3/20h with & without S9 and 20/20 without S9. Concurrent positive (±S9 specific) and negative controls were run. 100 metaphases per culture (300 per concentration) were evaluated.	Chromosomal aberration frequencies were similar to controls at all test item concentrations with or without S9 under either of the treatment/sampling times (note, maximum concentration of the *M. alba* leaf extract was equivalent to 10 µg/mL). Clear positive responses induced by all positive controls. Note: authors did not report or otherwise indicate statistical analysis of the CAT results; it is unclear what criteria were used to judge results.

		MT	Male ICR mice (7/group). Doses were 0, 1000, 3000, and 5000 mg/kg bw. An additional group was administered the positive control, mitomycin C, by IP injection. At 24 and 48h post administration, peripheral blood was prepared for evaluation of MPCE/1000 PCE.	No test item-related mortality or clinical signs were observed at any dose level. No SS or dose-related increases in MPCE at any dose at either time point (note maximum dose of the *M. alba* leaf extract was equivalent to 10 mg/kg bw). Positive control induced SS increases in MPCE.

Abbreviations: BMRT, bacterial reverse mutation test; CAT, in vitro mammalian chromosomal aberration test; GE, genetically engineered; IP, intraperitoneal; MPCE, micronucleated polychromatic erythrocytes; MT, in vivo mammalian micronucleus test; PCE, polychromatic erythrocytes; SS, statistically significant.
*The Vitotox® assay is based on a genetically engineered bioluminescent reporter signal for bacterial SOS response

Several oral toxicity studies on various *M. alba* leaf extracts were located but were considered inadequate for interpretation due to inconsistencies and/or inadequacies in reporting. An acute oral toxicity test in rats was reported by Abdulla et al. (2009) as conducted according to OECD TG 423 but the accompanying citation was of OECD TG 425 (with incorrect date) and, as reported, the study did not follow either guideline.[32] It appears that six rats/sex/group were administered an ethanolic extract of *M. alba* leaf at doses of 0, 2, and 5 g/kg bw and observed mortality and clinical signs for 24h only. It does not appear that body weights were determined or that necropsy was performed. As part of a 2011 master's thesis, Kunuru reported an OECD TG 425 acute oral toxicity tests of aqueous extract and successive petroleum ether, chloroform, and 90% ethanol Soxhlet extraction of *M. alba* leaf.[33] Due to no observed toxicity, only the limit test, at 2000 mg/kg bw, was conducted; however, it appears the animals were observed only for 24h and that body weights were not determined and necropsy was not performed. A study by Laddha and Vidyasagar (2012) reported only that "Oral administration of methanolic, ethyl acetate soluble fraction (EASF) and acetate insoluble fraction (EAISF) of Morus Alba leaves up to 2000 mg/kg did not produce any toxic effect and no mortality was observed in mice" and that no deaths, adverse clinical signs or behaviors, or statistically significant effects on body weight, food consumption, water intake, blood pressure, limited clinical pathology parameters, or organ weights were observed in a subacute oral toxicity study in rats administered 0 or 2000 mg/kg EASF.[34] No methods were reported. Aditya Rao et al. (2013) reported conducting acute toxicity studies on a "hot soxhlet extraction of *M. alba* leaves utilizing petroleum ether, chloroform and methanol sequentially ... per the stair case method" (described in "Ghosh MN, Fundamentals of Experimental Pharmacology, 2nd edn. Scientific book agency,

Calcutta, 1984").[35] The LD_{50}s were reported as 2 g/kg bw in rats and mice of both sexes. No additional details were reported.

In an acute toxicity study by de Oliveria et al., conducted as part of a test battery to evaluate both toxicity and efficacy of an ethanolic *M. alba* leaf extract, no mortality, abnormal behavior, or effects on body weight or food and water intake were observed at intraperitoneal doses of 300 and 2000 mg/kg bw; however, toxic effects on hematological and clinical chemistry parameters and histology of the liver, kidneys, and spleen were observed at both doses.[30] These effects were not considered relevant to the evaluation of the intended use of Reducose® 5% due to the differences in route of administration and extraction solvent. Oral toxicity studies on various *M. alba* leaf extracts that were considered at least minimally adequate for interpretation are summarized below.

A 90-day oral toxicity study in rats of a hydroethanolic (50%) extract of *M. alba* leaves containing 1.1% DNJ was reported by Miyazawa et al. (2003).[36]

Methods: The extract was administered in the diet at concentrations of 0.1, 0.4, and 1% to groups of 10 SPF SD (IGS) rats/sex/group for 90 days. The control group received basic feed (CE-2 (Japan CLEA)) and all four groups had access to feed and water ad libitum.

The animals were observed daily for mortality and clinical signs. Body weights and food consumption were measured weekly. Following 90 days of exposure, all animals were fasted overnight and blood samples were obtained under anesthesia for clinical pathology (white blood cell count and percent differentials, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, total protein, albumin, nonesterified fatty acids, free and total cholesterol, total cholesterol, triglyceride, phospholipid, glucose, blood urea nitrogen, creatinine, uric acid, total bilirubin, AST, ALT, gamma-glutamyl transferase , ALP, inorganic phosphorus, calcium, and magnesium). Following sacrifice by exsanguination, organs and tissues were examined for gross abnormalities, and the brain, pituitary gland, thymus, heart, lungs, liver, spleen, kidneys, testes, adrenal gland, prostate, ovaries and uterus were weighed. Histopathological examinations were conducted on all of the above organs as well as the tongue, eyeball, harderian glands, salivary glands, thyroid glands, trachea, esophagus, aorta, stomach, duodenum, jejunum, ileum, colon, pancreas, mesenteric lymph nodes, bladder, skin, femoral muscle, bone marrow, epididymis, vesicular and coagulating glands, and vaginas in animals from the control and 1% dietary groups. Statistical analyses were performed, and results were considered statistically significant if $P<0.05$.

Results: No deaths or abnormal clinical signs or behavior occurred within the study period. No statistically significant differences in body weights were observed in the treated groups when compared to the controls throughout the treatment period. The authors noted a non-significant trend towards reduced weight gain in high-dose (1%) males after 10 weeks and a non-significant dose-response in weight gain in the mid- and low-dose male groups and all female groups compared to their respective controls. Females in the mid-dose (0.4%) group had a statistically significant increase in food consumption in the final week, but no dose-responses were observed in any groups. Overall body weight development was not statistically significantly affected in the treated groups compared to controls.

No statistically significant differences were noted in the hematological or biochemical parameters tested. No statistically significant differences were found between the treated groups and the control group with respect to organ weights and no test item-related gross pathological lesions were observed during necropsy. Mucosal thickening of the glandular stomach, without correlating histopathology, was observed in one animal of each sex in each group, including the controls.

Table 21. Summary of Histopathology—90-day Study, *M. alba* leaf extract (1.1% DNJ)

Histopathologic Findings		Males		Females	
Organs	Observations	Control N=10	1% N=10	Control N=10	1% N=10
Heart	Cellular infiltration	2/10	2/10	0/10	0/10
Kidneys	Mineralization	0/10	0/10	3/10	2/10
Liver	Microgranuloma	0/10	0/10	3/10	2/10
Lung	Perivascular cellular infiltration	1/10	1/10	1/10	0/10
	Medial calcification, pulmonary artery	0/10	2/10	1/10	0/10
Pancreas	Cellular infiltration	1/10	2/10	0/10	0/10
Prostate	Cellular infiltration	3/10	4/10	N/A	N/A

Abbreviations: N/A, not applicable.
Data represent the number of animals with observation per number of animals observed.
Organs without lesions in 10/10 control or high-dose animals not shown.

A few lesions of slight degree were observed in various organs during the histological examination; however, these findings occurred with similar incidence in both treated and control animals and did not differ statistically (see Table 21).

Conclusions: The NOAEL was determined to be the highest dose group (1%), which equated to approximately 884.5 mg/kg bw/day for male rats and 995.7 mg/kg bw/day for female rats.

Wu et al. (2018) conducted a 28-day oral toxicity study in rats according to OECD TG 407 using a multiple ingredient test item of which an *M. alba* leaf extract (MLE; not further characterized) comprised 0.2 percent.[31] Ten Wistar rat/sex/group were administered dose of 0,1000, 3000, and 5000 mg/kg bw/day (equivalent to 2, 6, and 10 mg/kg bw/day MLE) by gavage for 28 consecutive days. No mortality, clinical signs of toxicity, ophthalmological lesions, or statistically significant differences in body weight or food consumption were observed. A statistically significant increase in mean alkaline phosphatase was observed in female animals at the mid-dose; however, the increase was within the physiological range and without correlating findings. No statistically significant changes in other clinical pathology parameters or absolute or relative organ weights were observed, and no histopathological lesions were observed. The NOAEL was determined to be 5000 mg/kg bw/day (equivalent to 10 mg/kg bw MLE).

6.3.2 Other DNJ-Rich Substances

The diet of the silkworm (*Bombyx mori*), a monophagous caterpillar, consists entirely of *M. alba* leaves.[37, 38] Silkworm extract powder (SEP) containing 1.25% DNJ has been subjected to a battery of genetic and oral toxicity tests.[38] These are summarized below in tabular format. SEP was prepared from silkworm (strain YeonNokJam) larvae reared on spring leaves of *M. alba*; the 5th instar 3rd day larvae were frozen, lyophilized, extracted with ethanol, and lyophilized again, and the resultant test item was dissolved in "sterile distilled water" to prepare the test solutions for all experiments except the in vitro chromosomal aberration test, in which the test item was dissolved in complete medium. The studies were conducted in compliance with GLP according to OECD (specific guidelines not reported) and Korean Ministry of Food and Drug safety test guidelines under approval of the IACUC of Chemon Nonclinical Research Institute. Under the conditions of the experiments, the extract did not exhibit genotoxic potential or acute or subchronic oral toxicity in rats.

Table 22. Genetic Toxicity Tests—Silkworm Extract Powder (1.25% DNJ)

Author	Test Item	Study Type	Design	Results
Heo et al. (2013)[38]	SEP	BMRT	*S. typhimurium* TA100, TA1535, TA98, TA1537 and E. coli WP2 *uvrA*. Concentrations were 5000, 1500, 500, 150, 50, and 15 µg/plate with and without S9. Concurrent positive (strain and ±S9 specific) and negative controls were run. All experiments conducted in triplicate.	No increase in mean revertants per plate in any strain at any concentration in the presence or absence of S9. Clear positive responses induced by all positive controls.
Heo et al. (2013)[38]	SEP	CAT	Chinese hamster lung cell cultures. Concentrations were 0, 150, 300, 600, and 700 µg/mL without S9 (treatment/sampling times, 6/18h and 24/24h). Concentrations were 0, 275, 550, 900, and 1100 µg/mL with S9 (treatment/sampling times, 6/18h). Concurrent positive (±S9 specific) and negative controls were run. 100 metaphases per culture (200 per concentration) were evaluated.	No statistically significant increases were observed in the number of chromosomal aberrations at any concentrations with or without S9 under any of the treatment/sampling times. Clear positive responses induced by all positive controls.
Heo et al. (2013)[38]	SEP	MT	Male SPF Hsd.IRC CD-1® mice (6 per dose). Doses were 0, 1250, 2500 and 5000 mg/kg bw/day for 2 consecutive days. An additional group was administered the positive control, cyclophosphamide, by IP injection. Animals were sacrificed 24-hours after final administration and bone marrow smears prepared for counting PCE:RBC ratio and MPCE/2000 PCE.	No mortality or abnormalities were observed at any dose level. No statistically significant increase in MPCE at any dose. No statistically significant differences in PCE:RBC ratio at any dose. Positive control induced statistically significant increase in MPCE and decrease in PCE:RBC ratio.

Abbreviations: BMRT, bacterial reverse mutation test; CAT, in vitro mammalian chromosomal aberration test; MPCE, micronucleated polychromatic erythrocytes; MT, in vivo mammalian micronucleus test; PCE, polychromatic erythrocytes; RBC, total erythrocytes; SEP, silkworm extract powder (1.25% DNJ); SPF, specific pathogen free.

Table 23. Oral Toxicity Studies—Silkworm Extract Powder (1.25% DNJ)

Author	Test Item	Study Type	Design	Results
Heo et al. (2013)[38]	SEP	AOTS	SPF Hsd.Sprague-Dawley®™SD®™ rats (5/sex/group). Doses were 0, 1250, 2500 and 5000 mg/kg bw administered once. 14-day observation period, body weight measurements, gross pathology.	No mortality was observed. Soft stool observed in a few mid- (males) and high-dose (males & females) on day 2. No body weight effects. No necropsy findings. The LD_{50} was concluded to be >5000 mg/kg bw.
Heo et al. (2013) [38]	SEP	90-day RDOTS + 28-day recovery	SPF Hsd.Sprague-Dawley®™SD®™ rats (10/sex/group + 5/sex/ control and high-dose recovery groups). Doses were 0, 500, 1000 and 2000 mg/kg bw/day. Mortality & clinical signs daily. Body weight, food, and water consumption measured daily. Ophthalmology and UA on ½ main and all recovery animals last week of respective periods. Hematology, clinical chemistry, gross pathology, and organ weights (absolute and relative to body weight) on all animals. No histological examination was reported in methods but was reported in results and discussion.	No mortality, abnormal clinical signs, or ophthalmological lesions were observed. SS, but WHCR, ↑ in bw compared to C were reported in HD M towards the end of the study and throughout the recovery period. This is not obvious in the figure as the LD M bw are > HD M bw. DR ↑ in WBC (F) and ALP (F) were SS at the HD, but WHCR, w/o CH, and were R. A few SS UA parameters in M were w/o CH and were R. SS ↑ in M adrenal and left kidney weights at the LD and HD and relative liver weight at the HD were not clearly DR and were w/o CH. SS ↑ in absolute kidney, lung, brain, and liver weights observed in M after the recovery period were WHCR and were attributed to the ↑ in bw. Gross lesions observed in main or recovery animals at necropsy with CH occurred with similar frequency in HD & C animals or were considered individual findings due to their low frequency of occurrence and appearance in only main or only recovery group animals and known occurrence in untreated Sprague-Dawley rats. The NOAEL was determined to be 2000 mg/kg bw/day.

Abbreviations: AOTS, acute oral toxicity study; bw, body weight(s); C, control(s); CH, correlating histopathology; DR, dose-related; F, female(s); HD, high-dose; LD, low-dose; M, males(s); R, not present at the end of the 28-day recovery period. RDOTS, repeated-dose oral toxicity study; SEP, silkworm extract powder (1.25% DNJ); SPF, specific pathogen free, SS, statistically significant; UA, urinalysis; WBC, white blood cell count; WHCR, within historical control range; w/o, without.

6.4 Additional Scientific Studies

6.4.1 In vitro Studies

Stannard et al. (1988) investigated the effects of DNJ on thyroid stimulating hormone (TSH) synthesis, degradation, and secretion in mouse thyrotropic tumor and non-neoplastic mouse hypothyroid pituitary cell lines.[39] At concentrations up to 5 mM, DNJ did not inhibit combining of the alpha and beta TSH subunits or synthesis or intracellular degradation of the proteins. However, secretion of TSH was markedly decreased at both 1 and 5 mM concentrations in the hypothyroid pituitary cells. No general, nonspecific, toxic effects were observed, and DNJ did not significantly interfere with secretion of other evaluated anterior pituitary hormones (10 unidentified hormones were evaluated as well as two (growth hormone and prolactin) specific nonglycosylated hormones; however, other glycosylated hormones were not specifically evaluated.

Our literature searches for effects of DNJ on pituitary hormones in general or with respect to hypothyroidism did not result in any additionally relevant studies although, in a follow-on study, Stannard et al. confirmed their results and that TSH secreted in DNJ treated mouse hypothyroid pituitary cells is bioactive.[40] Furthermore, as described in Subpart 6.1, Nakagawa et al. (2007) observed a C_{max} of 92 μM (9.2×10^{-2} mM) DNJ following oral administration of 110 mg/kg bw DNJ to rats.[10] In addition, Kim et al. (2010), evaluated absorption of DNJ in rats at much lower doses that more closely approximate human exposure (3.72–6.60 mg/kg bw/day using 100% presence probability and 0.718–1.41 mg/kg bw/day using 10% presence probability; see Subpart 3.2).[5] Following administration of 3 and 6 mg/kg bw pure DNJ, maximum plasma concentrations observed were 8 μM (as calculated by AIBMR) and 25.66 μM, respectively, while following administration of approximately 6 mg/kg bw DNJ as a constituent of 1.7 g/kg bw MLE, C_{max} was 12.01 μM DNJ. Finally, Nakagawa et al. (2008) investigated absorption of 6.3 mg DNJ from 1.2 g MLE in humans and observed a C_{max} of approximately 3.2 μM.[15] These concentrations are far below the concentrations (1 and 5 mM) of DNJ used by Stannard et al. to produce in vitro effects on TSH secretion; thus, the results observed by Stannard are unlikely to have any clinical significance following oral ingestion of DNJ from Reducose® 5%.

6.4.2 Animal Studies

Oral administration of 600 mg/kg bw/day MLE to streptozotocin-induced diabetic rats for 21 consecutive days did not affect liver function (as assessed by serum ALT and ALP measurement) compared to saline controls.[41] Following 21 days of MLE administration, rats were given metformin (Met) at doses of 25, 50, or 100 mg/kg bw and blood was collected over 12h fasting period. MLE statistically significantly

potentiated the effect of Met on fasting blood glucose as compared to saline controls.

To further investigate this effect, the pharmacokinetics of a 50 mg/kg bw dose of Met were assessed following 21 days of administration of 600 mg/kg bw/day MLE or saline. C_{max}, T_{max}, and $t_{1/2}$ were similar among the MLE-treated and saline-control groups. AUC_{0-24} was increased 1.7-fold in the MLE-treated group compared to the saline-control; however, the increase was not statistically significant. In contrast, clearance of Met during the elimination phase was statistically significantly decreased (~50%) in the MLE-treated group compared to the saline-control.

In order to investigate the lowered elimination rate of Met, human embryonic kidney cells (HEK-293 cells) over-expressing human organic cation transporter 2 (hOCT2) were incubated with MLE (0.1, 1, 10, and 100 μg/mL) in the presence or absence of Met (2.5 μM), and hOCT2 inhibitor metoprolol (25 μM) was used as a positive control. The expected results were observed with the positive control compared to Met alone and MLE statistically significantly, concentration-dependently inhibited Met uptake by HEK-293 cells at concentrations of ≥1 μg/mL. In addition to the demonstrated effect, the authors also hypothesized that hepatic metabolism of Met may have been inhibited by caffeic and chlorogenic acid constituents of MLE.

The MLE assessed in this study was prepared by extraction of *M. alba* leaves suspended in water under pressure followed by enzymatic fermentation, presumably to increase availability of compounds of interest, before freeze-drying. The MLE was not assessed for DNJ content, but was reported to contain trans-caffeic acid, syringaldehyde and chlorogenic acid; thus, it is uncertain how similar this MLE is to Reducose® 5%. To the best of our knowledge, there have been no reports of drug-herb interactions between Reducose® 5% and Met.

6.4.3 Human Studies

Thirteen out of 16 clinical trials investigating various uses of *M. alba* leaf preparations that were located (including two unpublished trials provided by the proponent) also included safety relevant outcomes and/or reporting of adverse events. These are summarized in Table 24 below.

Table 24 Summary of Corroborative Clinical Trials

Author, Date	Test Item	Dose	Duration	Subjects	Design	Results
Kimura et al. (2007)[7]	MLE (ethanol: water (20:80%); 1.5% DNJ)	0 or 1.2 g TID (3.6 g daily)	38 days	12 healthy adults	RCT	Administration of MLE for 38 days did not cause hypoglycemia.
Asai et al. (2011)[42]	MLE (ethanol: water (20:80%); 1.5% DNJ)	0, 200, 400, or 600 mg	Single dose	10 adult subjects with IGT or T2D M:F = 8:2	RCT crossover; 2-week washouts Per-protocol analysis	No AEs observed. No effect on BP or HR or safety-related biochemical measurements (specific tests performed not reported).
		0 or 400 mg TID (1200 mg/day)	12 weeks	76 adult subjects with IGT or T2D M:F=50:26	RCT	No AEs in MLE group. 2 AEs in placebo group. No SAEs. No per protocol (n= 65) SS differences in BP, HR, or safety-related blood measurements (specific tests performed not reported)
Aramwit et al., (2011)[43]	*M. alba* leaf tablet (0.14% DNJ)	764 mg TID (2.3 g/day)	12 weeks	23 adults M:F = 4:19	Open-label within-subjects study design.	Mild GI effects during 1st week of treatment only (diarrhea (26%), dizziness (8.7%), constipation and bloating (4.3%)). No SAE. No adverse effects on liver function tests, FPG, or HbA1c. No hypoglycemia.
Kim et al. (2012)[44]	MLE (aqueous) + ginseng powder + banaba leaf extract (1:1:1)	0 or 667 mg MLE TID (2 g MLE daily)	6 months	94 subjects with IGT (n=67) or T2D (n=27) 31 withdrawn for non-AE reasons (incidence similar between groups).	RCT 4-week run-in. Per-protocol analysis	One withdrawal dt to mild AEs (GI discomfort, nausea, muscle ache, dry lips)—no additional AEs were reported by the authors. No SS effects on liver and kidney function tests. No SS effects on BP.

Author, Date	Test Item	Dose	Duration	Subjects	Design	Results
Kim et al. (2015)[45]	MLE (aqueous; 0.36% DNJ))	0 or 1.667 g TID (5 g/day)	4 weeks	42 adult subjects with IGT	RCT 4-week run-in.	No SAE observed. No SS differences that were clinically relevant in measured safety parameters (i.e., hematology and clinical chemistry).
Gallagher et al., (2015, unpublished)	Reducose 5% + different test meals	250 mg	Single dose per arm	12 healthy adults M:F 8:4	Open-label, crossover; 2-day washouts	No AEs observed or reported. No adverse effects on BP.
Trimarco et al., (2015)[46]	MLE + RYR + berberine	200 mg/day MLE	4 weeks	23 adults M:F=11:12	Randomized, double-blind, crossover (2 different combination products)	No AE were reported. No adverse effects on FPG, HbA1c, or FPI. No clinically evident hypoglycemia.
Lown et al. (2017)[47]	Reducose 5%	0, 125, 250, or 500 mg	Single doses	37 healthy adults	RCT crossover 2-day washouts	No SS differences in GI AEs at any dose compared to placebo.
Riche et al. (2017)[48]	MLE	0 or 1000 mg TID (3000 mg/day)	3 months	24 adult T2D on stable Tx regimen	RTC. 2-week run-in	4 withdrawals dt AEs (MLE, 1 stomach upset, 1 bloating; placebo, 1 stomach upset, 1 influenza). No SS differences in reported GI AEs. 2 SAE in placebo group. No complaints of severe or symptomatic hypoglycemia, & no SS differences in documented hypoglycemia; cumulative incidence <1%. No SS differences in BW, BP, AST, ALT, HCO_3, or electrolytes. SS ↑ in creatinine in MLE group compared to baseline and placebo; SS ↑ in BUN in MLE group compared to baseline only (↑s were WNL).

Author, Date	Test Item	Dose	Duration	Subjects	Design	Results
Wang et al. (2018)[49]	Reducose 1% ÷ different test meals	750 mg	Single dose per arm	15 heathy adults M:F = 9:6	Randomized open-label, crossover; 2-day washouts	No AEs reported. No abnormal results on vital signs, hematology, liver or kidney function tests, or FBG. No abnormal UA, stool analyses, or ECG results attributable to the test item.
Wattanathorn et al. (2018)[50]	MLE (aqueous) + *Polygonum odoratum* leaf extract	0, 50 or 1500 mg total (ratio of extracts not reported)	8 weeks	45 healthy older adult Thai females	RCT	No AEs reported. No adverse effects on hematological or biochemical parameters. Slight SS ↑s compared to placebo in platelet count at the high-dose and albumin at both doses were WNL and without clinical significance.
Thiapitakwong et al. (2020) [51]	*M. alba* leaf powder (0.26% DNJ)	0, 2.3, 4.6 & 6.9 grange finding; 0 or 4.6 g main	Single dose-range finding; 12 weeks-main	85 healthy adults-range finding; 59 obese hyperglycemic	Randomized open-label trial with nutritional control (no placebo group)	Reported AEs were bloating and flatulence, loose stools, and constipation. One subject withdrew due to AEs. No serious AEs were reported. No adverse effects on liver or kidney function were observed.

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase ; BP, blood pressure; BUN, blood urea nitrogen; BW, body weight(s); DNJ, 1-deoxynojirimycin; dt, due to; ECG, electrocardiogram, F, female(s); FBG, fasting blood glucose (by finger stick); FPG, fasting plasma glucose; FPI, fasting plasma insulin; GI: gastrointestinal; HbA1c, glycosylated hemoglobin; HCO_3, bicarbonate; HR, heart rate; IGT, impaired glucose tolerance; M, males(s); MLE, mulberry leaf extract; RCT, randomized double-blinded placebo-controlled trial; RYR, red yeast rice; SAE, serious adverse event; SS, statistically significant; T2D, type 2 diabetes(ic); TID: three times daily; Tx, treatment; UA, urinalysis; WNL, within normal limits.

In addition to the above studies located in our searches, or provided by the proponent, a 2016 meta-analysis of 13 clinical trials (several of which are included in Table 24 above) detected no significant differences in relative risk and no heterogeneity in pooled analysis of adverse events reported in two of the 13 trials included, in which any adverse events were reported.[52] The reported adverse events were headache, nausea, unusual fullness, and diarrhea, and no serious adverse events were reported. In pooled analysis of laboratory results from three of the 13 trials, mean differences in blood urea nitrogen (BUN; assayed in only 2 of the 3

trials), creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were not statistically significance; additionally, the mean differences in creatinine, AST, and ALT were in the direction opposite of concern. Heterogeneity was detected between the trials for BUN, creatinine, and AST measurements. Nonetheless, the reported mean differences of the pooled analyses were small in magnitude. One of the three trials, Kim et al., 2015, included is reported in Table 24 while one was unpublished, and one was an evaluation of a six-ingredient test item of which mulberry leaf comprised 10%. In the trial by Kim et al., BUN was statistically significantly increased in the MLE group compared to placebo at both baseline and the 4-week evaluation; however, all values were within normal limits in both groups at both time points.[45] The other trial that evaluated BUN is unpublished and was not available for our direct review. No significant differences in creatinine, AST, or ALT were observed by Kim et al. or the multiple ingredient formulation trial[53]; thus, the meta-analysis, does not raise concerns with respect to effects of MLE on kidney or liver function.

6.5 Authoritative Safety Opinions

6.5.1 Food and Agriculture Organization of the United Nations

The Food and Agriculture Organization of the United Nations has reviewed the utilization of mulberry leaves and their potential for use as animal feed several times, most recently in 2000.[54] In this review, the organization cited various studies from around the world that found:

- In five-day-old dairy heifers reared for 112 days with restricted suckling, a replacement of commercial concentrate with up to 50% mulberry leaves did not affecting heifer performance, and the mulberry leaves improved total dry matter intake.
- Due to its superior palatability and lack of thorns, in central Italy, *M. alba* is preferred over other investigated shrubs for feeding cattle and sheep during the summer months when there are forage gaps.
- In India, 15–20 kg mulberry leaves as cattle fodder improved milk yield and quality. It was also reported that up to 6 kg of leaves per day did not adversely affect the health of animals or the yield and butter content of milk.
- In Japan, Haugh unit (a measure of the internal quality of the egg) and yolk color were higher and there was a greater proportion of yolk in eggs from domestic New Hampshire hens and guinea fowls fed mulberry compared to commercially available eggs from White Leghorn hens; mulberry leaf in the feed also increased the vitamin K1 content and decreased lipid peroxide content in yolks. Mulberry leaves used in poultry rations at levels up to 6%

do not have adverse effects on body weight or egg quality (egg production and yolk color were both improved).

- In growing pigs, mulberry leaf at 15% of diet increased daily gains compared to commercial concentrate.
- In Angora rabbits, supplementation of mulberry leaves up to 40% of dry matter in the diet was advantageous for wool production.

6.6 Allergenicity

Reducose® 5% does not contain or have added any of eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally, Reducose® 5% does not contain gluten, oats, celery, mustard, sesame seeds, or sulfur dioxide and sulfites.

Although allergy to *M. alba* fruit has been reported, such hypersensitivity reactions are considered rare. The potential for cross reactivity between Moraceae family members *M. alba* and fig (*Ficus carica*) fruits has been hypothesized.[55] One published case report indicated that a woman with hypersensitivity (extrinsic asthma and rhinitis) to several pollens and oral allergy syndrome caused by fruits (Rosaceae), reported several episodes of asthma when she was near mulberry leaves and an anaphylactic reaction after exposure to *M. alba* fruit.[56] No reports of primary allergy of *M. alba* leaves were discovered.

6.7 History of Consumption

M. alba leaves have an extensive history of consumption. Their use in China has been reported as early as A.D. 659.[57, 58] Traditional use of *M. alba* is also well-known in the Middle East and has been documented in other countries such as Japan, Chile, Spain, Turkey, Yugoslavia, Peru, and France,[59, 60] as well as South Korea where it is regulated as a permitted food ingredient.[61]

In some Asian countries, *M. alba* leaf is consumed as a tea; the leaf "juice" is served as a traditional drink, and the leaves are considered a food and used within the food industry.[1,10, 57] Indian cultures use *M. alba* leaves in traditional dishes such as curry, saag, pakoda, paratha, and dhokla and in the preparation of spices.[1, 62]

6.8 Past Sales and Reported Adverse Events

According to Phynova, 1015 kg of the company's Reducose 5% have been sold since its market introduction in 2018. Total sales within the U.S. are approximately 700 kg with the remaining 315 kg sold divided between China (250 kg) and the EU

(65 kg). Phynova states that no adverse event reports associated with the consumption of this ingredient to date have been received by the company.

No FDA letters regarding concern for safety to companies that market products containing mulberry leaf extracts in general, Reducose® specifically, or DNJ were located. A search of FDA's Recalls, Market Withdrawals, & Safety Alerts search engine, and FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System (CAERS) located four adverse event reports (AER) associated with mulberry containing products. There were no additional reports related to Reducose® specifically or DNJ located in our search. All databases were accessed on September 16, 2020.

CAERS currently contains records of 131,261 AERs submitted to FDA from January 2004 through March 31, 2020 (the date of the last data set release). Thus the frequency of occurrence within the CAERS data set is 0.003%. The two most recent reports were associated with use of an organic mulberry juice (note, mulberry fruit, from which juice is derived, is chemically dissimilar to mulberry leaf) and consisted of respiratory complaints (age and sex not reported) and feeling abnormal with increased blood lead and arsenic, insomnia, tremor, and memory impairment (71 year old male), respectively. The latter was reported as serious. The two earlier reports were both associated with mulberry leaf extracts (the most recent was a multiple ingredient supplement). Both occurred in elderly females (77 and 63 years old), and both involved serious adverse events. The first reported, renal disorder, hypotension, thrombosis, myocardial infarction, diabetes mellitus, and gallbladder disorder while the second reported hypoaesthesia.

Importantly, AERs are only associations, and reported products may not be causally related to the AE. CFSAN notes the following:

> *"The adverse event reports about a product and the total number of adverse event reports for that product in CAERS only reflect information AS REPORTED and do not represent any conclusion by FDA about whether the product actually caused the adverse events. For any given report, there is no certainty that a suspected product caused a reaction. Healthcare practitioners, firms, agencies, consumers, and others are encouraged to report suspected reactions; however, the event may have been related to a concurrent underlying condition or activity or to co-consumption of another product, or it may have simply occurred by chance at that time."*

Additionally, it is noted that AERs vary in quality and reliability and CAERS may contain duplicate reports.

6.9 Current Regulatory Status

A thorough search for the current regulatory status of *M. alba* or its extracts, relevant to their use in food in the United States, was conducted. A summary of the pertinent search results is shown below:

- An FDA GRAS notice (GRN No. 000013) was found in the FDA GRAS Notices Inventory database for use of nine botanical ingredients, one of which was *M. alba*, as flavoring agents in herbal tea beverages. The basis of the GRAS conclusion was through experience based on common use in food. GRN No. 13 received FDA's 'no questions' response letter with respect to three of the notified botanicals on June 2, 1999; however, *M. alba* was among the six botanicals that were considered by the Agency to have insufficient history of use data to establish reasonable certainty of no harm for their intended use.
- Pursuant to 21 CFR part §184.1444 maltodextrin is GRAS for human consumption with no limitation other than current good manufacturing practice.

6.10 Basis for the GRAS Conclusion

Reducose® 5% has been the subject of a thorough safety assessment as described above. The totality of evidence supporting safety is comprised of data and information that establish the safety of Reducose® 5% under the conditions of its intended use and data and information that is corroborative of safety. The general availability and general acceptance, throughout the scientific community of qualified experts, of the data and information that establish the safety of Reducose® 5% under its intended conditions of use establish the general recognition of this data and information. Together, the establishment of safety based on scientific procedures and its general recognition form the basis for Phynova's conclusion of GRAS status of Reducose® 5% for its intended use.

6.10.1 Data and Information that Establish Safety

The scientific data, information, and methods forming the basis of this conclusion are:

- The establishment of identity, demonstrating that Reducose® 5% is well characterized extract of *Morus alba* leaves containing 5 ± 0.5% DNJ, and spray dried on a maltodextrin carrier, which comprises approximately half of the final ingredient weight;
- The method of manufacture and specifications, demonstrating the safe production and robust quality control standards of Reducose® 5%;

- Known pharmacokinetic parameters of the DNJ marker, demonstrating reasonably similarities in laboratory animals and humans;
- The 28-day repeated-dose oral toxicity study in rats and dietary exposure estimate, establishing the lack of adverse health effects and or target organs of repeated exposure to Reducose® 5% in rats, and establishing an adequate margin of safety (MOS) for the intended conditions of use by humans of Reducose® 5% in food.

In the 28-day study, the NOAEL was 4000 mg/kg bw/day in male and female SPF Hsd.Han Wistar rats; the highest level tested. As the test item of the 28-day study contained L-leucine as a processing aid at an addition level of 6.5%, the equivalent NOAEL adjusted for the L-leucine content was 3740 mg/kg bw/day (4000 x 93.5%). Additionally, in terms of DNJ only, the NOAEL was 186 mg/kg bw/day (4000 x 4.65%) as the test item contained 4.65% DNJ. Based on the intended use of the ingredient in food in the categories and at the addition levels shown in Table 7 (also duplicated as Table 1), the NOAEL allows for an adequate MOS (NOAEL/Exposure; 4000 mg/kg/13.1 mg/kg) of approximately 305-fold in the general population when compared to the estimated human exposure level at the 90th percentile of consumers using a 10% presence probability factor, which supports a conclusion that the intended use of Reducose® 5% is reasonably certain to be safe. When adjusted for the added L-leucine, the MOS (3740 mg/kg/13.1 mg/kg) is approximately 258-fold and when expressed in terms of DNJ content (13.1 mg/kg x 4.5–5.5%) the MOS (186 mg/kg/0.590–0.722 mg/kg) ranges from approximately 258- to 315-fold. As Reducose® 5% is standardized to contain 5 ± 0.5% DNJ, the addition or removal of L-leucine does not impact the findings of the toxicology studies as the same amount of mulberry leaf extract (65.5%) and DNJ (4.65%) would have been present in the neat test item with or without the use of L-leucine, which would have been replaced with maltodextrin. Thus, regardless of L-leucine content, there is an adequate MOS and the conclusion that the intended use of Reducose® 5% is reasonably certain to be safe is supported.

6.10.2 Data and Information that is Corroborative of Safety

The safety of Reducose® 5% is corroborated by an acute oral toxicity study in mice in which the LD_{50} was >5 g/kg bw. The safety of Reducose® 5% is also corroborated by toxicological tests on Reducose® 1% (a related ingredient produced by Phynova with a lower DNJ content) in which a bacterial reverse mutation test and in vivo mammalian micronucleus test collectively demonstrated a lack of genotoxic potential of the ingredient, a sperm deformity test in mice in which no adverse effects on sperm morphology were observed at doses up to 10 g/kg bw for five days, and no general toxicity was observed in 14-day and 30-day repeated-dose oral

toxicity studies in rats in which the MTD and NOAEL were determined as ≥15 g/kg bw/day and 7.5 g/kg bw/day, respectively. Additionally, the safety of Reducose® 5% is corroborated by toxicological studies on other *M. alba* leaf preparations (with and without known DNJ contents) and other substances rich in DNJ. Finally, the safety of Reducose® 5% is further corroborated by the lack of serious adverse events reported in clinical trials using Reducose® 5% or other *M. alba* leaf preparations at daily dosages up to 5 g and durations up to 6 months, and the history of human consumption of approximately 1015 kg of Reducose® 5% over a one-year period with no adverse event reported.

6.10.3 General Recognition

The scientific data, information, and methods herein reported, that provide the basis of this GRAS conclusion by scientific procedures are published and available in the public domain. Part 7 of this GRAS notice contains the citations for the published studies. These publicly available data and information fulfill the requirement of the GRAS standard for general availability of the scientific data, information, and methods relied on to establish the safety of Reducose® 5% for its intended conditions of use. The peer-review of the published studies and lack of Letters to the Editor or other dissenting opinions provide ample evidence of general recognition among qualified experts that there is reasonable certainty that consumption of Reducose® 5% for its intended use is not harmful. The general availability and acceptance of these scientific data, information, and methods satisfy the criterion of the GRAS standard that general recognition of safety requires common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food that there is reasonable certainty that the substance is not harmful under the conditions of its intended use.

6.10.4 Data and Information that are Inconsistent with the GRAS Conclusion

In the diabetic rat drug interaction study by Huh et al. (2020), an MLE of unknown similarity to Reducose® 5% reduced clearance of Met in diabetic rats, possibly due to inhibition hOCT2 and/or hepatic cytochrome P450s. The study was discussed and placed in context in Subpart 6.4.2.

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

6.10.5 Information that is Exempt from Disclosure under FOIA

There are no data or information in this GRAS notice that are considered exempt from disclosure under FOIA as trade secret or commercial or financial information that is privileged or confidential.

Part 7: Supporting Data and Information

Initial literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted from October 2014 through November 2014. Additional literature searches were conducted from May 2015 through October 2015, January 2016 through October 2016, during March 2018, again from June 2019 through October 2019, and again on September 16, 2020.

7.1 Data and Information that are *not* Generally Available

Some of the data and information described in this GRAS Notice are unpublished and, therefore, are not generally available, as follows:

- The clinical trial PYN-IM-002a of Reducose 5% by Gallagher et al. (2015)
- The clinical trial PYN-IM-003 of Reducose 5% by Thondre et al. (2016)
- Sales and adverse event data reported by Phynova

The data and information cited above strengthen the weight of evidence and, thereby, corroborate the data and information that establish the safety of Reducose® 5% under the conditions of its intended use. We believe the safety conclusion can still be made even if qualified experts throughout the scientific community do not generally have access to this information.

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From: Tim Murbach
To: Hall, Karen
Subject: Re: [EXTERNAL] Re: Regarding GRN 000992
Date: Wednesday, August 18, 2021 10:49:34 AM
Attachments: Questions to Notifier_2021-08-02+Notifier Responses_2021-08-18.pdf

Hi Karen,

Please find our responses attached. I have set the responses in red font for ease of review (i.e., to help clearly delineate the responses from queries).

Please let me know should there be need of clarification or further queries.

Kind Regards,
Tim

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From: "Hall, Karen" <Karen.Hall@fda.hhs.gov>
Date: Thursday, August 12, 2021 at 2:58 PM
To: Tim Murbach <tim@aibmr.com>
Subject: RE: [EXTERNAL] Re: Regarding GRN 000992

Hi Tim,

I am good with August 20th.

Kind Regards,
Karen

From: Tim Murbach <tim@aibmr.com>
Sent: Thursday, August 12, 2021 3:21 PM
To: Hall, Karen <Karen.Hall@fda.hhs.gov>
Subject: [EXTERNAL] Re: Regarding GRN 000992

Hi Karen,

I am writing to ask if we can receive an extension on the requested 10 business day timeframe.

We are well along the road to preparing responses, but I have to send draft responses to the Notifier in Australia for input and review who also has to then forward aspects to various colleagues in the United Kingdom. So each draft has to go back and forth across the International Date Line twice, which takes time.

By my calculation, the original timeframe was to have responses back to you by end of business on Monday August 16. I think if we could have until the end of the week (i.e., August 20), it would be sufficient although I anticipate having in completed earlier.

Kind Regards,
Tim

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From: "Hall, Karen" <Karen.Hall@fda.hhs.gov>
Date: Monday, August 2, 2021 at 11:41 AM
To: Tim Murbach <tim@aibmr.com>
Subject: Regarding GRN 000992

Good Afternoon Dr. Murbach,

After reviewing Phynova's GRAS Notice 000992 for the intended use of white mulberry leaf extract, we noted some concerns attached to this email that need to be addressed. Responses may be sent in an email or in a separate document. Please do not send a revised copy of the notice. We respectively request a response within 10 business days. If you are unable to complete the response within that time frame or have questions, please contact me to discuss further options at 240-402-9195 or via email.

Kind Regards,
Karen
Karen Hall
Regulatory Review Scientist
Division of Food Ingredients
Office of Food Additive Safety

Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Karen.Hall@fda.hhs.gov

GRN 992 White Mulberry Leaf Extract Questions

Chemistry

1. On page 10 (Table 2), the notifier provides compositional data for mulberry leaf extract.

    - Please confirm that the total iminosugar content includes 1-deoxynojirimycin (DNJ).
      Notifier Response: We confirm that the total iminosugar content includes 1-deoxynojirimycin (DNJ)

    - The notifier reports that total carbohydrates are expected to comprise 30-55% of the finished extract formulation. However, in GRN 000984 (page 10, Table 2), the notifier reported that the finished extract formulation contains 30-50% maltodextrin in addition to 27-29% carbohydrates. Please clarify whether the total carbohydrate content of 30-55% as reported in GRN 000992 includes maltodextrin. We note that the notifier states on page 56 of GRN 000992 that approximately half of the weight of the finished extract formulation is maltodextrin.
      Notifier Response: We confirm that the total carbohydrate content of 30–55% includes the 28–50% maltodextrin.

    - The notifier reports the content of "Free amino acids/peptides/proteins" (i.e., total amino acids) in the finished extract formulation to be 25-35%. We note that in GRN 000894 the notifier reported the content of "Amino acids" to be 13-15%. Please confirm that the term "Amino acids" in GRN 000894 referred to free amino acids only, not the total amino acids. If this is not correct, please explain the increase in the total amino acid content in GRN 000992 compared to GRN 000894.
      Notifier Response: We confirm that the term "Amino acids" in GRN 894 referred to free amino acids only. At the time of submission of GRN 894, there were only limited nutritional data. As more data had become available since the submission of GRN 894, Table 2 was revised in GRN992.

2. On page 10 (Table 3), the notifier provides an incorrect CAS Registry Number for DNJ. Please provide the correct CAS number.
   Notifier Response: The CAS number for DNJ is 19130-96-2. The zero reported at the penultimate position in the GRN was a typo ('0' is next to '–' on the keyboard).

3. On page 13, the notifier states that "other raw materials" used in the manufacture of mulberry leaf extract are food grade. Please confirm that the only raw plant material used in the manufacture of the extract is the mulberry leaf and that by "other raw materials" the notifier means materials such as water, ammonia solution, filters, or ion-exchange resins used in the manufacture of the extract. In addition, please provide a statement that all materials used in the manufacturing process are approved for their respective uses *via* a regulation in Part 21 of the U.S. Code of Federal Regulations, are the subject of an effective food contact notification, or are GRAS for that use in the U. S.
   Notifier Response: Mulberry leaf is the only raw botanical material used in the manufacture of Reducose® 5%. Other raw materials referred to in the second paragraph of Subpart 2.2.3 of GRN 992 on page 13 mean materials such as water, ammonia solution, filters, or ion-exchange resins used in

the manufacture of the extract. These, with the exception of water, are approved for their respective uses via a regulation in 21 CFR as follows:

- Water: Except for the requirements for specific standardized beverages pertaining to bottled water, no direct regulation in Part 21 of the U.S. Code of Federal Regulations, effective food contact notification, or GRAS conclusion was located. Nonetheless, potable, distilled, and purified waters are considered foods appropriate for human consumption. As noted in Subpart 2.2.3 on page 13, potable water used for extraction is subject to monthly and annual testing and complies with regulations for human drinking water.
- Ion-exchange resins used in the manufacture of Reducose® 5% are approved secondary direct food additives permitted in food for human consumption pursuant to 21 CFR 173.25
- Filters used in the manufacture of Reducose® 5% are approved indirect food additives pursuant to 21 CFR 177.2910.
- Ammonium hydroxide (NH_4OH; CAS Reg. No. 1336-21-6) is a direct food substance that is GRAS for use as a pH control agent with no limitations except that use levels do not exceed cGMP pursuant to 21 CFR 184.1139.
- Sodium hydroxide (NaOH; CAS Reg. No. 1310-73-2) is a direct food substance that is GRAS for use as a processing aid with no limitations except that use levels do not exceed cGMP pursuant to 21 CFR 184.1763.
- Hydrochloric acid is a multi-purpose food substance that is GRAS for use as a buffer and neutralizing agent with no limitations except that use levels do not exceed cGMP pursuant to 21 CFR 182.1057

4. On pages 13-14 (Table 4), the notifier provides specifications for the mulberry leaf extract and identifies an analytical method for each parameter. Please provide a statement that all analytical methods used to test for each parameter are validated for that purpose.
   Notifier Response: All analytical methods used to test each parameter of the Reducose® 5% finished product specification are validated for their intended purposes.

5. On pages 15-16 (Tables 5 and 6), the notifier provides results of batch analyses for the extract either containing or not containing L-leucine, respectively. We note that only the extract that does not contain L-leucine is the subject of GRN 000992. According to Table 5, none of the four provided batch analyses for this extract include the results for all specification parameters (e.g., batch ML20110420 was not tested for mercury, cadmium, total coliforms, or aflatoxins; batch NB6556-l was not tested for moisture, acid insoluble ash, taste and odor, or solubility). In addition, none of the four batches in Table 5 were tested for pesticide residues (included as a specification), and three batches were tested according to the previous version of a specification for total aflatoxin that did not include aflatoxins G1 and G2. To demonstrate that the subject of GRN 000992 can be manufactured to meet the proposed specifications, please provide the results of the analyses for a minimum of three nonconsecutive batches for all parameters included in the specifications established by the notifier for the mulberry leaf extract.
   Notifier Response: Table 4 of GRN 992 represents the complete (i.e., safety-related and non-safety related) current product specifications applicable to the finished Reducose® 5% ingredient. These specifications were not altered by the removal of the use of L-leucine in the manufacturing process. Batch analyses detailed in Tables 5 and 6 are historic batches and demonstrate the reproducibility of the production process, with or without L-leucine. Removal of L-leucine does not change any specification attribute, rather it was used to increase spray drying yield (less sticking to walls). We

direct FDA to the introductory text of Subpart 2.3.1 for a more detailed discussion of the historic batch analyses provided. Due to production cycle of Reducose® 5%, new lots without L-leucine are not yet available. Nonetheless, if considering only the subset of specification parameters that bear directly on safety, batch analyses NB6556-1, NB6556-2, and NB6556-3 provide a complete analysis of three lots without L-leucine. Batch testing of lot ML20110420 was provided to demonstrate that lots without L-leucine meet the non-safety sensitive parameters set by the Notifier (i.e., moisture, ash, taste and odor, and solubility), which are tested on a skip-lot basis. As noted in our responses to chemistry queries 6 and 7 below, the specifications for pesticide residues and aflatoxins are customer-requested parameters that are unnecessary for food uses in the U.S. and do not bear on the safety of Reducose® 5%. Also, as noted in our response to Toxicology query #6, the GRAS review team for GRN 894 concluded the presence or absence of L-leucine has no bearing on safety (which includes the lack of effect/relevance to all ingredient specifications that bear on safety, as discussed above).

6. On pages 13-14 (Table 4), the notifier includes pesticide residues as a specification for mulberry leaf extract. Please clarify the basis for proposing this specification. We note that we generally ask that notifiers not include a specification for pesticide residues for ingredients manufactured using food-grade plant materials produced in accordance with good agricultural practices. Please clearly indicate that the notifier would not expect these impurities to be introduced by the controlled method of manufacture of mulberry leaf extract. We also note that limits specified in USP 561 are not applicable in the U.S. when articles of botanical origin are labeled for food purposes.
Notifier Response: We were not aware of the general advise above and highly appreciate the feedback. With respect to GRN 992 and FDA's query above, we do not expect any pesticide residue impurities to be introduced by the controlled method of manufacture of Reducose® 5%, and, additionally, do not consider the pesticide specification as a parameter necessary to ensure the safety of the finished ingredient. Rather, this specification for pesticide residue testing to ensure that the finished ingredient compiles with the limits of USP 561 has been incorporated at the request of specific customers. We additionally note that the raw botanical material's supplier specification requires analysis of pesticide residues, and as stated in the footnote to Table 4, all raw material is tested for pesticides prior to purchase and entering the supply chain.

7. On pages 13-14 (Table 4), the notifier includes aflatoxins B1 and total aflatoxins as specifications for mulberry leaf extract. Please clarify the basis for proposing these specification and state whether the notifier expects aflatoxins to be present in the finished mulberry leaf extract manufactured following current good manufacturing practices.
Notifier Response: As with the pesticide specification, the specifications for aflatoxins were added due to specific customer requests. Aflatoxins are not expected to be present in the finished ingredient—Reducose® 5%— manufactured following current good manufacturing practice and a hazard analysis and critical control point plan. Additionally, aflatoxin specifications are not considered to be parameters necessary to ensure the safety of the finished ingredient; aflatoxins are skip-lot tested only as their presence is not an identified risk.

8. On pages 18-19 (Table 7), the notifier provides use levels for mulberry leaf extract for all food categories included in the intended uses. On page 56, the notifier states that maltodextrin comprises approximately half of the weight of the finished extract formulation. Our understanding is that the use levels in Table 7 represent the use levels of the finished extract formulation containing approximately 50% maltodextrin. Please confirm that this is correct.
   Notifier Response: We confirm that the use levels in Table 7 represent the use levels of the finished extract formulation containing 28–50% maltodextrin.

Toxicology

1) There are many studies demonstrating that 1-DNJ is an inhibitor of alpha-glucosidase and have documented physiological effects regulating blood glucose levels.[12] We note that according to the notifier's website: "A single dose will be effective for an entire meal regardless of carbohydrate content."[3] Please provide a narrative as to why acute and/or chronic exposure to 1-DNJ from the intended use is not a safety concern, especially for those subpopulations that rely on medications to regulate blood glucose levels.

   Notifier Response: Carbohydrates, including sugars, are not essential/indispensable nutrients. Thus, there is no reason to assume that altering the glycemic index of foods/blocking digestion of dietary starches and disaccharides would present a safety concern any more so than would eliminating carbohydrates from the diet. Undigested carbohydrates in the gastrointestinal tract could be expected to cause transient increases in gastrointestinal side effects similar to increasing fiber in the diet.

   In the clinical trial by Kimura et al., cited above by FDA, the use of mulberry leaf powder containing 18 mg 1-DNJ did not cause hypoglycemia or alterations in average plasma glucose compared to placebo over 38 days in healthy subjects even though postprandial plasma glucose and insulin were statistically significant lower compared to placebo 60 minutes following acute administration of the powder at doses containing 12 or 18 mg 1-DNJ. Similarly, in the cited clinical trial by Lown et al., acute administration of Reducose® 5% at doses containing 12.5 or 25 mg 1-DNJ had statistically significant lowering effects compared to placebo on the postprandial glucose and insulin responses (as determined by positive incremental area under the respective curves) in healthy subjects. At no time during the 120 minutes of plasma sample collection did points along the glucose curve fall below lower limit of the normal range (i.e., 70 mg/dL (3.9 mmol/L) indicating that acute administration of the mulberry leaf powder, containing up 25 mg 1-DNJ, did not result in hypoglycemia. In addition, the occurrence of gastrointestinal side effects did not differ statistically among the Reducose® 5% and placebo groups.

   In the 28-day repeated dose study by Marx et al. (Marx et al., 2016), summarized in Subpart 6.2.2 of GRN 992 (pages 31–36) and incorporated here by reference, Reducose® 5% did not cause biologically or toxicologically significant or dose-related alterations in plasma glucose or other adverse effects in rats at doses containing up to 186 mg/kg bw/day 1-DNJ.

   Several clinical trials have administered mulberry leaf extracts (MLE) of varying 1-DNJ content to subjects with impaired glucose tolerance (IGT) and/or type 2 diabetes mellitus (T2D) (Asai et al., 2011; Kim et al., 2012; Kim et al., 2015; Riche et al., 2017; Thaipitakwong et al., 2020). These studies are summarized in Table 24 in Subpart 6.4.3 of GRN 992 (pages 50–52), which is incorporated here by reference.

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[1] Kimura T, Nakagawa K, et al. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses theelevation of postprandial blood glucose in humans. *J Agric Food Chem.* 2007;55(14):5869-74.

[2] Lown M, Fuller R, et al. Mulberry-extract improves glucose tolerance and decreases insulin concentrations in normoglycaemic adults: Results of a randomised double-blind placebo-controlled study. *PLoS One.* 2017;12(2):e0172239.

[3]https://www.bioriginal.com/products/reducose-mulberry-leaf-extract

Use of any medications to regulate blood glucose levels was an exclusion criterion in the trials by Asai et al. (2011), Kim et al. (2015), and Thaipitakwong et al. (2020). No serious adverse events or statistically significant differences compared to placebo in fasting blood glucose, HbA1C, glycated albumin, or safety measures monitored occurred in the studies by Asai et al. and Kim et al. in which subjects received MLEs containing 18 mg 1-DNJ daily for 12 or 4 weeks, respectively. In the study by Thaipitakwong et al., no hypoglycemia or serious adverse events were reported following 12-weeks of daily administration of MLE containing 12 mg 1-DNJ. Mild gastrointestinal symptoms were experienced by the majority of subjects receiving MLE (adverse events were not compared to the control group) but declined in incidence over the course of the study, as is typical with introduction of other nondigestible or poorly digestible carbohydrates, such as fiber and prebiotics, into the diet.

Use of insulin was an exclusion criterion in the study by Kim et al. (2012); however, the use of sulfonylureas, biguanides, or α-glucosidase inhibitors were not exclusion criteria. While it was not reported how many subjects were taking oral hypoglycemic agents or which agents they were using, it was reported that the type and dose was maintained throughout the study. The study intervention provided 2 g MLE daily (the 1-DNJ content was not reported) in combination with two other botanicals for six months. One subject receiving the intervention dropped out due to mild adverse effects; no other adverse events were reported, and no differences compared to placebo were observed in biochemical safety indices (creatinine, ALT, and AST). Fasting blood glucose and insulin did not differ between the intervention and placebo groups.

Specific inclusion criteria in the study by Riche et al. (2017) were a diagnosis of T2D with use of oral hypoglycemic single or combination therapy with no adjustments for at least two months and stable HbA1C. Exclusion criteria included the use of insulin therapy or an α-glucosidase inhibitor. 100% of enrolled subjects were taking metformin, 50% of intervention group subjects and 58% of placebo group subjects were taking a sulfonylurea, 25 and 42% of the respective group subjects were taking a dipeptidyl peptidase IV inhibitor, 17 and 33% were taking a thiazolidinedione , and 8 and 17% were taking either exenatide or colesevelam. Subjects ingested 3 g MLE daily (n = 12) or placebo (n = 12) for 3 months; however, the 1-DNJ content of the MLE intervention was not reported. As reported in Table 24, 1 subject in the placebo group and one subject in the MLE group withdrew due stomach upset and one subject in the MLE group withdrew due to bloating, and while gastrointestinal effects were the most commonly reported adverse effects, differences in incidence of gastrointestinal adverse events did not differ significantly between the groups. No serious adverse events occurred in the MLE group. The incidence of documented hypoglycemia did not differ between the groups (cumulative incidence < 1%) and no there were no complaints of severe or symptomatic hypoglycemia. There were no adverse effects on body weight, blood pressure, AST, ALT, bicarbonate, or serum electrolytes. While some statistically significant increases in kidney function tests (creatinine and BUN) were observed in the MLE group compared to placebo and/or baseline, they remained within normal ranges.

The above evidence, as well as the rest of the evidence reported in Part 6 of GRN 992, demonstrate that the intended use of Reducose® 5% by healthy individuals is not a safety concern. The above evidence also indicates that the intended use of Reducose® 5% by individuals with IGT or T2D who are not taking medications to regulate blood glucose levels is not safety concern. This is supported by the L-leucine-adjusted 258-fold margin of exposure (MOE) at the 90th percentile of consumers from the intended use of Reducose® 5% as compared to the NOAEL from the study by Marx et al. (2016). The above studies by Kim et al. (2012) and Riche et al. (2017) provide limited support for a lack of safety concern from the

intended use of Reducose® 5% by individuals take oral hypoglycemic medications although the 1-DNJ content of these MLEs is unknown.

To our knowledge, there have been no trials that have investigated the combine effects of insulin therapy and MLEs. Likewise, there have been no trials investigating the combined effects of pharmaceutical α-glucosidase inhibitors and MLEs. Nonetheless, as noted in GRN 992, Subpart 6.8 (pages 54–55), no adverse events associated with consumption of Reducose® 5% have been reported to Phynova since its market introduction in 2018 (note, Phynova independently concluded the GRAS status of the intended use of Reducose® 5% in late 2016) following sales of 1015 kg (700 kg of which were sold in the U.S.); since the submission of GRN 894, an additional 2435 kg have been sold (2345 of which was sold in the U.S.), also without any adverse event reports having been received by Phynova. While no data is available regarding the demographics of consumers, this still provides indirect evidence that there is not a safety concern in individuals taking medications to regulate blood glucose levels, either because no adverse events have occurred in individuals of this subpopulation consuming products containing Reducose® 5% or because individuals of this subpopulation actively avoid products containing Reducose® 5%. Furthermore, patients taking medications to regulate blood glucose levels should be under the care of a health care provider and instructed in potential adverse effects and interactions of the medications in use, proper self-monitoring of fasting and postprandial blood glucose, recognition and self-treatment of hypoglycemic symptoms (as well as when to seek medical intervention), and the need to advise their care provider of dietary changes and use of dietary supplements and functional foods intended for maintenance of healthy blood glucose.

2) Additionally, we have identified three derivatives of 1-DNJ, Miglitol, Miglustat and Migalastat, which are FDA-approved drugs for different indications. Furthermore, Miglitol (Glyset®) is contraindicated in certain patients[4]. In a paper by Reuser et al. (1994), the authors describe potential side effects of absorbable alpha-glucosidase inhibitors such as Miglitol. Importantly, they note that these inhibitors can accumulate in tissues in patients with renal impairment, rendering them susceptible to potential adverse effects such as glycogen accumulation. Please explain why exposure to 1-DNJ does not pose a safety concern in patients with renal disease or undiagnosed renal impairment. If 1-DNJ's absorption, distribution, metabolism, and excretion profile is expected to be different from derivates that are FDA-approved drugs, please provide data and information that support your conclusion.

Notifier Response: Pharmacokinetics of 1-DNJ were discussed in GRN 992, Subpart 6.1 (pages 26–30), which is incorporated here by reference. These data suggest that a fraction of orally administered 1-DNJ is rapidly absorbed and eliminated, intact, in the urine and does not bioaccumulate, while the unabsorbed fraction is eliminated in the feces; the absorbed fraction appears, based on limited data, to be greater in humans as compared to rats. Bioavailability appears to decrease with dose. The data further suggest that 1-DNJ is less bioavailable when administered as a constituent of an MLE compared to administration as a pure compound.

[4] Gylset® is contraindicated in patients with diabetic ketoacidosis; inflammatory bowel disease, colonic ulceration, or partial intestinal obstruction, and in patients predisposed to intestinal obstruction; chronic intestinal diseases associated with marked disorders of digestion or absorption, or with conditions that may deteriorate as a result of increased gas formation in the intestine (https://pfizermedicalinformation.com/en-us/glyset/contradictions)

To the best of our knowledge, accumulation of 1-DNJ has not been specifically investigated in subjects with renal impairment; however, because renal excretion is the primary route of elimination of absorbed 1-DNJ, it is expected that excretion would be reduced in people with advanced kidney disease, possibly leading to tissue accumulation. While we disagree that miglitol, a pharmaceutical derivative of 1-DNJ is comparable to 1-DNJ itself, as FDA's GRAS team has made the comparison above, we note that miglitol's package insert (https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/020682s010lbl.pdf) states, "Patients with creatinine clearance <25 mL/min taking 25 mg 3 times daily, exhibited a greater than two-fold increase in miglitol plasma levels as compared to subjects with creatinine clearance >60 mL/min." Use of miglitol is not recommended in people with creatinine clearance <25 mL/min; however, there is not contraindication or recommendation against its use in people with creatinine clearance >25 mL/min. Creatinine clearance <25 mL/min occurs in patients with stage 4 chronic kidney disease (CKD) or worse. Stage 4 and 5 CKD patients should be under the regular care of a nephrologist and a dietitian, and patients who have progressed to kidney failure require dialysis to remove metabolic waste products and other waste materials from circulation. In other words, individuals in whom theoretical accumulation of 1-DNJ might occur following exposure to Reducose® 5% are expected to be under close medical supervision to include what they are consuming dietarily. Thus, consuming foods containing Reducose® 5% is not expected to be a safety concern in this population any more than foods in general are.

Reuser et al. (1994), cited above by FDA, is a theoretical paper about potential, but unsubstantiated, adverse effects of pharmaceutical 1-DNJ derivatives that are not the equivalent of naturally occurring 1-DNJ or Reducose® 5%. The study relies on experimental data and theoretical scenarios, such as in vitro data using high concentrations of a pharmaceutical 1-DNJ derivative and that these artificially induced concentrations under acellular in vitro conditions are analogous to certain inherited disorders of metabolism followed by extrapolation that use of such pharmaceutical 1-DNJ derivatives by humans under prescribed conditions will mimic these disorders in individuals with **kidney failure**. Even in parenteral experiments in rats, extremely high doses of miglitol were unable to induce lysosomal α-glucosidase inhibition sufficient to result in lysosomal glycogen accumulation. Furthermore, under in vitro conditions, concentrations of 1-DNJ 1500x higher than the concentration of miglitol theoretically expected under normal oral dosing using the assumption that miglitol is freely distributed to all bodily tissues (i.e., a volume of distribution of 1; note, pharmacokinetic data provided in the miglitol package insert demonstrate "a volume of distribution of 0.18 L/kg, consistent with distribution primarily into the extracellular fluid") were required to induce delay of insulin receptor transport to the cell surface; thus, given the huge concentration required under the necessity of an apparent volume of distribution of 1 (which is not reality), we find this theoretical potential of 1-DNJ to induce a deficiency of insulin receptor at the cell surface to lack credible biological plausibility. Overall, we conclude that extrapolation from this paper to the real word is not credible and, rather, the scientific utility of the paper is that of hypothesis generation. We are not aware of any available data that has tested and substantiated any of the potential hypotheses of the paper.

In the real world, due to Reducose® 5%'s intestinal mechanism of action to reduce the glycemic index of carbohydrate foods and lack of evidence for any beneficial or adverse systemic effects in preclinical and clinical studies (including those cited and summarized in GRN 992) of Reducose® 5% and other related substances, bioaccumulation is not expected to occur or to be a major safety concern. Additionally, as noted above, it is expected that

individuals with severe kidney disease would be under the care of a nephrologist and dietitian with appropriate monitoring and instruction, and individuals with kidney failure would additionally be on dialysis.

Also, as noted above, Reducose® 5% has a wide MOE both as a whole ingredient and in terms of its 1-DNJ content. Additionally, using a 100x multiplicative uncertainty factor, the ADI of Reducose® 5% is 40 mg/kg bw/day and the ADI in terms of its 1-DNJ content is 1.86 mg/kg bw/day. The respective maximum EDIs in the general U.S. population (ages 2+ years) at the 90th percentile of consumers are 13.1 mg/kg bw/day for the whole ingredient and 0.722 mg/kg bw/day for 1-DNJ. As the EDIs are less than the ADIs, this supports a conclusion that the intended use of Reducose® 5% is reasonably certain to be safe. As the multiplicative uncertainty factor represents both rat to human extrapolation and interindividual variability, it is expected to compensate for the data gap of available scientific data in the subpopulation of individuals with renal impairment. Also, as noted above, no adverse events associated with consumption of the GRAS substance Reducose® 5% have been reported to Phynova since its market introduction in 2018 following sales of 3450 kg (3045 kg of which were sold in the U.S.).

3) In the Redbook 2000 (Chapter IV.C.3.a), it is stated that results from a sub-acute/28-day repeat dose oral toxicity studies in rodents:

> (1) can help predict appropriate doses for the test substance for future subchronic or chronic toxicity studies, (2) can be used to determine NOELs for some toxicology endpoints, and (3) allow future studies in rodents to be designed with special emphasis on identified target organs.

Several statistically significant differences were identified in Marx et al. (2016), using a test article that was identical to the article of commerce (pg. 30 of the notice). While these values were stated as lying within the historical range and reportedly were not associated with histopathological findings (pg. 32 of the notice), please provide an explanation as to why a longer study (such as 90-day oral repeat-dose studies) using the article of commerce at similar doses would not result in toxicologically relevant adverse effects.

Notifier Response: We note that Redbook 2000 is under revision, and FDA's GRAS team has previously advised that until revision is completed, referral to the Redbook should be avoided and to rely, instead, on OECD guidelines. Regardless, of the Redbook citation as prefix, we have addressed the main point of query 3 below.

Whether a 28-day study is suitable for extrapolation to lifetime exposure is a question to be answered on a case-by-case basis. Below we consider the factors pertaining to this determination beginning with the statistically significant observations of the study by Marx et al. referenced in FDA's query 3 above.

While a statistically significant decrease in body weight gain was observed transiently between Days 18 and 21 in high dose female rats, this had no effect on cumulative body weight development. Female body weight fluctuations during the study did not deviate from the controls at any point during the study by more than +2 or –1% and no fluctuations were statistically significant. Final mean body weight of the female control group was 183.7 ± 9.6 g while final mean body weight of the female control group was 181.6 ± 4.1 g, a difference of –1%. There were no statistically significant differences in mean body weight or body weight gain in male groups.

Neutrophil percent and lymphocyte percent differences were observed, in male and female animals, but were not dose related in the females or for neutrophils in the males and the magnitude of change in lymphocytes in males was low (–6%). In addition to the lack of a dose relationship, it is an important consideration that these changes remained within the range of the historical control data (HCD) of the laboratory. These HCD are data collected from control animals of the same strain and age, housed under the same conditions, provided the same feed and water, and having blood samples drawn from the same site under the same timing, and analyzed on the same equipment. Furthermore, these changes did not correlate with changes in total leukocyte count and their significance in terms of absolute neutrophil and lymphocyte counts, which is what matters biologically, is unknow as these were not measured or calculated and subjected to statistically analysis. There was an 18% decrease in percent reticulocytes compared to controls at the high dose in male rats. Again, this change was well within the range of the laboratory's HCD, which, again, is an important factor to be considered, and was not correlated with changes in erythrocyte count, hematocrit, hemoglobin, red blood cell indices, or total leukocyte count that could be indicative of anemia nor were there any trends suggestive of a move towards anemia. In fact, hemoglobin was non-significantly increased by 2% compared to controls, and histological alterations were not observed in bone marrow. Activated partial thromboplastin time (APTT) was statistically significantly increased compared to controls in males at the mid and high doses by 15 and 23%, respectively. Again, these changes were within normal range as determined by comparison against the laboratory's HCD. They were not correlated with change in prothrombin time (PT) or platelets, and we are not aware of any evidence to suggest or demonstrate that mulberry leaf, 1-DNJ, or maltodextrin are inhibitors of clotting factors, such as factors VIII, IX, Xa, or XII, that might result in a prolonged APTT in the presence of a normal PT.

Statistically significant increases compared to control in ALT and AST, the later without a dose relationship, in high-dose males were well within the HCD and well under a fold increase and, therefore, do not indicate damage to the liver, but were correlated to a dose responsive increase in liver weight relative to body weight. These changes could be indicative of enzyme induction, reflecting an adaptive and reversible change in response to increased metabolic demand. However, the absence of any histological changes in the livers of study animals indicates that a functional, let alone, potentially adverse, change has not been initiated. The statistically significant 9 and 10% decreased in creatinine in mid and high dose males with respect to controls are also well within normal range, and in the absence of muscle wasting, which was not observed, are in the direction opposite of biological or toxicological concern. Serum sodium was also statistically significantly decreased at the mid and high dose compared to control in males; however, sodium is under tight homeostatic control; thus, that the magnitude of the changes was –1% and they remained well within the range of HCD is an extremely important factor. Likewise, the –2% changes in sodium and calcium at the high dose and –3% change in calcium at the mid dose in females, remaining firmly within the respective HCD ranges are indicative of normal variation.

In addition to the changes in liver weight relative to body weight discussed above, there were several other minor, but statistically significant, changes in mean absolute and relative organ weights at the mid and high dose or high dose only. All of these changes remained well within the corresponding HCD ranges and none exceeded ± 30% of control values or were correlated (except liver relative to body weight as noted above) with changes in clinical, gross, or histopathology. Therefore, they were not toxicologically relevant.

In terms of whether any of the above changes would be expected to progress to a pathological degree if a study of longer duration were conducted, there are no obvious trends in the data discussed above nor are there potentially concerning findings based on correlation of parameters and/or biological plausibility of an underlying test item effect to suggest the likelihood of such progression. We also discussed this with the GRAS team at our March 29, 2018 pre-submission meeting and toxicology had no concerns. The 28-day study on the article of commerce is pivotal because it is the article of commerce and was tested up to the highest feasible dose (4000 mg/kg bw/day), which was determined as the NOAEL. However, we also note that this study does not stand alone in the safety evaluation as there are corroborative studies of longer duration on other related test substances as well as studies in humans and pharmacokinetic data, all of which form a bridge to suggest the use of Marx et al. as the pivotal study for risk characterization is appropriate.

In Subpart 6.3.1 of GRN 992, pages 43–44 we summarized a 90-day repeated-dose study in rats in which the test item was a hydroethanolic MLE containing 1.1% 1-DNJ. No test item related effects on body weight, clinical pathology parameters, organ weights, gross pathology or histopathology were observed. The high-dose was equivalent to 884.5 and 995.7 mg/kg bw/day (equivalent to 9.7 and 11.0 mg/kg bw/day 1-DNJ, respectively) in male and female rats, respectively. In Table 23 of Subpart 6.3.2 of GRN 992 on page 47 we summarized a 90-day repeated-dose study in rats with a 28-day recovery period in which the test item was a silkworm extract containing 1.25% 1-DNJ. While some statistically significant changes in clinical pathology parameters were observed, they were within the HCD ranges and were not statistically significant at the end of the recovery period. Body weight was also significantly increased in high-dose males, remaining increased at the end of the recovery period, but was within the HCD range at all times. Some significant increases in absolute organ weights compared to controls were considered due to the increased body weight, and the NOAEL was determined to be 2000 mg/kg bw/day (equivalent to 25 mg/kg bw/day 1-DNJ); the highest dose tested. These trials corroborate the lack of toxicologically relevant changes from longer term exposure to MLE and/or 1-DNJ although the doses were lower compared to the 28-day study by Marx et al.

Studies in humans, also corroborate the results of the 28-day rat study by Marx et al. as pivotal in determining an MOE and ADI for the intended use of Reducose® 5%. No serious safety concerns were raised by the clinical trials reported in Table 24 on pages 50–53 of GRN 992, Subpart 6.4.3 with exposure durations to MLE up to 6 months at 2 g daily or doses up to 5 g (18 mg 1-DNJ) for 4 weeks. One trial gave 3.6 g MLE containing 54 mg 1-DNJ for 38 days. The maximum EDI at the 90th percentile of consumers in the general U.S. population (2+ years) using a 10% presence probability factor is 839.0 mg/day containing up to 46.1 mg 1-DNJ.

In addition, the use of Marx et al. is corroborated by the pharmacokinetics and pharmacodynamics of 1-DNJ. 1-DNJ is active on carbohydrate in the intestinal lumen with no known systemic activities and is metabolically inert. 1-DNJ is rapidly absorbed and rapidly excreted intact in the urine without bioaccumulation following oral administration to rats, suggesting 28-days should be adequate to investigate its systemic effects.

4) You state on pg. 30 "… the test items evaluated by Marx et al. and Li et al. are identical to the article of commerce that is subject to this GRAS Notice, except that, due to manufacturing process change in order to comply with US regulations governing GRAS substances, L-leucine is no longer used as a processing aid." However, in comparing the description of the test article

described in the two studies, it is not clear that the two test articles are identical. For example, Marx et al. state:

> "The dried mulberry leaf then undergoes a water extraction and ion exchange chromatography to enrich the alkaloid components. The eluent is reduced under vacuum to allow optimum spray drying."

Li et al. state:

> "Dried leaves were extracted by water. The extraction solution was filtered with a 10KD membrane and then ultra-filtered with a 3KD membrane. The filtrate was concentrated and spray dried to obtain MLE."

Given that ion exchange chromatography and filtration are different manufacturing/purification steps, please provide an explanation as to why you concluded that the two test articles are identical without analytical methods to confirm identity and composition.

Notifier Response: The referred to statement on page 30 is inaccurate. We now amend this statement to read as follows:

> "… the test item evaluated by Marx et al. is identical to the article of commerce that is subject to this GRAS Notice, except that, due to a manufacturing process change in order to comply with US regulations governing GRAS substances, L-leucine is no longer used as a processing aid. L-leucine is still present in the ingredient at approximately 1–2% as a naturally occurring constituent of the white mulberry leaf extract. The test item evaluated by Li et al. is Reducose® 1%, a related product produced as a simple extract from the same raw botanical starting material and standardized to 1% 1-DNJ."

5) On pg. 43-44, the notifier cites a reference for a 90-day oral toxicity study in rats that is only available in Japanese. This study is listed within references that are generally available, and it was noted that an English translation was used. On pg. 44, the notifier states: "A few lesions of slight degree were observed in various organs during the histological examination; however, these findings occurred with similar incidence in both treated and control animals and did not differ statistically …" Please provide a) an explanation for why this study in Japanese is considered generally available; b) how the presented experimental data and the authors' conclusions can be evaluated by qualified experts who are not literate in Japanese and may not have access to an English translation.

Notifier Response: a) This is corroborative data (see GRN 992 Subpart 6.10.2, pages 57–58) that is not required to be generally available according to the 2016 GRAS Final Rule on pages 49 and 50 as well as preexisting regulation at 21 CFR 170.30(b).

b) We inadvertently included this English translation of a Japanese study in the list of references that are generally available provided in Subpart 7.2 of GRN 992, pages 60—64. We now amend GRN 992 to list the citation for this study (Miyazawa M, Miyahara C, et al. Ninety-day dietary toxicity study of mulberry leaf extract in rats [English translation]. *Shokuhin Eiseigaku Zasshi*. 2003;44(4):191-7) as data and information that are not generally available in Subpart 7.1, page 60, and we amend Subpart 7.2 of GRN 992 on page 62 to remove the citation for this study (number 36).

6) The notifier has provided exposure estimates for both Reducose 5% as well as 1-DNJ. Additionally, they have calculated these exposure estimates utilizing a 100% presence probability factor as well as a 10% presence probability factor; however, margin of safety (MOS) calculations were only performed using the exposure estimates based on the 10% presence probability factor. The MOS for Reducose 5% (3740mg/kg/67.8mg/kg) is approximately 55-fold

and for DNJ content (67.8mg/kg x 4.5-5.5%) the MOS (186mg/kg/3.05-3.73mg/kg) ranges from 50 to 61-fold when utilizing the 100% presence probability factor. These calculations are based on the NOAEL determined from the 28-day oral tod xicity study (Marx et al.), adjusted for the added L-leucine. Please explain why this MOS is adequate to support the conclusion that the intended use of Reducose 5% is reasonably certain to be safe.

Notifier Response: Exposure calculations were also discussed at the March 29, 2018 pre-submission meeting. It was noted that the number of food categories contained in the intended use, would by de facto result in a huge exposure using 100% presence probability. Mike DiNovi (FDA's GRAS team exposure expert at the time of the meeting) joked with the Notifier saying, "you would love to be able to sell that much, right?" and further implied that he would consider a 10% presence probability analysis to be highly conservative. As such, the exposure estimates for the purpose of conducting the risk characterization (i.e., MOS) were conducted using a 10% presence probability factor. We included the exposure (but not risk characterization) at 100% presence probably as Mike DiNovi had informed us that even though only the 10% presence probability is used for the risk characterization, we should still provide FDA with the 100% presence probability data along with the explanation of why it is a gross overestimate of exposure and the reasons why the 10% presence probability is conservative. This was all provided, as advised, in GRN 992 and is expanded on below.

As discussed on page 22 of GRN 992, Subpart 3.2, 100% presence probability assumes that every single product in the intended use categories would contain Reducose® 5% at the maximum addition levels shown in Table 7 (pages 18 and 19, Subpart 3.1) and that every time an individual consumed any product from any of the categories, they would be exposed to the maximum amount of Reducose® 5%/serving. As shown in Table 7, the intended use contains a wide range of food categories that are widely consumed, such as breads, pastas, beverages, deserts, and snack foods, and as noted on page 21, the exposure analysis using 100% presence probability estimates that 100% of the U.S. consumers of these categories would be exposed to Reducose® 5% at the maximum addition level per serving.

In reality, the above assumptions are highly unrealistic. Reducose® 5% would not be a characterizing ingredient (e.g., flour in bread) in any of the intended use categories nor would it be required for a physical or technical functional effect (e.g., yeast in bread) in any of the intended use categories. Rather, Reducose® 5% is a functional ingredient, with a significant cost consideration for food producers, and as such is likely to be use only in the 'functional foods' market segment, and only in a subset of foods in that category, such as foods intend for people interested in following a low glycemic index diet yet not wanting to give up high glycemic index foods (the function of Reducose® 5% is, essentially, to lower the glycemic index of foods containing the ingredient).

The intent of the 10% presence probably was to represent a 10% market share in each of the categories. This is still highly conservative. Consider, for example, the likelihood that even 10% of all products in the coffee, bread, or potato chip markets (or any of the other categories listed in Table 7) would contain Reducose® 5%. Such an assumption is still highly conservative. It would be much more realist, yet still conservative, to assume that Reducose® 5% could capture 10% of the market share of the low glycemic index subset of the subset of 'functional foods' contained within each and every one of the intended use categories (note, such a limited and realistic, yet conservative, assumption is not possible to analyze within the NHANES data set).

In addition, the 10% presence probability analysis is much more conservative than a 10%, across the board, market share assumption in which the aggregate total of the 100% presence probability analysis is simply multiplied by 10%, which would result in an absolute exposure to

Reducose® 5%, at the 90th percentile of consumers, of 400.6 mg/day and an exposure relative to body weight of 6.8 mg/kg bw/day (approximately ½ the exposures estimated using the 10% presence probability factor). The 10% presence probability factor assumes that a consumer would randomly pick a product containing the ingredient 10% of the time they consumed any product from any food category listed in Table 7. As shown in Tables 10 and 11 on pages 22 and 23, respectively, and discussed on page 23, approximately 66% of the general U.S. population (2+ years) of consumers of products in the intended use categories were identified as consumers of Reducose® 5% when using the 10% presence probability factor, a highly conservative assumption.

In summary, the exposure estimate was performed using 10% presence probability and was considered reasonable for the reasons explained in GRN 992 and expanded on above. This estimate was used to calculate the MOS, which was considered reasonable with regard to supporting a conclusion that the ingredient is reasonably certain to be safe under the conditions of its intended use. The 100% presence probability data is NOT the exposure estimate for the intended use of Reducose 5% and was provided for informational purposes only, as it is a step in the process of deriving the actual exposure estimate.

Finally, pertaining to this query, Chemistry query #5 above, and Toxicology query #3 above, we note that the intended use, exposure estimates, and MOS calculations of GRN 992 are almost identical to the intended use, exposure estimates, and MOS calculations contained in GRN 894. We further note that we were asked, at the end of an August 31, 2020 teleconference, to request FDA cease evaluation of GRN 894 only because one member of the GRAS team believed that the amendments to GRN 894 that would be required to explain the minor, non-significant manufacturing change to remove L-leucine as a processing aid for regulatory compliance reasons and its effect on the MOS calculation and safety assessment would be too confusing as an amendment and, therefore, required a new submission. Neither chemistry or toxicology had any safety concerns regarding the intended use, exposure estimates, or MOS calculations presented in GRN 894 during FDA's review nor were there any safety concerns when considering the effect that removal of L-leucine as a processing aid would have on overall safety or the MOS calculations. This lack of safety concern, including that the removal of L-leucine has no bearing on safety, was clearly stated by Ron Chanderbhan during our August 31, 2020 teleconference with the GRAS team during the review of GRN 894 and confirmed by the review team's toxicology and chemistry members.

7) The Notifier describes the composition of Reducose 5% within the identification section (pg. 10), stating that white mulberry leaf extract contains flavonoids (kaempferol), stilbenoids (resveratrol), and anthocyanins, as well as other iminosugars and proteins. Please provide a narrative addressing the safety of the other components of white mulberry leaf extract.
Notifier Response: We note that GRN 992 is a safety assessment (i.e., GRAS conclusion) of Reducose® 5%, an iminosugar-rich extract of white mulberry (*Morus alba* L.) leaves that is standardized to a concentration of 5% 1-deoxynojirimycin (DNJ). It is not a safety assessment of purified flavonoids (kaempferol), stilbenoids (resveratrol), anthocyanins, or any particular iminosugar or protein.

Furthermore, while we stated on page 10 that raw *M. alba* leaves contain the above mentioned constituents, we did not state that Reducose® 5% contains these constituents. Flavonoids (kaempferol), stilbenoids (resveratrol), anthocyanins are not expected to be present in the extract as they are removed by the ion exchange process. Proteins are removed by filtration (see chemistry query 1 above and minor point 2 below) using a ultrafiltration membrane with a

cut-off of 3 kDa (average protein molecular weight is 55 kDa and even small proteins would be in the range of ≥ 5kDa). Iminosugars other than 1-DNJ are present only at low levels. Cumulatively, other iminosugars are expected to be present in the finished Reducose® 5% ingredient at 2–3% (see Table 2, Subpart 2.1 on page 10 of GRN 992 as well as chemistry query #1 above. Individually, other iminosugars, such as fagomine (commonly found in buckwheat) or DAB (1,4-dideoxy-1,4-imino-D-arabitol) are present in the finished ingredient at less than 1%, a level considered insignificant and inconsequential, especially given, as noted below and above, that toxicological testing was performed on the ingredient as a whole.

Reducose® 5% is a *M. alba* leaf extract spray-dried on a maltodextrin carrier, which comprises 28–50% of the finished ingredient, for standardization. The extract is filtered to remove large components such as proteins, purified, and concentrated and filtered multiple times. We discuss some data concerning 1-DNJ only because it is present in the ingredient at levels we consider to be greater than insignificant/trace and because it is used as the marker for standardization. Additionally, pharmacokinetic data on MLEs are available only in terms of 1-DNJ and it may contribute to the functional effect of the ingredient and, was, therefore, considered relevant in identification of corroborative safety data on related ingredients. In terms of the pivotal study for purposes of establishing an MOS (Marx et al.), the test item was Reducose® 5% and was identical to the current Reducose® 5% substance that is the subject of GRN 992 except for the removal of L-leucine as a processing aid. Thus, the study by Marx et al. is a toxicological investigation of Reducose® 5% as a whole ingredient including its extract and carrier components as well as all constituents of the extract component at the levels present in the finished ingredient. Furthermore, the finished ingredient, being standardized to 1-DNJ content, the extract proportion of finished ingredient is identical to the extract proportion of Reducose® 5% containing added L-leucine as a processes aid, the finished ingredients differing, other than L-leucine content only in maltodextrin content, which is adjusted to compensate for the absence of L-leucine.

8) In Section 6.4.2, the notifier discusses studies by Huh et al. (2020), in which MLE was shown to potentiate the effect of and decrease the clearance of metformin in an experimentally induced diabetic rat model. The notifier states:

> "The MLE was not assessed for DNJ content, but was reported to contain trans-caffeic acid, syringaldehyde and chlorogenic acid; thus, it is uncertain how similar this MLE is to Reducose® 5%."

Please provide data and/or other information that support your implicit conclusion that these observed effects are due to constituents (i.e., trans-caffeic acid, syringaldehyde and chlorogenic acid) that are not present in your article of commerce. If they are not present, please provide generally available and accepted evidence that there are no drug-herb interactions between your article of commerce and metformin.

Notifier Response: We did not implicitly conclude that the observed effects of Huh et al. (2020) were due to trans-caffeic acid, syringaldehyde, or chlorogenic acid. In making this statement, we were only pointing out that there are dissimilarities, as well as unknowns, between Huh et al.'s test item and Reducose® 5% and it is uncertain for what specific effects any differences might be responsible. While Reducose® 5% does not contain trans-caffeic acid, syringaldehyde and chlorogenic acid, there may also be other unknown dissimilarities between Reducose® 5% and Huh et al.'s test item. Thus, the quoted statement above should be taken to imply that these are the constituents of Huh et al.'s test item that were responsible for the observed

effects.

The search string “((("metformin"[MeSH Terms] OR "metformin"[All Fields] OR "metformine"[All Fields] OR "metformin s"[All Fields] OR "metformins"[All Fields]) AND ("drug elimination routes"[MeSH Terms] OR ("drug"[All Fields] AND "elimination"[All Fields] AND "routes"[All Fields]) OR "drug elimination routes"[All Fields] OR ("drug"[All Fields] AND "clearance"[All Fields]) OR "drug clearance"[All Fields]) AND ("caffeic acid"[Supplementary Concept] OR "caffeic acid"[All Fields] OR "trans caffeic acid"[All Fields])) OR (("metformin"[MeSH Terms] OR "metformin"[All Fields] OR "metformine"[All Fields] OR "metformin s"[All Fields] OR "metformins"[All Fields]) AND ("drug elimination routes"[MeSH Terms] OR ("drug"[All Fields] AND "elimination"[All Fields] AND "routes"[All Fields]) OR "drug elimination routes"[All Fields] OR ("drug"[All Fields] AND "clearance"[All Fields]) OR "drug clearance"[All Fields]) AND ("syringaldehyde"[Supplementary Concept] OR "syringaldehyde"[All Fields]))) OR (("metformin"[MeSH Terms] OR "metformin"[All Fields] OR "metformine"[All Fields] OR "metformin s"[All Fields] OR "metformins"[All Fields]) AND ("drug elimination routes"[MeSH Terms] OR ("drug"[All Fields] AND "elimination"[All Fields] AND "routes"[All Fields]) OR "drug elimination routes"[All Fields] OR ("drug"[All Fields] AND "clearance"[All Fields]) OR "drug clearance"[All Fields]) AND ("chlorogenic acid"[MeSH Terms] OR ("chlorogenic"[All Fields] AND "acid"[All Fields]) OR "chlorogenic acid"[All Fields])) did not return any hits in PubMed.

As expected, due to the known metabolism of 1-DNJ, the search string “((metformin) AND (drug clearance)) AND (1-deoxynojirimycin)” also did not return any hits.

Nonetheless, Huh et al. do discuss the biological plausibility of caffeic acid and chlorogenic acid (which as noted above, are not constituents of Reducose 5%) affecting the clearance of metformin by altering its hepatic metabolism. They cite studies by Geng et al. (2015)[*] and Xu et al. (2016)[†] stating, “plant extracts of caffeic acid and chlorogenic acid could inhibit the activities of CYP2C11 and CYP3A1” as well as a study by Jung et al. (2019)[‡] demonstrating “Met[formin] is metabolized by hepatic CYP2C11, 2D1, and 3A1/2, and eliminated via the kidneys in rats.”

We also note that Riche et al. (2017) evaluated the effects of 3 g daily for 3 months of an MLE in T2D humans taking metformin in which no significant adverse effects were observed (see response to Toxicology query #1 above), suggesting that MLEs in general do not present a safety concern if consumed by diabetic individuals taking metformin. Importantly, this was a study in human subjects.

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[*] Geng, T.; Si, H.; Kang, D.; Li, Y.; Huang,W.; Ding, G.;Wang, Z.; Bi, Y.; Zhang, H.; Xiao,W. Influences of Re Du Ning Injection, a traditional Chinese medicine injection, on the CYP450 activities in rats using a cocktail method. J. Ethnopharmacol. 2015, 174, 426–436.

[†] Xu, X.; Geng, T.; Zhang, S.; Kang, D.; Li, Y.; Herbal, G.D.C. Inhibition of Re Du Ning Injection on enzyme activities of rat liver microsomes using cocktail method. Chin. Herb. Med. 2016, 8, 231–241

[‡] Jung, S.-H.; Han, J.-H.; Park, H.-S.; Lee, D.-H.; Kim, S.J.; Cho, H.S.; Kang, J.S.; Myung, C.-S. E_ects of unaltered and bioconverted mulberry leaf extracts on cellular glucose uptake and antidiabetic action in animals. BMC Complement. Altern. Med. 2019, 19, 55.

Minor Points:

1) On pg. 49, the notifier refers to two unpublished clinical trials using white mulberry leaf preparations. Please discuss whether any effects of these preparations on glucose levels were observed and if so, please indicate why this is not a safety concern.
Notifier Response: The unpublished study cited as "Gallagher et al. (2015, unpublished)" in Table 24 on page 51 of GRN 992, Subpart 6.4.3 (and also listed as data and information that are not generally available in Subpart 7.1 on page 60 as, "The clinical trial PYN-IM-002a of Reducose 5% by Gallagher et al. (2015)") was an open-label 5-arm trial to evaluate the effect of Reducose® 5% on the glycemic index of 4 common carbohydrate test foods. Glycemic indexes of the test foods in combination with 250 mg Reducose® 5% (test arms) were determined by the test arm postprandial incremental area under the glucose curves versus that of a glucose meal (reference arm). Results were compared to the glycemic index values of the test foods recorded in the literature. Adverse events were monitored as secondary outcomes. As noted on page 51 in Table 24, there were no adverse effects reported by participants or observed by the investigators. All subjects had normal blood pressure at baseline and blood pressure was not affected by administration of the test meals with Reducose® 5%. Study data indicates that administration of the test meals with Reducose® 5% did not adversely affect glucose levels at any of the collection timepoints or the overall postprandial glucose response curve over the 120-minute monitoring period.

   The unpublished study, cited on page 60, Subpart 7.1 as "The clinical trial PYN-IM-003 of Reducose 5% by Thondre et al. (2016)" was inadvertently left out of Table 24 (note, while the study was cited in both GRNs 894 and 992 as quoted here, in responding to this query it came to our attention that the study number was incorrectly cited; PYN-IM-003 is actually the published study cited in Table 24 as Lown et al., 2017. We now amend the citation in GRN 992 to read PYN-IM-004). This study (PYN-IM-004) has recently (April 2021) been published (Thondre et al., 2021). The study was a randomized double-blinded placebo-controlled crossover trial conducted in 37 healthy adults to assess the effects of Reducose® 5% on postprandial glycemic and insulinemic responses. Data was reported for 36 subjects as insulin data were not available for one subject. A single bolus of 250 mg Reducose® 5% was administered with 75 g sucrose as the test meal, and 75 g sucrose alone was administered as the placebo meal. The study was powered to detect statistically significant differences in mean incremental area under the glucose curve. The authors reported that no adverse effects occurred during the study; however, the methods did not describe how adverse effects were assessed. Neither glucose nor insulin levels fell below baseline (fasting levels) during the 120-minute monitoring period following ingestion of the test meal.

2) The notifier states that 25-35% of Reducose 5% is composed of free amino acids, peptides, or proteins. Given the relatively high protein content, please address why bioinformatics analyses and/or other analytical methodologies[5] to support your case report analysis are not needed to ensure that the risk for allergenicity from the intended use is low.
Notifier Response: Reducose® 5% contains an MLE that does not contain proteins due to extensive filtration (see response to Toxicology query #7 above). We now amend GRN 992 Table 2 column 1 of row 4, Subpart 2.1 on page 10 to read, "Free amino acids/peptides". Free amino acids naturally present in mulberry leaf make up the majority of the 23–35% free amino acids/peptides reported in Table 2, comprising approximately 85% of this total while peptides ≤ 27 amino acid residues make up the remaining 15%.

As noted on page 54 in GRN 992, Subparts 6.6 and 6.7, no reports of allergic reactions to *M. alba* leaves were located despite an extensive history of consumption dating back to at least 659 A.D. We now amend Subpart 6.6 to further include a recent publication by Papia et al. 2020. This paper cites respiratory allergies to mulberry pollen and food allergies to mulberry fruit (Papia et al., 2020). We also note that the paper incorrectly reports that Navarro et al. (1997) reported a case of allergy to *M. alba* leaves. As reported in GRN 992, Subpart 6.6, page 54, Navarro et al. (1997) reported an allergic reaction to ingestion of *M. alba* fruit in a female who also reported a history of several episodes of asthma when near *M. alba* leaves. As this is a vague description without an official diagnosis it should not be over-interpreted to presume an allergy to *M. alba* leaves (e.g., "near *M. alba* leaves" could mean near an *M. alba* tree that was releasing pollen). Papia et al. further report potentially allergenic proteins identified a in mulberry leaf extract of 18 kDa, in mulberry fruit with molecular weights of 10*, 18*, and 17 kDa, in mulberry pollen with molecular weights of 72, 15, 10, 10*, 8*, and 7* kDa, and in mulberry species without specific identification of plant part of 17, 18, and 9 kDa (* indicates proteins specifically identified in the noted plant parts of *M. alba*; other proteins were identified in other mulberry species/genera or their sources were identified only by the common name). If any of these, or other, potentially allergenic proteins are present in raw mulberry leaves used for the production of Reducose® 5%, either naturally or by cross contamination, they would be removed during manufacture by the 100 kDa and 3 kDa ultrafiltration cut offs. Thus, given the absence of intact proteins in Reducose® 5% and the long history of use of mulberry leaves without confirmed reports of allergic reactions, the allergenic potential of Reducose® 5% is considered very low, and it was not considered necessary to perform bioinformatics analyses and/or other analytical methodologies for this extract.

3) Please identify the sources (databases) and search parameters used for the literature searches performed for the safety assessment of this GRAS notice.

Notifier Response: Sources for the literature searches included PubMed, Google Scholar, National Toxicology Program, toxplanet (including its indexed databases, such as the former TOXNET databases), websites of US FDA, EFSA, WHO, and FAO, medical libraries of University of Washington and University of Arizona, and AIBMR's internal library.

Search parameters included Reducose; Iminonorm; *Morus alba*; 1-deoxynojirimycin; toxicity; toxicology; toxicity tests—subacute, subchronic, chronic, acute; mutagenicity; mutagenic; genotoxic; genotoxicity; genetic toxicity; clastogenic; carcinogenicity; carcinogenic; safety; no observed adverse effect level; NOAEL; no observed effect level; NOEL; Lowest observed adverse effect level; LOAEL; lowest observed effect level; LOEL; chromosome aberrations; micronucleus; bacterial reverse mutations; Ames test; comet; pharmacokinetics; ADME; absorption; distribution; metabolism; excretion; elimination; bioavailability; and biological availability. The searches included MESH terms associated with these terms when and where applicable. The terms were put together in various Boolean search strings used when and where applicable. On some search occasions databases were searched more specifically, such as for a specific paper (for example, a reference cited in another article). Some databases were searched using primarily key words related to the name of the substance rather than Boolean strings (e.g., toxplanet, FDA's Food Ingredient and Packaging Inventories).

4) On pg. 49, the notifier states “thirteen out of 16 clinical trials…are summarized in Table 24” yet only 12 studies are listed. Please provide the summary for the missing study in Table 24.
Notifier Response: The study missing from Table 24 is the unpublished study cited on page 60, Subpart 7.1 as “The clinical trial PYN-IM-003 (now corrected to PYN-IM-004; see response to Minor Point #1 above) of Reducose 5% by Thondre et al. (2016)”. The study has now been published (as of April of this year) as Thondre et al. (2021) and is summarized above at Minor Point #1.

[5] Moreno FJ. Gastrointestinal digestion of food allergens: effect on their allergenicity. *Biomed Pharmacother*. 2007;61(1):50-60.

References:

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Kim, J. Y., et al., 2015. Mulberry leaf extract improves postprandial glucose response in prediabetic subjects: a randomized, double-blind placebo-controlled trial. J Med Food. 18**,** 306-13.

Marx, T. K., et al., 2016. A 28-Day Repeated Dose Toxicological Study of an Aqueous Extract of Morus Alba L. Int J Toxicol. 1-9.

Papia, F., et al., 2020. Allergic reactions to genus Morus plants: a review. Clin Mol Allergy. 18**,** 1.

Riche, D. M., et al., 2017. Impact of mulberry leaf extract on type 2 diabetes (Mul-DM): A randomized, placebo-controlled pilot study. Complement Ther Med. 32**,** 105-108.

Thaipitakwong, T., et al., 2020. A randomized controlled study of dose-finding, efficacy, and safety of mulberry leaves on glycemic profiles in obese persons with borderline diabetes. Complement Ther Med. 49**,** 102292.

Thondre, P. S., et al., 2021. Mulberry leaf extract improves glycaemic response and insulaemic response to sucrose in healthy subjects: results of a randomized, double blind, placebo-controlled study. Nutr Metab (Lond). 18**,** 41.