#894

AIBMR Life Sciences, Inc.

December 3, 2019

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,

[redacted]

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

#894

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
2800 E. Madison, Suite 202
Seattle WA 98112

December 3, 2019

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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.
2800 E. Madison, Suite 202
Seattle, WA 98112
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

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]

3 December 2019

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%
Amino acids	13–15%
Carbohydrates	27–29%
Maltodextrin	30–50% (used for standardization)
L-leucine	5–10%

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 Overview

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 spray dried to produce a powder. During the spray-drying process, maltodextrin and L-leucine (or other similar GRAS excipients) are added. 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.

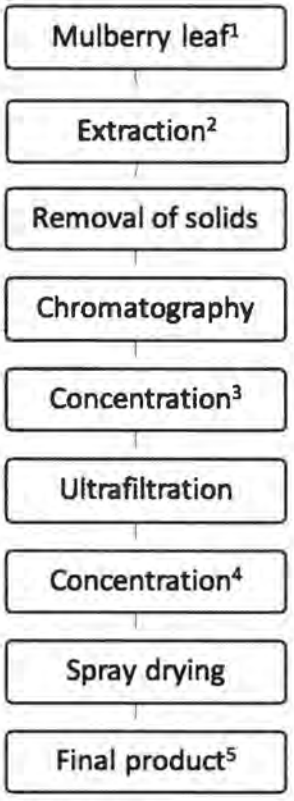

Figure 2. Manufacturing Flowchart

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
Marker Compounds		
1-Deoxynojirimycin (DNJ)	5.0% ± 0.5%	Internal Method (HPLC-ELSD)
Physical Characteristics		
Appearance	Fine powder without aggregates	GB16740-2014
Color	Light brown/brown powder	
Taste	Slightly bitter malt-taste	
Odor	Characteristic	
Solubility	Easily dissolved in water & 50% EtOH; not soluble in oil.	CP2015 / General notices 15.2
Chemical Characteristics		
Moisture	<7.0%	GB5009.3-2016 method 2
Acid insoluble ash	<2.0%	CP2015/2302
Heavy Metals		
Arsenic	<1 ppm	GB5005.268-2016 (ICP-MS)
Cadmium	<0.5 ppm	
Lead	<1 ppm	
Mercury	<0.1 ppm	
Microbiological Tests		
Total Aerobic Plate Count	$<10^3$ cfu/g	Ph. Eur 2.6.12 / ISO 4833-1:2013
Total Yeast & Mold	$<10^2$ cfu/g	Ph. Eur 2.6.12 / ISO 21527:2008
Escherichia coli	<10 cfu/g	Ph. Eur 2.6.12, 2.6.31 / ISO 16649-2:2001

Salmonella	Absent in 25 g	Ph. Eur 2.6.12, 2.6.31 / ISO 6579-1:2017
Pesticide Residues	Complies with EU 396/2005	USP<561> / Ph. Eur 2.8.13
Mycotoxins*	Complies with EU 1881/2006	DIN EN 14123 [modified]; NY/T 1970-2010; DIN EN 14132 [modified]
PAH*	Complies with EU 1881/2006	EN 16619:2015

Abbreviations: cfu, colony forming units; CP, Chinese Pharmacopeia; DIN, German Institute for Standardization; EN, European Standards; EU, European Union; 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; NY/T, agricultural voluntary standards; PAH, polycyclic aromatic hydrocarbons; Ph. Eur, European Pharmacopoeia; ppm, parts per million; USP, United States Pharmacopoeia.
*Skip-lot testing of mycotoxins and PAHs is performed in accordance with the HACCP plan.

2.3.1 Batch Analysis

Production conformity and consistency of Reducose® 5% are tested in production lots. Batch analyses of four non-consecutive lots, representing approximately 3.5 years of production, are shown in Table 5 below and are reasonably consistent and met the product specifications, except as indicated.

Table 5. Reducose® 5% Batch Analyses

Tested Parameters	Specification	Lot No./Date of Manufacture			
		IM150525 2015-05-25	A1701191 2017-07-15	A1701493 2017-09-07	181102 2018-11-06
Marker Compounds					
1-Deoxynojirimycin (DNJ)	5.0% ± 0.5%	5.02%	5%	5.08%	5.3%
Physical Characteristics					
Appearance	Fine powder without aggregates	Conforms	Conforms	Conforms	Conforms
Color	Light brown/brown powder	Conforms	Conforms	Conforms	Conforms
Taste	Slightly bitter malt-taste	Conforms	Conforms	Conforms	Conforms
Odor	Characteristic	Conforms	Conforms	Conforms	Conforms
Solubility	Easily dissolved in water & 50% EtOH; not soluble in oil.	Conforms	Conforms	Conforms	Conforms
Chemical Characteristics					
Moisture	<7.0%	6.5%	5.5%	4.3%	5.4%
Acid insoluble ash	<2.0%	0.84%	0.3%	0.5%	0.4%

Heavy Metals					
Arsenic	<1 ppm	0.98 ppm	0.970 ppm	1.104 ppm*	0.458 ppm
Cadmium	<0.5 ppm	ND	0.026 ppm	0.032 ppm	0.016 ppm
Lead	<1 ppm	0.12 ppm	0.158 ppm	0.124 ppm	0.083 ppm
Mercury	<0.1 ppm	ND	0.003 ppm	<0.001 ppm†	<0.001 ppm†
Microbiological Tests					
Total Aerobic Plate Count	$<10^3$ cfu/g	<10 cfu/g	7.5×10^2 cfu/g	1.45×10^3 cfu/g*	40 cfu/g
Total Yeast & Mold	$<10^2$ cfu/g	<10 cfu/g	40 cfu/g	85 cfu/g	Y: <10 cfu/g M: <10 cfu/g
Escherichia coli	<10 cfu/g	ND	ND	ND	<10 cfu/g
Salmonella	Absent in 25 g	ND	ND	ND	ND
Pesticide Residues					
Panel per EU 396/2005	Complies with EU 396/2005	Complies	Complies	Complies	Complies
Mycotoxins‡					
Panel per EU 1881/2006	Complies with EU 1881/2006	NT‡	NT‡	NT‡	Complies
PAH‡					
Panel per EU 1881/2006	Complies with EU 1881/2006	NT‡	NT‡	NT‡	Complies

Abbreviations: cfu, colony forming units; EU, European Union; M, mold; ND, not detected; NMT, not more than; NT, lot not tested in accordance with the skip-lot procedure; OOS, out of specification result; PAH, polycyclic aromatic hydrocarbons; ppm, parts per million; Y, yeast.

*The product specifications were amended in June 2018 to lower the limits for arsenic (from <2 ppm to <1 ppm) and total aerobic plate count (from $<10^4$ cfu/g to $<10^3$ cfu/g). Lot A1701493 was manufactured on September 7, 2017 and was fully compliant with the specifications in place at that time.

†The limit of quantification for the mercury assay is 0.001 ppm.

‡Skip-lot testing of mycotoxins and PAHs is performed in accordance with the 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 6 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 6. 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)
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Bars	537	40	3.1	5	6.2	248
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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 7 and 8.

Table 7. 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)	2445.0	30.1	4099.4	73.9	1.8
Total Population	2+	6530 (100)	2341.3	25.1	4005.5	50.5	1.3

Creme run #427

Table 8. 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	120.0	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	1.9
Total Population	2+	6530 (100)	37.2	0.4	67.8	1.5	2.2

Creme run #427

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 4005.5 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 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 9 and 10 below.

Table 9. 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	1004 (68.7)	292.4	15.0	689.3	51.9	7.5
Adolescents	13–19	515 (63.0)	394.3	25.8	928.8	114.7	12.3
Adults	20+	2712 (65.1)	367.6	12.2	874.5	43.2	4.9
Total Population	2+	4231 (65.4)	358.5	9.2	843.4	36.5	4.3

Creme run #428

Table 10. 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	1004 (68.7)	11.4	0.6	25.7	1.5	5.8
Adolescents	13–19	515 (63.0)	6.2	0.5	15.6	1.9	12.2
Adults	20+	2712 (65.1)	4.6	0.2	10.6	0.5	4.7
Total Population	2+	4231 (65.4)	5.8	0.2	13.0	0.5	3.8

Creme run #428

According to the estimates using a 10% presence probability factor in the tables above, approximately 65.4% 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 843.4 mg/day (13.0 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 689.3 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 (3.72 mg/kg bw/day) and 46.4 mg/day (0.718 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 37.9 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 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 cages 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 rat cecal microbiota culture increased DNJ content by 115.5% suggesting a slight protential 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 in 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.

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.[14] 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 unpublished acute oral toxicity study in mice performed by the Drug and Safety Evaluation Centre, Beijing Municipal Institute for Drug Control, Beijing, China. 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.[15] 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.[16] These studies are summarized below.

6.2.1 Acute Oral Toxicity Study in Mice (Reducose® 5%, unpublished)

Methods: The study was conducted in compliance with GLP (Order No. 2 of the State Food and Drug Administration, 2003) in reference to OECD Test Guideline (TG) 401 (1987), US EPA OPPTS Harmonized Test Guideline 870.1100 (1998), Technical Guidelines for Acute Toxic Studies of TCM and Natural Drug (Center for Drug Evaluation, SFDA, GPT2-1), and Technical Specifications for Health Food Testing Evaluation (2003).

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 on Study Day 0. Mice were monitored for general observations, in and out of the cage, including appearance, activity, reaction to stimuli, secretions, excretions, and death, once prior to administration of the test item, continuously for 4 hours following administration, and once daily thereafter for 14 days. Body weight was recorded prior to test item administration on Study Day 0, and body weight and food intake were recorded on Study Days 1, 4, 7, 11, and 14. Animals were sacrificed (4% chloral hydrate solution IP) on Study Day 14, and the heart, liver, spleen, lung, kidney, adrenal, thymus, brain, stomach, intestine, trachea, esophagus, cervical lymph node, testis, epididymis, uterus, and ovaries were inspected for gross pathological findings at necropsy. Body weight differences were evaluated statistically by univariate analysis of variance and a T-test using SPSS software.

Results: No mortality or abnormal signs or reactions 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; therefore, no microscopic examination of organs and tissues was conducted.

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%)[15]

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[17] 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)[18] 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 11 and 12). 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 13–15).

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 16 and 17).[19-25]

Table 11. Summary of Hematology—28-day Study, Reducose 5%

Group (mg/kg bw/d)	WBC $\times 10^3$/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	86.70 ± 3.54	2.26 ± 0.80	0.64 ± 0.37	0.08 ± 0.04	8.97 ± 0.24	167.90 ± 4.62	0.457 ± 0.015	51.00 ± 1.84	18.74 ± 0.64	367.38 ± 3.02	922.0 ± 96.32	4.83 ± 0.53	22.11 ± 1.15	18.53 ± 2.85
1000	9.83 ± 1.87	13.13 ± 3.04	84.04 ± 3.15	1.96 ± 0.44	0.81 ± 0.32	0.06 ± 0.05	8.88 ± 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 ± 0.85	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	21.60 ± 1.61	20.47 ± 2.27
4000	9.50 ± 1.91	**15.11 ± 4.93***	**81.79 ± 5.02***	2.15 ± 0.46	0.88 ± 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 Range§	6.59-18.37	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	17.9-20.3	350-375	478-1119	3.52-7.97	18.9-25.8	14.2-22.2

Group (mg/kg bw/d)	WBC $\times 10^3$/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 ± 0.49	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	806.00 ± 53.89	4.68 ± 0.90	20.33 ± 0.82	17.93 ± 0.99
1000	**8.11 ± 1.39***	**12.23 ± 2.64***	84.91 ± 2.94	1.91 ± 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 ± 3.24
2000	8.34 ± 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 ± 0.48	354.30 ± 4.14	778.10 ± 56.54	4.56 ± 0.79	20.83 ± 0.95	**20.62 ± 1.82****
4000	7.33 ± 1.14	**13.97 ± 2.12****	**83.05 ± 2.33***	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 Range§	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	0.27-0.46	48.9-59.1	18.2-20.6	346-376	609-1096	3.33-6.19	15.3-23.9	14.6-22.8

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; NEU, neutrophils; PLT, platelet; PT, prothrombin time; RBC, red blood cell; RET, reticulocyte; TE, total erythrocytes; WBC, white blood cell.

Table 12. 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 ± 28.9	2.03 ± 0.23	23.13 ± 2.14	7.57 ± 0.92	6.03 ± 0.68	1.93 ± 0.25	2.65 ± 0.14	2.66 ± 0.05	139.8 ± 0.9	4.12 ± 0.19	102.53 ± 0.69	33.49 ± 1.08	57.91 ± 1.43	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	1.75 ± 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 ± 2.07****	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 ± 1.26	**6.80 ± 0.91***	1.67 ± 0.27	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 ± 1.86	1.42 ± 0.08
4000	**50.96 ± 8.29****	**108.26 ± 12.28***		148.8 ± 17.5	1.85 ± 0.32	**20.79 ± 1.31***	8.02 ± 1.36	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 ± 0.64	34.19 ± 0.58	58.94 ± 3.01	1.39 ± 0.16
Historical Range§	42.4-76.7	66.3-144.8	0.1-1.9	112-321	0.64-2.76	17.7-30.3	5.27-11.12	4.66-7.59	1.32-2.74	2.11-3.23	2.49-2.89	132.0-143.0	3.66-4.94	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	46.42 ± 6.77	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 ± 0.31	24.54 ± 2.03	7.23 ± 1.30	5.35 ± 0.99	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	**2.54 ± 0.07***	139.70 ± 1.06	3.99 ± 0.30	104.58 ± 0.59	34.00 ± 1.09	56.56 ± 1.80	1.53 ± 0.09
4000	46.13 ± 8.65	93.95 ± 8.20	-	102.90 ± 21.06	1.72 ± 0.23	24.54 ± 1.29	7.79 ± 1.40	5.84 ± 1.09	1.72 ± 0.19	1.99 ± 0.26	**2.58 ± 0.04****	**138.20 ± 1.03****	4.07 ± 0.29	104.30 ± 1.04	34.21 ± 0.80	56.95 ± 1.36	1.51 ± 0.07
Historical Range§	36.8-86.4	76.8-272.1	0.1-2.6	56-192	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	55.2-65.2	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; Cl^-, chloride; CREA, creatinine; GGT, gamma glutamyl transferase; K^+, potassium; Na^+, sodium; PI, inorganic phosphate; TBIL, total bilirubin; TPROT, total protein

"-" no data; the quantitation limit of GGT: 7 U/L

Table 13. 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 ±	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 14. 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 15. 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 16. 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 17. 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%)[16]

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%)[16]

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%)[16]

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%)[16]

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%)[16]

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 18).

Table 18. 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 19. Genetic Toxicity Tests—Other *Morus alba* Leaf Preparations

Author	Test Item	Study Type	Design	Results
Kim et al. (2007) [26]	*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)[27]	*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)[28]	*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)[29]	*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.[30] 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.[31] 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.[32] 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 staircase method" (described in "Ghosh MN, Fundamentals of Experimental Pharmacology, 2nd edn. Scientific book agency, Calcutta, 1984").[33] 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.[28] 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).[34]

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 20. 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.[29] 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.[35, 36] Silkworm extract powder (SEP) containing 1.25% DNJ has been subjected to a battery of genetic and oral toxicity tests.[36] 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 21. Genetic Toxicity Tests—Silkworm Extract Powder (1.25% DNJ)

Author	Test Item	Study Type	Design	Results
Heo et al. (2013)[36]	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)[36]	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)[36]	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 22. Oral Toxicity Studies—Silkworm Extract Powder (1.25% DNJ)

Author	Test Item	Study Type	Design	Results
Heo et al. (2013)[36]	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) [36]	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.[37] 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.[38] Furthermore, as described in Subpart 6.1, Nakagawa et al. (2007) observed a C_{max} of 92 μM (9.2 x 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.[14] 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 Human Studies

Twelve out of 15 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 23 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.

Author, Date	Test Item	Dose	Duration	Subjects	Design	Results
Asai et al. (2011)[39]	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)[40]	*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)[41]	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.
Kim et al. (2015)[42]	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).

Author, Date	Test Item	Dose	Duration	Subjects	Design	Results
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)[43]	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)[44]	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)[45]	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).
Wang et al. (2018)[46]	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.

Author, Date	Test Item	Dose	Duration	Subjects	Design	Results
Wattanathorn et al. (2018)[47]	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.

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.[48] 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 significant; 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.[42] 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[49]; 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.[50] 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 affect 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.[51] 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.[52] 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.[53, 54] 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,[55, 56] as well as South Korea where it is regulated as a permitted food ingredient.[57]

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, 53] Indian cultures use *M. alba* leaves in traditional dishes such as curry, saag, pakoda, paratha, and dhokla and in the preparation of spices.[1, 58]

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 three 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 August 26, 2019.

CAERS currently contains records of 160,193 AERs submitted to FDA from January 2004 through March 31, 2019 (the date of the last data set release). Thus the frequency of occurrence within the CAERS data set is 0.0019%. The most recent report was of respiratory complaints (age and sex not reported) associated with use of an Organic Mulberry Juice. 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 a 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. Based on the intended use of the ingredient in food in the categories and at the addition levels shown in Tables 6 (also duplicated as Table 1), the NOAEL allows for an adequate MOS (NOAEL/Exposure; 4000 mg/kg/13 mg/kg) of approximately 308-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.

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

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

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, and again from June 2019 through October 2019.

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 acute oral toxicity study in mice of Reducose® 5%
- 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.

7.2 References that *are* Generally Available

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