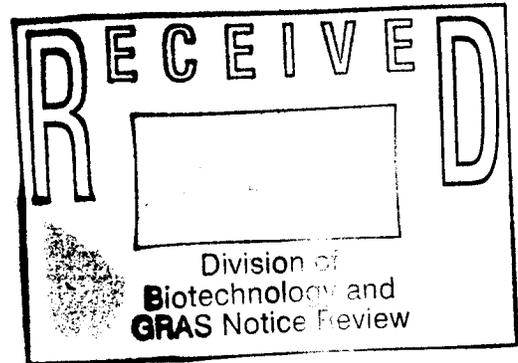




GRAS Notice (GRN) No. 497

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

ORIGINAL SUBMISSION



GRM 000497

December 3, 2013

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

Re: GRAS Exemption Claim for Oligonol®

Dear Dr. Gaynor:

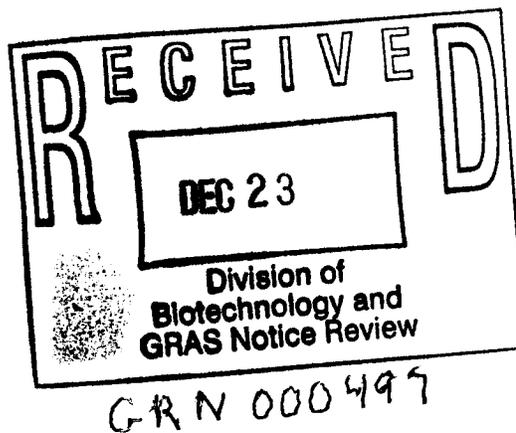
In accordance with proposed 21 CFR §170.36 [Notice of a claim for exemption based on a Generally Recognized as Safe (GRAS) determination] published in the *Federal Register* [62 FR 18938 (17 April 1997)], I am submitting one hard copy and one electronic copy (on CD), as the Notifier [Amino Up Chemical Co., Ltd., 363-32 Shin-ei, Kiyota-ku, Sapporo, 004-0839, Japan], a Notice of the determination, on the basis of scientific procedures, that Oligonol® produced by Amino Up Chemical Co., Ltd., as defined in the enclosed documents, is GRAS under specific conditions of use as a food ingredient, and therefore, is exempt from the premarket approval requirements of the *Federal, Food, Drug and Cosmetic Act*. Information setting forth the basis for the GRAS determination, which includes a comprehensive summary of the data available that have been reviewed by an independent panel of experts (the Expert Panel) qualified by scientific training and experience to evaluate the safety of Oligonol® under the intended conditions of use, also are enclosed for review by the agency.

The enclosed electronic files have been scanned for viruses prior to submission and are certified as being virus-free using McAfee VirusScan 8.8. Should you have any questions or concerns regarding this GRAS Notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

(b) (6)

Kohei Homma, Ph.D.
R&D Manager, Global Strategy Group
Amino Up Chemical Co., Ltd.
Telephone: +81-11-889-2233
Email: kohei.homma@aminoup.co.jp



Generally Recognized as Safe (GRAS) Exemption Claim for Oligonol®

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

Submitted by: Amino Up Chemical Co., Ltd.
363-32 Shin-ei,
Kiyota-ku,
Sapporo 004-0839
Japan

December 3, 2013

Generally Recognized as Safe (GRAS) Exemption Claim for Oligonol®

Table of Contents

	Page
I. GRAS EXEMPTION CLAIM	3
I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1) [62 FR 18938 - U.S. FDA, 1997]	3
I.B Name and Address of Notifier	3
I.C Common Name of the Notified Substance	3
I.D Conditions of Intended Use in Food	3
I.E Basis for the GRAS Determination	5
I.F Availability of Information	5
II DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE	6
II.A Identity	6
II.B Method of Manufacture	7
II.C Product Specifications	8
II.D Stability	9
II.D.1 Bulk Stability	9
II.D.2 Stability under Intended Uses	10
II.D.3 Degradation Pathways and Products	11
III SELF-LIMITING LEVELS OF USE	12
IV BASIS FOR GRAS DETERMINATION	12
IV.A Probable Consumption of Oligonol®	13
IV.A.1 Estimated Consumption of Polyphenols from Natural Occurrences in Food	13
IV.A.2 Estimated Consumption of Oligonol® from Intended Food Uses	15
IV.A.3 Estimated Consumption of Polyphenols from the Intended Food Uses of Oligonol®	17
IV.A.4 Summary	19
IV.B Absorption, Distribution, Metabolism, Excretion	19
IV.B.1 Polyphenol Constituents of Oligonol®	19
IV.B.2 Polyphenols from Oligonol®-like Product	21
IV.B.3 Summary	22
IV.C Toxicological Studies Conducted with Oligonol®	22
IV.C.1 Acute Toxicity	22
IV.C.2 Sub-Chronic Toxicity	22
IV.C.3 Mutagenicity and Genotoxicity	27
IV.D Human Studies Conducted with Oligonol®	28
IV.E Safety of an Oligonol®-like Product	29
IV.E.1 Acute Toxicity	29
IV.E.2 Short-Term Toxicity	30

IV.E.3	Mutagenicity and Genotoxicity.....	30
IV.E.4	Human Studies	30
IV.F	Safety of the Green Tea Extract Component of Oligonol®	31
IV.F.1	14-Week Toxicity Study of Green Tea Extract.....	31
IV.F.2	Nasal Toxicity Study with Oligonol®	32
IV.G	Summary of Studies Supporting the Safety of Oligonol®	33
IV.H	Nutritional Considerations.....	33
IV.H.1	Allergenicity.....	33
IV.I	Summary and Basis for GRAS.....	34
V	REFERENCES.....	36

Appendix A Expert Panel Consensus Statement Concerning the GRAS Status of Oligonol®

List of Tables

Table I.D-1	Summary of the Individual Proposed Food-Uses and Use Levels for Oligonol® in the United States	4
Table II.A-1	Compositional Analysis of Three Sample Lots of Oligonol®	7
Table II.C-1	Product Specifications for Oligonol®	8
Table II.C-2	Batch Analyses for 3 Non-Consecutive Lots of Oligonol®.....	9
Table II.D.1-1	Composition of Monomeric Flavan-3-ol and Procyanidins in Oligonol® After One Year of Storage.....	10
Table II.D.2-1	Total Polyphenol Content (%) in Oligonol® Following Storage at Various pH and Temperatures for Up to 3 Months	10
Table IV.A-1	Examples of the Major Classes of Dietary Polyphenols.....	14
Table IV.A.2-1	Summary of the Estimated Daily Intake of Oligonol® from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data).....	16
Table IV.A.2-2	Summary of the Estimated Daily Per Kilogram Body Weight Intake of Oligonol® from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)	16
Table IV.A.2-3	Summary of the Estimated Daily Intake of Oligonol® from Proposed Food-Uses and Dietary Supplement Use in the U.S. by Population Group (2009-2010 NHANES Data)	17
Table IV.A.3-1	Intake of Monomeric Flavan-3-ols from Consumption of Oligonol® Compared to Drinking Green Tea	18
Table IV.A.3-2	Intake of Procyanidins from Consumption of Oligonol® Compared to Selected Dietary Sources.....	18

I. GRAS EXEMPTION CLAIM

I.A Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1) [62 FR 18938 - U.S. FDA, 1997]

Amino Up hereby claims that the use of Oligonol® [polyphenols obtained from a 5:1 mixture of lychee fruit extract (*Litchi chinensis* Sonn.) and green tea leaf extract (*Camellia sinensis* (L.) Sinensis)] in foods, as described in Section I.D below, is exempt from the requirement of premarket approval of the *Federal Food, Drug, and Cosmetic Act* because we have determined that such uses are Generally Recognized as Safe (GRAS).

Signed,

(b) (6)

Kohei Homma, Ph.D.
R&D Manager, Global Strategy Group
Amino Up Chemical Co., Ltd.

December 3, 2013

Date

I.B Name and Address of Notifier

Amino Up Chemical Co., Ltd.
363-32 Shin-ei,
Kiyota-ku,
Sapporo, 004-0839
Japan
Email: kohei.homma@aminoup.co.jp

I.C Common Name of the Notified Substance

Polyphenols from lychee fruit and green tea leaves

I.D Conditions of Intended Use in Food

(i) Foods in Which the Substance is to be Used

Amino Up intends to market Oligonol® for use as an ingredient in multiple food categories at use levels ranging from 12 to 192 mg/serving, as described in Table I.D-1.

Table I.D-1 Summary of the Individual Proposed Food-Uses and Use Levels for Oligonol® in the United States

Food Category	Proposed Food-Use	Oligonol® Use Level (mg/serving)	Serving Size (g/mL) ^a	Use Level (%)
Breakfast Cereals	Ready-to-Eat Cereals	12, 24, or 44	(Puffed) 15 (Fiber) 30 (Biscuit) 55	0.08
Coffee and Tea	Tea	100	240	0.042
Dairy Product Analogs	Soy and Imitation Milk	24	240	0.01
Grain Products and Pastas	Granola, Meal Replacement, and Breakfast Bars	32	40	0.08
Milk Products	Dairy-Based Drinks (one shot) ^b	100	100	0.10
	Flavored Milk and Milk Drinks	24	240	0.01
	Milk-Based Meal Replacement Beverages	192	240	0.08
	Yogurt	45	225	0.02
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks and Ades (RTD and Powder)	100	240	0.042
	Fruit Juices	100	240	0.042
Soups and Soup Mixes	Soups with Legumes or Potatoes as Major Ingredients ^c	100	245	0.041
	Tomato and Vegetable Soups ^c	100	245	0.041

Abbreviations: RTD = Ready-to-Drink

^a Serving sizes based on the US FDA Reference amounts customarily consumed per eating occasion (RACCs), April 1, 2013, 21 CFR §101.12 (U.S. FDA, 2013a). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

^b Food codes representative of one-shot dairy based drinks were not identified. Fruit smoothie drinks and milk with acidophilus were used as surrogates for one-shot dairy-based drinks.

^c Excluding meat/poultry-based soups

(ii) Purpose for Which the Substance is Used

Oligonol® is intended to serve as a source of polyphenols when added to foods. The polyphenols that are present in Oligonol® also occur naturally in various plant-derived dietary sources. It should be noted that although the polyphenols present in Oligonol® gives the ingredient a reddish-brown color, the intended use levels of Oligonol® will not reach concentrations at which the color imparted would be significant with regard to the appearance, value, marketability, or consumer acceptability of the resulting food products. Any slight color effect that may result from the addition of Oligonol® to foods would be considered incidental to its nutritional use, and not from the intentional use for the purpose of imparting color. Therefore, Oligonol® is not intended to function as a color additive, as defined under Section 201(t)(1) of the Federal Food, Drug, and Cosmetics Act and FDA's implementing regulations in 21 CFR Part 70.

(iii) Description of the Population Expected to Consume the Substance

Oligonol[®] is expected to be consumed by members of the general United States (U.S.) population who may be reasonably expected to consume at least one food within the aforementioned food categories. Food and beverage products containing Oligonol[®] will be targeted to consumers seeking foods enriched in polyphenols.

I.E Basis for the GRAS Determination

Pursuant to Title 21, Section 170.30 of the *Code of Federal Regulations* (CFR) §170.30 (U.S. FDA, 2013b), Oligonol[®] has been determined as GRAS on the basis of scientific procedures. This GRAS determination is based on data pertaining to the safety of Oligonol[®] that are generally available in the public domain, and on consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of Oligonol[®] as a component of food [see Appendix A, entitled, "**Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Oligonol[®]**"].

The Expert Panel consisted of the following qualified scientific experts: John A. Thomas, Ph.D. (Indiana University School of Medicine); Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell); and Stephen L. Taylor, Ph.D. (University of Nebraska). The Expert Panel, convened by Amino Up, independently and critically evaluated all data and information presented herein, and concluded that Oligonol[®] is GRAS for use as an ingredient in food and beverage products as described in Table I.D-1.

I.F Availability of Information

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

Amino Up Chemical Co., Ltd.
363-32 Shin-ei
Kiyota-ku
Sapporo, 004-0839
Japan

Attn:
Kohei Homma,
R&D Manager, Global Strategy Group
Telephone: +81-11-889-2233 (office); +81-90-1528-7316 (cell)
Email: kohei.homma@aminoup.co.jp

Should the FDA have any questions or additional information requests regarding this notification, Amino Up Chemical Co., Ltd. will supply these data and information.

II DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED SUBSTANCE

II.A Identity

Common or Usual Name: Polyphenols derived from lychee fruit and green tea leaves

Trade Name: Oligonol[®]

Composition:

Oligonol[®] is a reddish-brown powder composed of a mixture of oligomerized polyphenols derived from extracts of the *Litchi chinensis* (lychee) fruit and *C. sinensis* (green tea) leaf. The compositional analysis of three non-consecutive sample lots of Oligonol[®] is presented in Table II.A-1. The total polyphenol content of Oligonol[®], as measured using the Folin-Denis assay, accounts for >80% of the total mixture. Oligonol[®] consists mainly of monomeric flavan-3-ols, as well as procyanidins formed from the condensation of these monomeric units. Specifically, Oligonol[®] contains four different flavan-3-ol monomers, (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin gallate (EGCG), which combined constitute approximately 16% of Oligonol[®]. In addition, 5 polyphenol dimers [procyanidin A1, procyanidin A2, procyanidin B1, procyanidin B2, and (-)-epicatechin-(4 β →8)-(-)-epigallocatechin gallate] and a trimer [(-)-epicatechin-(4 β →8, 2 β →O→7)-epicatechin-(4 β →8)-epicatechin] have been identified in Oligonol[®] and quantified using high-performance liquid chromatography (HPLC). These dimers and trimer constitute approximately 14 and 4% of Oligonol[®], respectively. Longer oligomers, composed of varying combinations of monomers, also are present in Oligonol[®]; however, due to technical limitations, these cannot be readily identified or quantified.

Table II.A-1 Compositional Analysis of Three Sample Lots of Oligonol[®]				
Constituents	Lot Number^a			
	OLF0703	OLF0705	OLF0804	
Material Balance Analysis				
Monomeric Flavan-3-ols + Procyanidins	92.5	95.6	92.7	
Sugars (%)	3.5	3.7	3.5	
Moisture (%)	0.0	0.0	0.0	
Protein (%)	1.6	1.6	1.6	
Total fat (%)	0.5	0.6	1.0	
Ash (%)	0.2	0.3	0.2	
Total (%)	98.3	101.8	99.0	
Phenolic Composition				
Monomeric Flavan-3-ols (%)				
(+)-Catechin and (-)-Epicatechin	8.2	7.9	6.4	
(-)-Epicatechin-3-gallate	2.1	2.0	2.3	
(-)-Epigallocatechin gallate	6.0	6.2	6.4	
Total Monomeric Flavan-3-ols (%)	16.3	16.1	15.1	
Procyanidins (%)				
Dimers	Procyanidin A1	4.1	4.0	3.5
	Procyanidin A2	5.0	4.9	5.3
	Procyanidin B1	1.3	1.3	0.4
	Procyanidin B2	3.1	3.0	2.5
	(-)-Epicatechin-(4 β →8)-(-)-epigallocatechin gallate	0.3	0.3	0.5
	Total Dimers (%)	13.8	13.5	12.2
Trimer	(-)-Epicatechin-(4 β →8, 2 β →O→7)-epicatechin-(4 β →8)-epicatechin	3.8	3.8	1.9
Other procyanidins	Not further classified	58.6	62.2	63.5
Total Procyanidins (%)	76.2	79.5	77.6	
Total Monomeric Flavan-3-ols + Procyanidins	92.5	95.6	92.7	

^a Analyses were conducted on freeze-dried samples of Oligonol[®].

II.B Method of Manufacture

Oligonol[®] is manufactured using an oligomerization process whereby the polyphenols present in a 5:1 mixture of extracts from the lychee fruit (*Litchi chinensis* Sonn.) and green tea leaves [*Camellia sinensis* (L.) Kuntze] are cleaved into monomers and lower molecular weight oligomers in the presence of citric acid (Tanaka *et al.*, 2007). The reaction mixture then undergoes a series of filtration and purification steps, and is spray-dried to produce the final product in powdered form. Oligonol[®] is manufactured in accordance with Good Manufacturing Practices (GMP) for dietary supplements, a standard that is based on guidelines prepared by

the Japan Ministry of Health, Labour and Welfare and is certified by the Japan Health and Nutrition Food Association. Oligonol® is also manufactured in accordance with ISO 9001:2008 and ISO 22000:2005. All of the raw materials and processing aids used in the manufacture of Oligonol® are food grade and suitable for use in accordance with U.S. regulations.

II.C Product Specifications

Food-grade specifications have been established for Oligonol®. Each batch of Oligonol® is specified to contain at least 70% procyanidins and 10% of the monomeric flavan-3-ols. Limits for the levels of heavy metals and microbial contamination have also been set. The product specifications for Oligonol® are presented below in Table II.C-1.

Table II.C-1 Product Specifications for Oligonol®		
Specification Parameter	Specification	Reference/Test Methodology Performance of Test
Identity		
Characteristic	Reddish-brown powder, Astringent taste	Sensory analysis
Moisture (%)	Not more than 5.0	Oven drying at 70°C for 6h under reduced pressure
Total Procyanidin (%)	More than 70	Porter method
Monomeric flavan-3-ols (%)	More than 10	HPLC method
Heavy Metals		
Lead (Pb) (ppm)	Not more than 0.2	Atomic absorption spectrophotometry
Arsenic (as As ₂ O ₃) (ppm)	Not more than 1.0	Colorimetric method (arsenic limit test)
Microbial Specifications		
Number of bacteria (CFU/g)	Not more than 1,000	Microbial Limit test (pour plate method)
Mold and Yeast (CFU/g)	Not detected	Microbial Limit test (spread plate method)
Coliforms (CFU/g)	Not detected	Microbial Limit test (spread plate method)

Abbreviations: As₂O₃ = arsenic oxide; CFU = colony forming units; HPLC = high-performance liquid chromatography; ppm = parts per million.

Analysis of 3 non-consecutive lots of Oligonol® demonstrates that the manufacturing process produces a consistent product meeting physical, chemical, and microbiological specifications. The results of these batch analyses are presented in Table II.C-2.

Table II.C-2 Batch Analyses for 3 Non-Consecutive Lots of Oligonol®

Specification Parameter	Specification	Manufacturing Lot ^a		
		OLF0703	OLF0705	OLF0804
Identity				
Characteristic	Reddish-brown powder, characteristic rough taste	Satisfied	Satisfied	Satisfied
Moisture (%)	Not more than 3.0	0.0	0.0	0.0
Total Procyanidin (%)	More than 70	76.2	79.5	77.6
Monomeric flavan-3-ols (%)	More than 10	16.3	16.1	15.1
Heavy Metals				
Lead (Pb) (ppm)	Not more than 0.2	Not detected	Not detected	Not detected
Arsenic (as As ₂ O ₃) (ppm)	Not more than 1.0	≤1.0	≤1.0	≤1.0
Microbial Specifications				
Number of bacteria (CFU/g)	Not more than 1,000	0	0	0
Mold and yeast	Not detected	Not detected	Not detected	Not detected
Coliforms (presence/absence)	Absent	Absent	Absent	Absent

Abbreviations: As₂O₃ = arsenic oxide; CFU = colony forming units; HPLC = high-performance liquid chromatography; Pb = lead; ppm = parts per million.

^a Analyses were conducted on freeze-dried samples of Oligonol®.

II.D Stability

II.D.1 Bulk Stability

The stability of the monomeric and polyphenol constituents of Oligonol® was assessed after one year of storage. A sample lot of the Oligonol® powder was stored at room temperature, in an aluminum bag to prevent light and moisture from affecting the sample. The content of the polyphenolic constituents of Oligonol® were measured in the sample at baseline and after one year of storage. No significant differences in the polyphenol composition of Oligonol® were observed (see Table II.D.1-1).

Table II.D.1-1 Composition of Monomeric Flavan-3-ol and Procyanidins in Oligonol® After One Year of Storage

Phenolic Constituents of Oligonol®	Initial Composition (%)	Composition after 1 year (%)
(+)-catechin and (-)-epicatechin	8.4	8.4
(-)-epicatechin-3-gallate	2.2	2.3
(-)-epigallocatechin gallate	6.2	6.3
Procyanidin A1	4.3	4.5
Procyanidin A2	5.2	5.2
Procyanidin B1	1.2	1.2
Procyanidin B2	2.6	3.0
(-)-epicatechin-(4 β - \rightarrow 8)-(-)-epigallocatechin gallate	0.2	0.3
(-)-epicatechin-(4 β - \rightarrow 8, 2 β - \rightarrow O - \rightarrow 7)-epicatechin-(4 β - \rightarrow 8)-epicatechin	4.2	4.2
Total (%)	34.5	35.4

II.D.2 Stability under Intended Uses

The stability of Oligonol® under different pH conditions has been evaluated. Solutions containing 50 ppm of Oligonol®, adjusted to pH of 3, 5, or 7, were stored at 4°C, room temperature, or 40°C for 3 months. The Oligonol® solutions were analyzed for total polyphenol content at study initiation, and at 1, 2, and 3 months of storage. The results of this stability study are summarized in Table II.D.2-1.

Table II.D.2-1 Total Polyphenol Content (%) in Oligonol® Following Storage at Various pH and Temperatures for Up to 3 Months

pH	Period	Storage Temperature		
		4°C	Room temperature	40°C
3	At baseline	100	100	100
	After 1 month	100	100	100
	After 2 months	100	100	100
	After 3 months	100	100	100
5	At baseline	100	100	100
	After 1 month	100	98	80
	After 2 months	97	88	75
	After 3 months	96	88	71
7	At baseline	100	100	100
	After 1 month	100	93	62
	After 2 months	90	77	58
	After 3 months	85	72	45

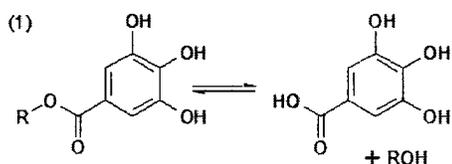
Oligonol is proposed for use mainly in foods that are acidic (e.g., fruit and vegetable-based beverages). Accordingly, the total polyphenol content of the Oligonol[®] was not altered for the duration of the storage period (up to 3 months) under acidic conditions (pH 3). Although Oligonol[®] is less stable under alkaline conditions, such as dairy-based foods, these products are usually refrigerated, which would reduce the extent of degradation. Furthermore, as discussed in Section II.D.3, there are no safety concerns anticipated with the degradation products that may be formed from Oligonol[®] under alkaline conditions.

A study was also conducted to investigate the sensitivity of Oligonol[®] to light. Oligonol[®], at a concentration of 100 ppm in tap water, was maintained at 4°C either in the dark or exposed to light (3,000 to 3,500 lux) for 4 weeks. Total polyphenol content was measured at baseline, 2 and 4 weeks using the Folin-Denis method. No decline in total polyphenol content was observed under the dark or lit conditions.

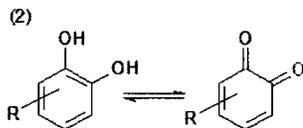
II.D.3 Degradation Pathways and Products

There are a number of well-established mechanisms by which the polyphenolic compounds found naturally in foods can degrade under conditions of high pH (>7), exposure to air and elevated temperatures (DeMan, 1999; Francis, 1999). It is anticipated that similar processes would be observed with Oligonol[®]. Examples of these typical degradation processes are outlined below:

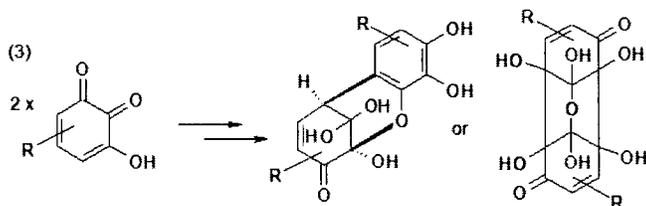
1. Hydrolyzable systems such as (-)-epicatechin-3-gallate and (-)-epigallocatechin gallate may break down under alkaline conditions to release free gallic acid.



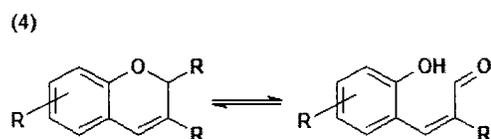
2. Oxidation of dihydroxybenzene systems results in the formation of quinones. In particular, alkaline hydrolysis of procyanidins may result in the formation of quinone intermediates which are then converted to anthocyanins.



3. Oxidative coupling mechanisms will result in polymerization to form polymers. In buffered solutions of pH 7.4, (-)-epigallocatechin gallate forms the dimer theasinensin A by auto-oxidation (Tanaka *et al.*, 2003).



4. Anthocyanins and some flavanoid derivatives may undergo ring opening under strongly alkaline conditions (pH 12) to form chalcones.



R = other substituents

The degradation products of Oligonol[®] under alkaline conditions will be the same to those found in other polyphenol-containing foods. For example, the oxidation products of procyanidins and the (-)-epigallocatechin gallate dimers (*i.e.*, theasinensins A and D) are constituents of black tea (Tanaka *et al.*, 2003). Background consumption of these degradation products from dietary sources are far greater than those that may potentially arise from the proposed uses and use-levels of Oligonol[®]. Given the historical consumption of these degradation products in the diet, they are not considered to pose a safety concern to humans.

III SELF-LIMITING LEVELS OF USE

The use of Oligonol[®] is largely limited by the desired astringency (*i.e.*, drying or puckering sensation in the mouth) for a particular food or beverage product. Thus, the use of Oligonol[®] is self-limiting based on its organoleptic properties.

IV BASIS FOR GRAS DETERMINATION

The data and information summarized below demonstrate that Oligonol[®] [polyphenols obtained from a 5:1 mixture of lychee fruit extract (*Litchi chinensis* Sonn.) and green tea leaf extract (*Camellia sinensis*)] under the conditions of proposed use described in Table I.D-1, is GRAS, based on scientific procedures. Both lychee fruit and green tea have a long history of safe consumption in the diet. Furthermore, the polyphenol constituents in Oligonol[®] (*i.e.*, monomeric

flavan-3-ols and procyanidins) are widely consumed in the diet through plant-derived food sources, as discussed in Section IV.A. Oligonol[®] is expected to undergo similar metabolic fates as polyphenols that are consumed in the diet, as summarized in Section IV.B. The safety of Oligonol[®] is supported by data from acute and repeat-dose oral toxicity studies conducted in rodents, and Oligonol[®] is not mutagenic or genotoxic when tested using the standard battery of *in vitro* and *in vivo* assays. Oligonol[®] has also been orally administered to human subjects at doses ranging from 200 to 600 mg/person/day, for durations as long as 3 months, without adverse effects. The safety of Oligonol[®] can be further corroborated by toxicity studies and a clinical study conducted with an Oligonol[®]-like product that is manufactured using the same oligomerization process (but with different starting materials) and contains a similar composition of polyphenols (*i.e.*, 15 to 20% monomers, 8 to 12% dimers, and 5 to 10% trimers) as Oligonol[®]. The studies conducted with Oligonol[®] and “Oligonol[®]-like” products are summarized in Sections IV.C to IV.E. Even though green tea has been consumed for centuries by large numbers of the international population, and it continues to be one of the most widely consumed beverages in the world, there have been some concerns raised over the safety of highly concentrated, purified forms of green tea extracts being marketed primarily as dietary supplements for weight loss purposes, including liver toxicity, as presented in Section IV.F. Although evidence of hepatotoxicity and nasal toxicity were reported following administration of green tea extracts in rodent studies conducted by the National Toxicology Program (NTP), these findings were not observed in product-specific toxicity studies conducted with Oligonol[®] at dose levels up to 1,000 mg/kg body weight/day. Moreover, it is important to note that the intakes of green tea catechins from the intended uses of Oligonol[®] are less than the amount that would be consumed from a standard 200 mL serving of green tea.

Finally, the totality of data and information were reviewed by a Panel of Experts, qualified by scientific training and experience to evaluate the safety of ingredients as components of food, who similarly concluded that the intended uses of Oligonol[®] are GRAS based on scientific procedures.

IV.A Probable Consumption of Oligonol[®]

IV.A.1 Estimated Consumption of Polyphenols from Natural Occurrences in Food

Polyphenols are bioactive compounds that are present in various dietary sources, including fruit, vegetables and beverages of plant origin (such as teas). Polyphenols can be divided into various classes according to their basic chemical structures; the classification of the major dietary polyphenols is summarized below in Table IV.A-1. Polyphenols are highly complex chemical entities; for example, flavonoids can be further divided into 6 major subclasses, which consist of over 5,000 identified compounds (Martin and Appel, 2010).

Table IV.A-1 Examples of the Major Classes of Dietary Polyphenols^a

Class	Subclass	Examples
Flavonoids	Flavan-3-ols (<i>main polyphenols in Oligonol[®]</i>)	Catechins (monomers) Procyanidins (oligomers)
	Flavonols	Quercetin, myricetin
	Flavanones	Naringenin
	Flavones	Apigenin
	Isoflavones	Daidzein
	Anthocyanins	Cyanidin, peonidin
Phenolic acids	Hydroxycinnamic acids	Caffeic acid, ferulic acid, sinapic acid, <i>p</i> -coumaric acid
	Hydroxybenzoic acids	Gallic acid
Stilbenes	--	Resveratrol
Lignans	--	Secoisolaicresinol

^a Modified from McKay and Blumberg (2007) and Martin and Appel (2010).

Among the different classes of polyphenols, the flavonoids are one of the most common in the diet, accounting for nearly two-thirds of the total dietary intake of polyphenols (Aron and Kennedy, 2008; Song and Chun, 2008; Martin and Appel, 2010; Tsao, 2010). Specifically, flavan-3-ols and their polymeric condensation products (*i.e.*, proanthocyanidins¹) are one of the most commonly consumed flavonoids, occurring in various dietary sources such as fruits, vegetables, plant-derived beverages (*e.g.*, tea, coffee, wine, beer), and chocolate (Santos-Buelga and Scalbert, 2000; Beecher, 2003; Gu *et al.*, 2004; Manach *et al.*, 2004; Aron and Kennedy, 2008; Thilakarathna and Rupasinghe, 2013).

A number of studies have been conducted to provide estimations of the dietary intake of polyphenols, specifically flavonoids, in various countries across the world (reviewed in Beecher, 2003; Chun *et al.*, 2007). In general, consumption of the recommended 5 servings of fruits and vegetables per day is estimated to provide a total polyphenol intake of >500 mg, of which flavonoid intake accounts for approximately 150 to 300 mg/day (Williamson and Holst, 2008; Martin and Appel, 2010). Other dietary sources such as cocoa, coffee or tea are also rich in flavonoids. For example, under typical brewing times, a 235 mL serving of tea contains between 137 to 141 mg of flavonoids (Lakenbrink *et al.*, 2000). Accordingly, the total daily intakes of total polyphenols from the diet have been reported to range from less than 100 mg to more than 2,000 mg among certain individuals, depending on dietary habits (Clifford, 2004). It has been proposed that total polyphenol intake levels of approximately 1,000 mg/day can be expected among individuals who consume a balanced diet (Williamson and Holst, 2008; Martin and Appel, 2010).

¹ Proanthocyanidins that are composed exclusively of epi(catechin) are known as procyanidins. Procyanidins are the most abundant form of proanthocyanidins in plants.

IV.A.2 Estimated Consumption of Oligonol[®] from Intended Food Uses

Estimates for the intake of Oligonol[®] were based on the proposed food-uses and use levels in conjunction with food consumption data included in the US National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) from the 2009-2010 cycle (CDC, 2011; USDA, 2012).

Calculations for the mean and 90th percentile all-person and all-user intakes were performed for each of the individual proposed food-uses of Oligonol[®] and the percentage of consumers were determined. Similar calculations were used to estimate the total intake of Oligonol[®] resulting from all proposed food-uses of Oligonol[®] combined. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

- infants, ages 0 to 2;
- children, ages 3 to 11;
- female teenagers, ages 12 to 19;
- male teenagers, ages 12 to 19;
- female adults, ages 20 and up;
- male adults, ages 20 and up; and
- total population (all age and gender groups combined).

Estimates for the total daily intakes of Oligonol[®] from all intended food-uses are provided in Table IV.A.2-1 and Table IV.A.2-2 on a per person and per kilogram body weight basis, respectively. Approximately 86.2% of the total U.S. population was identified as potential consumers of Oligonol[®] from proposed food-uses (7,194 actual users identified). Consumption of proposed food-uses by the total U.S. population resulted in an estimated mean all-person and all-user intakes of Oligonol[®] of 164 mg/person/day (2.9 mg/kg body weight/day) and 190 mg/person/day (3.3 mg/kg body weight/day), respectively. The 90th percentile all-person and all-user intakes of Oligonol[®] from proposed food-uses by the total population were 386 mg/person/day (6.8 mg/kg body weight/day) and 412 mg/person/day (7.3 mg/kg body weight/day), respectively.

Of all population groups, children had the greatest percentage of users at 97.5%, followed by female teenagers at 90.4%. The greatest mean all-person and all-user intakes of Oligonol[®] on an absolute basis were determined to occur in male adults at 183 mg/person/day (2.1 mg/kg body weight/day) and 222 mg/person/day (2.6 mg/kg body weight/day), respectively. On a body weight basis, the mean all-person and all-user intakes of Oligonol[®] were highest in infants, at 6.8 and 9.6 mg/kg body weight/day, respectively.

Table IV.A.2-1 Summary of the Estimated Daily Intake of Oligonol® from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/day)		All-Users Consumption (mg/day)			
		Mean	90 th Percentile	% Users	n	Mean	90 th Percentile
Infants	0 to 2	85	226	71.4	570	120	257
Children	3 to 11	140	275	97.5	1,397	143	277
Female Teenagers	12 to 19	154	304	90.4	487	170	326
Male Teenagers	12 to 19	166	390	87.8	516	189	397
Female Adults	20 and up	164	395	87.0	2,305	188	416
Male Adults	20 and up	183	475	82.5	1,919	222	512
Total Population	All Ages	164	386	86.2	7,194	190	412

When high consumers (*i.e.*, intakes at the 90th percentile) were examined, the estimate for the all-person and all-user intakes of Oligonol® from proposed food-uses were determined to be greatest in male adults at 475 and 512 mg/person/day, respectively (Table IV.A.2-1). The lowest 90th percentile all-person and all-user intake estimates were observed to occur in infants, with values of 226 and 257 mg/person/day, respectively, on an absolute basis (Table IV.A.2-1). On a body weight basis, infants were determined to have the greatest all-person and all-user 90th percentile intakes of Oligonol®, with values of 18.3 and 19.7 mg/kg body weight/day, respectively (Table IV.A.2-2).

In terms of contribution to total mean intake of Oligonol®, tea (contributed 36.5% to total mean intakes), fruit-flavored drinks and ades (contributed 22.7% to total mean intakes), and fruit juices (contributed 19.7% to total mean intakes) were the 3 largest sources of intake across all population groups on both an absolute and on a mg/kg body weight basis.

Table IV.A.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Oligonol® from Proposed Food-Uses in the U.S. by Population Group (2009-2010 NHANES Data)

Population Group	Age Group (Years)	All-Person Consumption (mg/kg bw/day)		All-Users Consumption (mg/kg bw/day)			
		Mean	90 th Percentile	%	n	Mean	90 th Percentile
Infants	0 to 2	6.8	18.3	71.4	570	9.6	19.7
Children	3 to 11	5.5	11.1	97.5	1,397	5.7	11.2
Female Teenagers	12 to 19	2.6	5.4	90.4	487	2.8	5.9
Male Teenagers	12 to 19	2.5	6.0	87.8	516	2.9	6.1
Female Adults	20 and up	2.3	5.4	87.0	2,305	2.6	5.7
Male Adults	20 and up	2.1	5.5	82.5	1,919	2.6	6.0
Total Population	All Ages	2.9	6.8	86.2	7,194	3.3	7.3

In addition to the proposed food uses, Oligonol® is approved as a New Dietary Ingredient in the U.S. and thus, exposure may also occur through consumption of dietary supplements where Oligonol® is intended for use by adults at maximum recommended use levels of 200 mg/day. The exposure to Oligonol®, under worst-case scenario where Oligonol® dietary supplements are consumed at the maximum recommended amount in addition to proposed food uses, is presented in Table IV.A.2-3. Since Oligonol® dietary supplements are not intended for use by children, only the intake levels for teenagers and adults (males and females) are presented. The 90th percentile intake of Oligonol® is estimated at 616 and 712 mg/person/day for female and male adults, respectively. This corresponds to intakes of 8.5 and 8.3 mg/kg body weight/day for female and male adults, respectively.

Population Group	Age Group (Years)	All-Users Consumption ^a (mg/day)		Mean Body Weight ^b (kg)	All-Users Consumption ^c (mg/kg bw/day)	
		Mean	90 th Percentile		Mean	90 th Percentile
Female Teenagers	12 to 19	370	526	60.7	6.1	8.7
Male Teenagers	12 to 19	389	597	65.2	6.0	9.2
Female Adults	20 and up	388	616	72.3	5.4	8.5
Male Adults	20 and up	422	712	85.4	4.9	8.3

^a Mean intake of Oligonol® from proposed food uses plus intake from dietary supplements at 200 mg/day.

^b Mean body weight for each population group was estimated by dividing the mean all-users consumption from proposed food uses of Oligonol® on an mg/day basis by the amount consumed on a kg/body weight/day basis.

^c Intake of Oligonol® from proposed food uses plus dietary supplements at 200 mg/day, calculated based on the mean body weight estimated for each population group.

IV.A.3 Estimated Consumption of Polyphenols from the Intended Food Uses of Oligonol®

Oligonol® is intended for use as an ingredient in foods to supplement the levels of polyphenols that are already consumed as part of a normal diet. As discussed above in Section IV.A.1, the monomeric flavan-3-ols and oligomeric procyanidins present in Oligonol® are consumed naturally through various plant-derived dietary sources. One of the major dietary source of monomeric flavan-3-ols is green tea; the estimated intake of the monomeric flavan-3-ols even among the high consumers of Oligonol® is generally less than the amount that would be obtained from drinking 1 cup of green tea (see Table IV.A.3-1). Similarly, the estimated intake of procyanidins even among the high consumers of Oligonol® is comparable to the amount that would be consumed from one serving of fruit that is rich in procyanidins (Table IV.A.3-2).

	Oligonol [®]			Green tea
	Composition (%)	Intake ^a (mg/day)		Intake from 200 mL serving ^b (mg)
		Total population	Male adults	
(+)-Catechin and (-)-EC	8	33.0	41.0	21.6
(-)-EGC	--	--	--	33.4
(-)-ECG	2	8.2	10.2	39.5
(-)-EGCG	6	24.7	30.7	155.6
Total monomeric flavan-3-ol content	16	65.9	81.9	250.1

Abbreviations: (-)-EC = (-)-Epicatechin; (-)-EGC = (-)-Epigallocatechin; (-)-ECG = (-)-Epicatechin-3-gallate; (-)-EGCG = (-)-Epigallocatechin gallate

^a Intake was calculated based on estimated 90th percentile intake of Oligonol[®] among all-users of 412 mg/day for the total population and 512 mg/day for male adults.

^b Calculated based on data taken from USDA, 2007.

	Type of Interflavan Linkages	Total Procyanidin Content (mg/100 g food) ^b	Serving Size (g)	Estimated Daily Consumption (mg/day)	
Choke berry	B	663.7	80	531.0	
Cranberry	A, B	418.8	80	335.0	
Blueberry	B	255.8	80	204.6	
Plum	A, B	215.9	80	172.7	
Apple	B	104.4	80	83.5	
Peach	B	67.3	80	53.8	
Green Pear	B	42.3	80	33.8	
Peanut	A, B	15.6	25	3.9	
Cocoa ^c	B	1635.9	--	--	
Sorghum, Sumac ^c	B	1919.5	--	--	
	Type of Interflavan Linkages	Total Procyanidin Content (%w/w)	Serving Size (g)	Estimated Daily Consumption (mg/day) ^d	
				Total population	Male Adults
Oligonol [®]	A, B	70	--	288.4	358.4

^a Estimated daily consumption was calculated using the data on proanthocyanidin content in foods that was published in Prior and Gu (2005), and the typical serving sizes that was published by Lewis *et al.* (2012). The foods selected are rich in procyanidins, and includes those that contain both the A-type and B-type linkages (e.g., cranberries, plums, and peanuts), similar to the procyanidins present in Oligonol[®]. Foods that contain other forms of proanthocyanidins in addition to procyanidins are not included here.

^b On wet weight basis.

^c Procyanidin content was reported for unprocessed cocoa and sumac sorghum (Gu *et al.*, 2004); therefore, the estimated daily amount consumed is not calculated.

^d Intake was calculated based on estimated 90th percentile intake of Oligonol[®] among all-users of 412 mg/day for the total population and 512 mg/day for male adults, and assuming the procyanidin content of Oligonol[®] is 70%.

IV.A.4 Summary

In summary, consumption data and information pertaining to the individual intended food-uses of Oligonol[®] were used to estimate the all-person and all-user intakes of Oligonol[®] for specific demographic groups and for the total U.S. population. On an all-user basis, the mean intake of Oligonol[®] by the total U.S. population from proposed food-uses was estimated to be 190 mg/person/day (3.3 mg/kg body weight/day). The high consumer (90th percentile) all-user intake of Oligonol[®] by the total U.S. population from proposed food-uses of Oligonol[®] was estimated to be 412 mg/person/day (7.3 mg/kg body weight/day). Oligonol[®] is also marketed as dietary supplements for adults in the U.S.; the 90th percentile intake of Oligonol[®] from intake of dietary supplements (200 mg/day) in addition to proposed food-uses is estimated at 616 and 712 mg/person/day for female and male adults, respectively. This corresponds to intakes of 8.5 and 8.3 mg/kg body weight/day for female and male adults, respectively.

This type of intake methodology is generally considered to be “worst case” as a result of several conservative assumptions made in the consumption estimates. For example, it is often assumed that all food products within a food category contain the ingredient at the maximum specified level of use. In addition, it is well-established that the length of a dietary survey affects the estimated consumption of individual users. Short-term surveys, such as the typical 2- or 3-day dietary surveys, may overestimate the consumption of food products that are consumed relatively infrequently. It is also highly unlikely that individuals consuming foods enriched with Oligonol[®] will also consume Oligonol[®] dietary supplements. Therefore, it is anticipated that the actual intake of Oligonol[®] from the intended conditions of use will be less than estimated.

IV.B Absorption, Distribution, Metabolism, Excretion

IV.B.1 Polyphenol Constituents of Oligonol[®]

The metabolic fate of Oligonol[®] can be extrapolated from studies conducted with its polyphenolic constituents (*i.e.*, monomeric flavan-3-ols and oligomeric procyanidins). A large number of pre-clinical and clinical studies have been conducted to investigate the metabolic fate of the polyphenol constituents in Oligonol[®]; a brief discussion of these processes is provided below.

IV.B.1.1 Monomeric Flavan-3-ols

Although the monomeric flavan-3-ols are present in diverse food sources, the majority of the bioavailability studies have been conducted using cocoa or tea as the test article. In general, monomeric flavan-3-ols are absorbed to a greater extent than dimers, trimers, and larger oligomers (Manach *et al.*, 2005; Yang *et al.*, 2008). Unlike other flavonoids, flavan-3-ols are not glycosylated, and therefore they do not require hydrolysis in the gastrointestinal tract prior to absorption (Manach *et al.*, 2004; Yashin *et al.*, 2012).

(-)-Epicatechin, epigallocatechin (EGC), ECG, and EGCG have been reported to be absorbed from the gastrointestinal tract following oral administration in mice and rats (Okushio *et al.*, 1995, 1996; Nakagawa and Miyazawa, 1997; Suganuma *et al.*, 1998; Donovan *et al.*, 2001). Both the intact form and metabolites of these monomeric flavan-3-ols have been identified in the plasma of rodents following oral administration (Piskula and Terao, 1998; Harada *et al.*, 1999; Kim *et al.*, 2000). Studies conducted in humans suggest that the bioavailability of catechins (*i.e.*, epicatechin, EGC, ECG, and EGCG) is generally low. Manach *et al.* (2005) used data from 97 human studies that investigated the kinetics and extent of polyphenol absorption, and determined the mean relative urinary excretion (as % of intake) to be 18.5% for (epi)catechin (range: 2.1 to 55.0%), 11.1% for EGC (range: 4.2 to 15.6%), and 0.06% for EGCG (range: 0.0 to 0.1%). In these studies, relative urinary excretion was used as an indicator of absorption though it is possible that underestimation may have occurred for compounds (such as EGCG) that are highly excreted in the bile (Manach *et al.*, 2005). There is some evidence from both animal and human studies to suggest that the bioavailability of catechins may be enhanced if they are consumed under fasting compared to fed states (Chow *et al.*, 2005; Isbrucker *et al.*, 2006; Kapetanovic *et al.*, 2009).

Following absorption, monomeric flavan-3-ols predominantly undergo methylation, glucuronidation, and/or sulfation (Yang *et al.*, 2008; Yashin *et al.*, 2012). These conjugated metabolites have been detected in the plasma and urine of animals and humans. For example, the major circulating metabolites of epicatechin has been identified as epicatechin-3'-*O*-glucuronide, 4'-*O*-methylepicatechin-3'-*O*-glucuronide, 4'-*O*-methylepicatechin-5- or 7-glucuronide, and the aglycones epicatechin and 4'-methylepicatechin (Manach *et al.*, 2005). Although catechins occur mostly as conjugated forms in systemic circulation, EGCG is a notable exception in that a large proportion (77 to 90%) has been detected in the plasma as the free form (Manach *et al.*, 2005; Yashin *et al.*, 2012).

Monomeric flavan-3-ols that are not absorbed in the upper gastrointestinal tract may be subjected to metabolism by the colonic microflora in the lower gastrointestinal tract into metabolites that may then be subsequently absorbed (reviewed in Manach *et al.*, 2005). Accordingly, the conjugated forms of the microbial metabolites of catechins [*e.g.*, 5-(3', 4', 5'-trihydroxyphenyl) valerolactone, 5-(3',4'-dihydroxyphenyl) valerolactone, and 5-(3',5'-dihydroxyphenyl) valerolactone] have been detected in the plasma and urine of human volunteers following ingestion of green tea (Meng *et al.*, 2002). These metabolites were present in the urine at levels that were 8 to 25 times higher than those of the unchanged catechins, and accounted for 6 to 38% of the ingested dose of EGC and epicatechin (Li *et al.*, 2000). Overall, catechins and their metabolites are known to be rapidly eliminated through the urinary, fecal, and biliary routes (Manach *et al.*, 2005).

IV.B.1.2 Proanthocyanidins

Among the flavonoids, the proanthocyanidins are the most poorly absorbed, with absorption being 10- to 100-fold lower than their monomeric constituents in both preclinical and clinical studies (reviewed in Manach and Donovan, 2004; Manach *et al.*, 2005; Aron and Kennedy, 2008). Although data from *in vitro* studies suggest that oligomeric procyanidins could be hydrolyzed to smaller monomeric and dimeric units under conditions simulating those in the stomach (Kühnau, 1976; Spencer *et al.*, 2000), this finding has not been supported by studies conducted in animals or humans (Donovan *et al.*, 2002; Rios *et al.*, 2002). As such, the majority of ingested proanthocyanidins are expected to escape gastric degradation and absorption in the small intestines, but instead, become metabolized by the colonic microflora in the lower gastrointestinal tract. *In vitro* incubation of purified, radiolabeled proanthocyanidin oligomers with isolated human colonic microflora resulted in the production of various phenolic acids, including *m*-hydroxyphenylpropionic acid, *m*-hydroxyphenylacetic acid, and their *p*-hydroxy isomers, *m*-hydroxyphenylvaleric acid, phenylpropionic acid, phenylacetic acid, and benzoic acid (Déprez *et al.*, 2000). Some of these metabolites have also been detected in the urine of humans fed chocolate (which are rich in procyanidins) (Rios *et al.*, 2003), as well as animals that were orally administered purified catechins, procyanidin B3 (dimer), procyanidin C2 (trimer), and larger oligomeric procyanidins (Gonthier *et al.*, 2003).

IV.B.2 Polyphenols from Oligonol®-like Product

A human bioavailability study has been conducted using the Oligonol®-like product (Fujii *et al.*, 2007). This Oligonol®-like product was manufactured using a similar oligomerization process as Oligonol®, but using different starting materials (*i.e.*, fruits rich in proanthocyanidins such as grape seed extracts, apples, and persimmons). Similar to Oligonol®, the Oligonol®-like product is composed of 15 to 20% monomers, 8 to 12% dimers, and 5 to 10% trimers.

Thirty subjects (23 to 62 years of age) were randomly divided into 3 groups (5/sex/group) and given capsules containing 200 mg grape seed extract (control), 100 mg of an Oligonol®-like product, or 200 mg of the Oligonol®-like product, to be taken orally for a period of 92 days (Fujii *et al.*, 2007). Blood samples were collected at 0, 2, 4, and 6 hours after dosing, as well as on Days 28 and 92, and total polyphenol concentrations in the serum were measured. A peak in total polyphenol concentration in the serum was observed at 2 hours following ingestion of the Oligonol®-like product (at 200 mg/day dose), whereas no clear peak was observed in the group consuming the control grape seed extract product. Furthermore, the steady-state levels of polyphenols in the serum at Day 92 were dose-dependently higher in subjects administered the Oligonol®-like product. Compared to controls, the serum levels of polyphenols were 4 and 10 times higher in subjects receiving the 100 and 200 mg/day dose of the Oligonol®-like product, respectively. Overall, this study demonstrates that the polyphenols in the Oligonol®-like product are bioavailable.

IV.B.3 Summary

Given that Oligonol[®] consists mainly of monomeric flavan-3-ols and procyanidins, it is expected to undergo similar absorption, distribution, metabolism, and excretion processes as naturally occurring polyphenolic compounds. In general, the flavan-3-ol monomers are absorbed to a greater extent than its oligomeric forms, with the absorption of procyanidins reported at 10 to 100-fold less than the monomers (Manach and Donovan, 2004; Manach *et al.*, 2005; Aron and Kennedy, 2008; Yang *et al.*, 2008). Following absorption, the majority of monomeric flavan-3-ol, with the exception of EGCG, becomes conjugated *via* methylation, glucuronidation, and/or sulfation (Yang *et al.*, 2008; Yashin *et al.*, 2012). The monomeric flavan-3-ols, as well as the oligomeric procyanidins, that escapes absorption and passes into the lower intestinal tract intact may be metabolized by the colonic microflora into metabolites (*e.g.*, phenylvalerolactones and phenolic acids) that can be subsequently absorbed. Similar to other polyphenols, flavan-3-ols and their metabolites are rapidly excreted through the urine, feces, and bile (Manach *et al.*, 2005). Additionally, one human study conducted with an Oligonol[®]-like product, which is similar to Oligonol[®] with respect to the manufacturing process and composition, indicate that total polyphenol levels in the serum are elevated following consumption of the Oligonol[®]-like product at doses of 200 mg/day (Fujii *et al.*, 2007).

IV.C Toxicological Studies Conducted with Oligonol[®]

IV.C.1 Acute Toxicity

Sprague-Dawley rats (10/sex/group) were administered a single dose of 2,000 mg/kg body weight of Oligonol[®] in water by gavage, and the vehicle (water) was administered to control animals (Fujii *et al.*, 2008). All animals were observed for signs of toxicity, including mortality and moribundity, for 14 days. At the end of the 14-day observation period, all animals were terminated and the organs were removed and examined macroscopically. There were no deaths during the study period, and the body weight of animals administered Oligonol[®] did not differ significantly from animals in the control groups. In 4 females administered Oligonol[®], mucous in the feces was observed within the first day, and salivation was observed in 1 female at 30 minutes after administration. These findings were not reported in any of the males. No abnormalities were revealed upon macroscopic examination. Based on the results of this study, the authors concluded the oral median lethal dose (LD₅₀) for Oligonol[®] to be greater than 2,000 mg/kg body weight in male and female rats.

IV.C.2 Sub-Chronic Toxicity

Oligonol[®] has been administered to rats by gavage in 2 conventional 90-day oral toxicity studies, and one 90-day oral toxicity study has been conducted where Oligonol[®] was added to the diet of mice. The results of these studies are summarized below.

IV.C.2.1 Rats

Gavage Study #1

In a subchronic oral toxicity study conducted according to Good Laboratory Practice (GLP), groups of 6 male and 6 female Sprague-Dawley rats (3 weeks of age) were administered 0 (control), 100, 300, or 1,000 mg/kg body weight Oligonol[®] by gavage once daily for a period of 90 days (Fujii *et al.*, 2008). Rats were acclimatized for 2 weeks prior to initiation of dosing and were provided with a commercial pelleted diet and tap water *ad libitum*. Animals were observed for general appearance twice daily, and food consumption and body weights were measured twice weekly. During Week 13, urine samples were collected immediately after dosing from non-fasted animals using metabolic cages. Urine collected during the first 3 hours was measured for pH, protein, glucose, ketone bodies, urobilinogen, bilirubin, and occult blood. The accumulated urine samples (collected for 21 hours) were measured for urine volume and specific gravity. On the day of necropsy, blood samples were collected and analyzed for red blood cell count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration, platelet count, white blood cell count, reticulocyte count, and differential white blood cell count, and prothrombin time and activated partial thromboplastin time were measured in the plasma. Clinical chemistry parameters were measured including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), *gamma*-glutamyl transpeptidase (GGPT), and glucose. Serum from blood samples was analyzed for total cholesterol, triglycerides, total bilirubin, urea nitrogen, creatinine, calcium, inorganic phosphorus, total protein, sodium, potassium, chloride, protein fraction, and albumin/globulin ratio. On Day 90, all animals were sacrificed and organs and tissues were examined macroscopically. The brain, pituitary gland, thyroid, thymus, adrenal glands, spleen, heart, liver, kidneys, testes, epididymides, and ovaries were weighed, and relative organ weights were calculated. In addition, all gross lesions, lung, mesenteric lymph node, pancreas, tongue, mandibular lymph node, salivary glands, mammary gland, skin, eyeballs, Harderian glands, sternum, right femur bone, spinal cord, skeletal muscle, thoracic aorta, trachea, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, prostate, seminal vesicle, uterus, vagina, and peripheral nerve were resected with a border of normal tissue. Tissue samples from all animals were histopathologically examined.

There were no significant differences in body weight or food consumption between the control group and the treated groups throughout the 90-day period. There were no abnormal findings in general appearance or clinical observations for animals in the 100 and 300 mg/kg body weight dose groups in either sex. However, in the 1,000 mg/kg body weight group, bloody feces was observed in 1 male on Days 86, 87, 90, and on the day of necropsy. Urinalysis parameters were within normal ranges for all animals with the exception of an increase in urine volume in 2 female animals in the 100 mg/kg body weight/day dose group. The authors suggested that the high values may have resulted from contamination of the urine samples with the drinking

water bottles. In the 1,000 mg/kg body weight/day group the urine was orange-yellow in males and females.

There were no treatment related effects observed in clinical chemistry parameters. A significant increase in activated partial thromboplastin time in the males of the 1,000 mg/kg body weight/day dose group occurred. This result was not observed in the female rats, and there was no dose-response relationship observed in males. In female rats, the reticulocyte count was significantly decreased in the 100 and 1,000 mg/kg body weight/day dose groups when compared to the control group. This was not observed in the male rats at any doses or in females in the 300 mg/kg body weight/day dose group. All other hematological parameters in animals receiving Oligonol[®] did not differ significantly from controls. The blood biochemistry parameters were mostly normal with the exception of a significant decrease in urea nitrogen in males in the 1,000 mg/kg body weight/day dose group compared to the control group. This effect was not noted in females, and was not considered to be test-article related. In females at the 1,000 mg/kg body weight/day dose group, total protein and triglycerides were significantly decreased compared to the control group. This effect was not considered to be treatment related because it was not seen in males and did not follow a dose-response relationship.

There were no abnormal gross necropsy findings in either sex at the 100 mg/kg body weight/day dose group. One male in the 300 mg/kg body weight/day dose group was observed to have gray discoloration of the mucosa of duodenum and focal red discoloration of mucosa of the jejunum. In the 1,000 mg/kg body weight/day dose group, all males and females had gray discoloration of the mucosa of the duodenum. Males in the 300 mg/kg body weight/day dose group had significantly lower absolute thymus weights than the control animals, however, there were no effects observed in the 1,000 mg/kg male groups or in the females. Females in the 1,000 mg/kg body weight/day dose group had significantly lower absolute ovary weights than the control group. However, the relative weight was not significantly different from the control and no histological changes were observed, and so, these findings were not considered to be toxicologically significant. There were no significant histopathological findings observed in any of the tissues examined with the exception of a dose-dependent deposition of brown pigment in the lamina propria of the duodenum following staining. Slight deposition was observed in all rats in the 300 mg/kg body weight/day dose group and the intensity increased to moderate in rats in the 1,000 mg/kg body weight/day dose group. The pigment was not observed in any other tissues in the gastrointestinal tract and was not accompanied by an inflammation or changes in the tissue. Although this finding was considered to be treatment related, it was not considered to be toxicologically significant as there was no change in the tissues or inflammation. The authors suggested that the brown pigment was probably an accumulation of oxidized phenolics, resulting in the positive staining. The authors considered the no-observed-adverse-effect level (NOAEL) to be 1,000 mg/kg body weight/day, the highest dose tested.

Gavage Study #2

In an unpublished study conducted under GLP and in accordance with the Organisation for Economic Co-operation and Development (OECD) Guidelines, CD-rats (10/sex/group) were administered Oligonol® at 0, 100, 300, or 800 mg/kg body weight/day by gavage for 90 days (Leuschner, 2011). A separate set of animals in the control and high-dose group (5/sex/group) underwent an additional 6-week recovery period following the 90-day treatment period. Rats were acclimatized for 7 days prior to initiation of dosing, and commercial pelleted diet and tap water were available *ad libitum*. Animals were observed for general appearance at least once daily, while detailed clinical observations, food consumption and body weights were measured weekly. A neurological screening test was conducted at study termination (Week 13) or at the end of the recovery period (Week 19), and included sensory reactivity to various types of stimuli, as well as assessment of grip strength and motor activity. Blood samples were collected following an overnight fast at study termination or at the end of the recovery period for determination of hematological and clinical biochemistry parameters. Urine samples were collected following overnight fast at the study termination or at the end of the recovery period for urinalysis. Ophthalmological examination was performed prior to the start of the study, at study termination, and at the end of the recovery period. Necropsy was performed at study termination, and organs/tissues were examined for macroscopic and histopathological changes.

No treatment-related changes were noted in mortality, clinical signs (*i.e.*, behavior, external appearance, or feces), or functional observation tests throughout the duration of the study. No treatment-related effects were found during the neurological screen test (*i.e.*, fore- and hind limb grip strength, spontaneous motility) and ophthalmological examination at Week 13 or 19. The body weight of male animals treated with 800 mg/kg body weight/day of Oligonol® was approximately 9% less than the body weight of control animals at Week 2 of treatment ($p \leq 0.01$). However, no other significant changes in body weight were reported over the course of the study. No treatment-related changes in food or water intake were reported by the study authors.

Hematological parameters (*i.e.*, hemoglobin, red blood cell count, white blood cell count, reticulocytes, platelet count, differential blood count, hematocrit, thromboplastin time, activated partial thromboplastin time, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) did not vary by treatment. A significant increase in plasma total bilirubin (by 26%) was observed at the end of the study in females treated with 800 mg/kg body weight/day dose compared to controls, although levels returned to those observed in controls upon cessation of treatment with Oligonol® at the end of the 6-week recovery period. No other treatment-related changes in clinical biochemistry parameters (*i.e.*, albumin, cholesterol, creatinine, glucose, total protein, triglycerides, blood urea, ALT, AST, ALP, lactate dehydrogenase, and electrolytes) were reported. Specific gravity of the urine was increased significantly by 2% ($p \leq 0.01$) at the end of the study in females treated with 800 mg/kg

body weight/day of Oligonol[®] compared to controls. This effect subsided upon cessation of Oligonol[®] treatment at the end of the recovery period. No other treatment-related changes in urinalysis parameters (*i.e.*, urine pH, urine volume, analyte concentrations of nitrite, protein, glucose, ketones, urobilinogen, bilirubin and hemoglobin, urine color and microscopically analyzed urine sediments) were observed.

Macroscopic examination at necropsy did not reveal any treatment-related abnormalities. A small number of macroscopic changes were noted in the lungs, liver, thymus, stomach, adrenals and ovary. However, these changes were considered to be spontaneous and within the normal range of variation as they were not dose-dependent and occurred in animals in the control group. No treatment-related changes in the relative and absolute organ weights were found, and histopathological examination did not reveal any treatment-related changes. Examination of the testicles in males treated with 800 mg/kg body weight/day of Oligonol[®] revealed normal spermiogenesis, sperm count, and no evidence of degenerating spermatocytes and spermatids. Based on these results, the authors concluded the no-observed-effect level (NOEL) of Oligonol[®] to be 300 mg/kg of Oligonol[®], and the NOAEL to be 800 mg/kg.

IV.C.2.2 Mice

In a non-GLP study, the safety of Oligonol[®] and lychee fruit extract (a starting material in Oligonol[®]) were assessed in ddY male mice (Fujii *et al.*, 2008). The mice were 5-weeks old and acclimatized for 1 week before the start of the study. Groups of 5 mice were fed CE-2 feed powder for rodents diet supplemented with 0 (control), 200 mg lychee fruit extract/kg body weight, 2 mg Oligonol[®]/kg body weight, 20 mg Oligonol[®]/kg body weight, or 200 mg Oligonol[®]/kg body weight for a 90-day period. Animals were housed 5 per cage in polycarbonate cages. Body weights and food consumption were measured twice weekly, and diets were prepared daily to provide the exact dosage. General status of the animals was recorded twice weekly. On Day 90, all mice were terminated, blood samples were collected for analysis of biochemical parameters, and organs including brain, heart, liver, kidney, and spleen were removed for macroscopic examination and weighing. Serum was obtained and glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glucose, blood urea nitrogen, creatinine, triglyceride, total cholesterol, total protein, albumin, and albumin/glucose ratio were measured. All animals survived until the end of the study period and there were no adverse effects observed. Body weights were not significantly different between mice fed lychee fruit extract or Oligonol[®] compared with mice fed the control diet. Similarly, there were no significant differences in absolute and relative organ weights between the groups. Analysis of biochemical parameters did not reveal any abnormalities and the lack of toxicity in this study supports the findings in the rat studies.

IV.C.3 Mutagenicity and Genotoxicity

Fujii *et al.* (2008) conducted an *in vivo* micronucleus assay, a bacterial reverse mutation test, and an *in vitro* chromosomal aberration assay to assess the mutagenic/genotoxic potential of Oligonol[®]. In the micronucleus assay, 7-week-old male SPF mice were randomly assigned to 5 groups of 6 animals, representing a control group, a positive control group, and 3 treatment groups (500, 1,000, or 2,000 mg/kg body weight). A commercial pelleted diet and tap water were available *ad libitum* for all animals throughout the study period. Mitomycin C was the positive control and administered at a dose of 1 mg/kg body weight, and carboxymethylcellulose sodium solution was used as the negative control, as it was the vehicle for Oligonol[®]. With the exception of the positive controls treatments conducted twice by gavage at a 24-hour interval, each animal was observed for general appearance before and after the first and second administration. Animals were weighed before the first administration and 24 hours after the final administration. All animals were killed 24 hours after the final administration of the control or test substances and bone marrow smears were prepared for each animal following the extraction of marrow from both femurs. The ratio of polychromatic to normochromatic erythrocytes, the incidence of micronuclei, and the percent of polychromatic erythrocytes were measured. The general appearance of the mice in all groups was normal before and after administration, and body weights were not significantly different between groups. There were no statistically significant differences between the control and Oligonol[®] treated groups in the micronuclei counts or in the percent of polychromatic erythrocytes. The percent of polychromatic erythrocytes to total erythrocytes was normal, indicating there was no suppression of bone marrow function. The positive control group had a significant increase in micronuclei compared to the control group, which validated the test system. Based on the results of this study, it can be concluded that Oligonol[®] is not clastogenic *in vivo*.

A bacterial reverse mutation test also was conducted with Oligonol[®] using *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, and *Escherichia coli* WP2uvrA, with and without metabolic activation. Distilled water was used as the negative control and commonly accepted mutagens [*i.e.*, 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, sodium azide; and 9-Aminoacridine hydrochloride hydrate] were used as the positive control. An initial concentration range finding test was carried out with doses from 1.5 to 5,000 µg/plate, and from those results, 156, 313, 635, 1,250, 2,500, and 5,000 µg/plate were selected as the doses to be used for the test. In the concentration range finding test and the main test the average number of revertant colonies in the Oligonol[®] treated groups was less than twice that of the negative control group, and no concentration-dependent increases in the number of revertant colonies were observed either without or with metabolic activation. Therefore, the findings from both the range-finding test and the main test demonstrate that Oligonol[®] is not mutagenic in the bacterial strains tested.

A chromosome aberration test was conducted with Oligonol[®] using cultured Chinese hamster lung cells. Mitomycin C was used as the positive control without metabolic activation at concentrations of 0.1 and 0.05 µg/mL in the 6- and 24-hour treatments, respectively. Benzo[a]pyrene was used as the positive control substance in the assays with metabolic activation. Incubation with the test substance with and without metabolic activation for 6 hours, and without metabolic activation for 24 hours was followed as the protocol for the preliminary test. In the main test, Oligonol[®] concentrations were used at 19.5, 39.1, 78.1, 156, 313, 469, and 625 µg/mL for the 6-hour test with metabolic activation, 9.8, 19.5, 39.1, 78.1, 104, 130, and 156 µg/mL for the 6-hour test without metabolic activation, and at 4.9, 9.8, 19.5, 29.3, 39.1, 58.6, 78.1 µg/mL for the 24-hour treatment without metabolic activation.

In the 6-hour preliminary test, inhibition of cell growth was observed at 156 µg/mL and above without metabolic activation, and at 635 µg/mL and above with metabolic activation. In the 24-hour test, inhibition of cell growth was observed at a dose level of 78.1 µg/mL. In the main test, after 6 hours of incubation, inhibition of cell growth was seen at 130 µg/mL and above without metabolic activation, and at 469 µg/mL and above with metabolic activation. Following 24 hours of incubation, inhibition of growth was observed at 39.1 µg/mL and above. There was no precipitation of the test substance and there were no effects on the pH of the culture solution in any test series. In both the 6- and 24-hour treatment groups, the incidences of structural aberrations were less than 5% at all concentrations tested. In both the 6- and 24-hour test groups, with or without metabolic activation the incidence of numerical aberration (polyploidy) of chromosomes increased in a concentration-dependent manner. The authors concluded that Oligonol[®] caused numerical, but not structural aberrations under the conditions tested. The increased polyploidy observed was considered to be a non-genotoxic event.

Together, the results of these micronucleus, reverse mutation, and chromosome aberration tests provide evidence that Oligonol[®] is not genotoxic or mutagenic.

IV.D Human Studies Conducted with Oligonol[®]

Three unpublished human studies have been conducted with Oligonol[®]. In the first study, 6 healthy volunteers (5 male and 1 female) consumed 200 mg Oligonol[®] twice a day (400 mg/day) for 3 months. Blood biochemistry parameters were evaluated on Days 0, 30, and 92, and included indicators of liver and kidney function such as GOT, GPT, GGPT, blood urea nitrogen (BUN) and creatinine. No changes occurred over the treatment period and no significant adverse events were reported. One volunteer experienced transient diarrhea for the first week that resolved without treatment.

In another study, Oligonol[®] was administered at a daily dose of 300 mg twice a day (600 mg/day) for 14 days to 29 healthy subjects (16 females and 13 males). Parameters evaluated included hematology (white blood cells, hemoglobin, hematocrit, and platelets), serum biochemistry (calcium, creatinine, total protein, albumin, globulin, bilirubin, BUN, glucose,

AST, ALT, ALP, Na, K, Cl, CO₂), serum malondialdehyde as a measure of lipid peroxidation, and electrocardiogram (ECG) readings. There were no changes in any parameter between baseline and the final visit. Twenty-one subjects (72.4%) reported no adverse symptoms during the trial at the interim or final assessment. Three subjects (10.3%) had abdominal discomfort and bloating at both interim and final visits and 5 subjects (17.3%) reported other minor transient symptoms including dry mouth, increased appetite, fatigue and headache, mild nausea, and loose stools at either the interim or the final visit.

A double-blind, randomized, placebo-controlled study was conducted in 76 male and female subjects with pre-hypertension or hypertension (Stage 1 or Stage 2) to determine the efficacy of Oligonol[®] in reducing blood pressure. Twenty-three subjects were assigned to receive placebo, while 28 and 25 subjects received 100 and 200 mg/day of Oligonol[®], respectively, for 60 days. Fasting blood samples were collected at baseline and at the end of the study (Day 60). Safety-related endpoints examined included hematology (*i.e.*, WBC count with differential, hemoglobin, hematocrit, platelet count) and serum biochemistry parameters (*i.e.*, glucose, total bilirubin, total protein, ALP, ALT, AST, BUN, creatinine, calcium, sodium, potassium, chloride, bicarbonate, lipid profile). No adverse events were reported in subjects treated with Oligonol[®], and no treatment-related changes in hematology and serum biochemistry parameters were observed. Subjects treated with Oligonol[®] at both doses had fasting mean blood glucose levels greater than 100 mg/dL following the 60 days of treatment (106.1±3.9 mg/dL for 100 mg/day dose and 112.1±49.0 mg/dL for 200 mg/day dose); however, this finding was also observed during the screening visit, and may be driven by individuals who did not comply to the overnight fast prior to blood sample collection.

IV.E Safety of an Oligonol[®]-like Product

As mentioned in Section IV.B.2, Amino Up also manufactures an Oligonol[®]-like product using the same oligomerization process as Oligonol[®], but with different starting materials (*i.e.*, fruits rich in proanthocyanidins such as grape seed extracts, apples, and persimmons). Similar to Oligonol[®], the Oligonol[®]-like product is composed of 15 to 20% flavan-3-ol monomers, 8 to 12% dimers, and 5 to 10% trimers. Based upon the similarity in composition, the data from toxicity studies (*i.e.*, acute and short-term) and human clinical studies conducted with the Oligonol[®]-like product can be used to corroborate the safety of Oligonol[®].

IV.E.1 Acute Toxicity

The acute oral toxicity of an Oligonol-like product was assessed in single dose studies in rats and mice (Fujii *et al.*, 2007). Five-week-old male and female Sprague-Dawley rats (5/sex/group) were administered 2,000 mg/kg body weight of the Oligonol-like product by gavage. No mortalities were observed and the LD₅₀ was determined to be >2,000 mg/kg body weight. In another study, 8-week-old male and female ddY mice were administered a single dose of Oligonol-like product at 2,500, 5,000, 7,500, and 10,000 mg/kg body weight by gavage.

Mortality was observed in 1/8, 2/7, 3/3, and 17/18 mice in the 2,500, 5,000, 7,500, and 10,000 mg/kg body weight dose, respectively, and the LD₅₀ was determined to be 5,000 mg/kg body weight.

IV.E.2 Short-Term Toxicity

In a 1-month study conducted by Fujii *et al.* (2007), 7-week-old ddY mice were randomly allocated to 4 groups of 6 mice each and fed diets supplemented with either grape seed polyphenols at 200 mg/kg body weight/day, or an Oligonol-like product at 3.33, 24.6, or 200 mg/kg body weight/day. A control group fed a basal diet also was included. Food consumption and body weights were measured every 2 days and at the end of the 4-week study period all mice were terminated and serum was collected for the measurement of biochemical parameters including: GOT, GPT, triglyceride, total cholesterol, BUN, total protein, albumin, and albumin/globulin. There were no visible signs of adverse effects or toxicity and there was no significant changes in body weight gain or food consumption between the groups. Serum biochemistry parameters were all within normal limits and did not reveal any abnormalities. The authors concluded that the Oligonol-like product administered at a level of 200 mg/kg body weight/day, for a 1-month period, was not associated with adverse effects in mice.

IV.E.3 Mutagenicity and Genotoxicity

In a GLP study, an Oligonol-like product was tested in a reverse mutation test in *S. typhimurium* strains TA98, TA100, TA104, TA1535, and TA1537 with or without metabolic activation at concentrations increasing from 156 to 5,000 µg/mL. This Oligonol-like product also was tested in *E. coli* strain wp2uvra at concentrations increasing from 156 to 5,000 µg/mL. All tests were negative and the authors concluded that the Oligonol-like product is not mutagenic (Fujii *et al.*, 2007).

IV.E.4 Human Studies

In a bioavailability study by Fujii *et al.* (2007), volunteers (15 men and 15 women) aged 23 to 62 years were randomly divided into 3 groups (5/sex/group) and given capsules containing either 200 mg grape seed extract (as control), 100 mg of an Oligonol-like product, or 200 mg of an Oligonol-like product to be taken daily for a period of 92 days (Fujii *et al.*, 2007). Blood and urine samples were collected on Days 0, 28, and 92 for the measurement of hematology parameters (white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets); liver function (GOT, GPT, ALP, and GGPT); diabetic index; fasting-blood glucose; lipid index (total cholesterol, high-density lipoprotein cholesterol, and triglycerides); and renal function (uric acid, BUN, and creatine). All the measured parameters were within normal ranges and did not indicate any signs of toxicity.

IV.F Safety of the Green Tea Extract Component of Oligonol®

Product-specific studies conducted with Oligonol® and the Oligonol®-like product clearly indicate a lack of adverse findings, with no toxicity observed at levels up to 1,000 mg/kg body weight/day in 90-day oral toxicity studies in rodents, no evidence of genotoxicity/mutagenicity, and no adverse effects reported in humans at doses up to 600 mg/day. However, due to the fact that green tea catechins, especially EGCG, have been scrutinized for potential liver toxicity, and that these catechins are a minor constituent of Oligonol® (accounting for approximately 16% of the product), Amino Up undertook a thorough evaluation of the literature pertaining to the adverse effects of green tea extracts to determine their implication, if any, on the safety of Oligonol®.

IV.F.1 14-Week Toxicity Study of Green Tea Extract

Recently, preparations of green tea extracts and/or its individual components have been marketed in a number of dietary supplements and promoted for its many reported health benefits. However, case reports of hepatotoxicity from use of these concentrated, purified forms of green tea have been identified primarily in females for weight management purposes, which have raised concerns over their safety despite the long history of safe consumption of green tea (Bonkovsky *et al.*, 2006; Sarma *et al.*, 2008; Mazzanti *et al.*, 2009). Evidence from animal studies suggests that green tea catechins, particularly EGCG, may be associated with hepatotoxicity, especially when it is administered during the fasting state (Isbrucker *et al.*, 2006; Kapetanovic *et al.*, 2009). Given these published adverse liver effects, the National Cancer Institute, who has a substantial research interest on the potential benefits of green tea extracts and their constituents, nominated EGCG for toxicity testing. Since purified forms of EGCG are costly, and that human exposure through supplemental form occurs mostly through consumption of green tea extracts, the National Toxicology Program (NTP) decided to conduct toxicological evaluations using a green tea extract preparation (containing 48.4% EGCG, 12.8% ECG, 4.6% gallic acid gallate, 2.26% EGC, 2.83% EC, 0.51% catechin, 0.45% catechin gallate, and 5% caffeine), rather than a purified EGCG material.

The NTP therefore conducted a 14-week toxicity study where a green tea extract was administered *via* oral gavage at doses of 0 (control), 62.5, 125, 250, 500, and 1,000 mg/kg body weight/day to F344 rats (10/sex/group) and B6C3F1 mice (10/sex/group) for 14 weeks (Chan *et al.*, 2010). In mice, the highest dose of green tea extract produced mortality in 6 males and 4 females before the end of the study, with the cause of death determined to be liver necrosis. Evidence of hepatotoxicity was also observed in some of the female rats administered the highest dose of the green tea extract. On this basis, the authors determined the NOAEL for liver toxicity to be 500 mg green tea extract/kg body weight/day in both rats and mice. In addition, both rats and mice developed lesions in the nasal cavity over the course of the study. The authors determined a NOAEL for nasal toxicity at 62.5 mg/kg body weight/day for male rats, while a NOAEL for nasal toxicity could not be determined for female rats or mice of both sexes (Chan *et*

al., 2010). Evidence of nasal toxicity has not been previously reported following the oral administration of green tea extracts. Nasal toxicity is not commonly observed for compounds administered *via* non-inhalation routes; nevertheless, the authors acknowledged that, in contrast to humans, the rodent nasal mucosa is an organ enriched with cytochrome P450 enzymes, as well as other xenobiotic metabolizing enzymes that can potentially contribute to the metabolism of systemically absorbed compounds within this tissue, which is likely to have impacted the effects noted following green tea extract administration (Chan *et al.*, 2010).

IV.F.2 Nasal Toxicity Study with Oligonol®

In light of the nasal toxicity observed in a 90-day study conducted by the NTP in which green tea extract was administered to rats and mice by gavage, an additional study was conducted to evaluate the effect of Oligonol® administration on the nasal cavity since this tissue was not collected in the previous 90-day studies described above in Section IV.C.2. Sprague-Dawley rats (10/sex/group) at 5 weeks of age were administered 0 (control), 100, 300, and 1,000 mg/kg body weight/day Oligonol® by gavage once daily for a period of 13 weeks (Kitadate *et al.*, 2013). The study was conducted in accordance with GLP. Rats were acclimatized for 7 days prior to initiation of treatment and were provided with a commercial pelleted diet and tap water *ad libitum*. The animals were observed once daily for clinical signs and twice daily for mortality and moribundity. Body weights were recorded prior to treatment, once weekly during treatment, 1 day prior to necropsy and on the day of necropsy, while daily food intake was calculated from the amount consumed every 7 days. Necropsy was conducted and gross abnormalities were recorded. The nasal cavity was collected from all of the animals for histopathological examination.

There were no mortalities observed, and the only treatment-related clinical sign was compound-colored stool in 2 of the males in the mid-dose group, and in all of the animals of the high-dose group. Treatment with Oligonol® did not affect body weight throughout the study, though males in the high-dose group had significantly higher food intake (by 7 to 13%) during Weeks 5, 6, and 11 of the study compared to controls. However, given the increased food consumption occurred transiently, and there was no significant differences in food intake in the other 90-day rat study administered 1,000 mg/kg body weight/day of Oligonol®, these changes were not considered to be toxicologically meaningful. No gross abnormalities were reported in any of the animals upon examination at necropsy. Minor microscopic changes were reported in the nasal cavity, though these occurred at similar frequency and severity in both the controls and treated animals. Thus, they were considered to be incidental findings and not toxicologically relevant. Based on these results, the authors concluded that treatment with Oligonol® does not produce toxicity in the nasal cavity at doses up to 1,000 mg/kg body weight/day.

IV.G Summary of Studies Supporting the Safety of Oligonol®

The safety of Oligonol® has been established through the conduct of acute and sub-chronic toxicity studies in rats and mice, as well as the standard battery of genotoxicity assays (including an *in vivo* micronucleus assay, a bacterial reverse mutation test, and *in vitro* chromosome aberration assay), and 3 human studies including a total of over 100 subjects. The oral LD₅₀ in the rat was determined to be greater than 2,000 mg/kg body weight, indicating that Oligonol® is not acutely toxic. The NOAEL from 2 conventional 90-day oral toxicity studies conducted in rats is at least 1,000 mg/kg body weight/day, as no toxicologically relevant adverse effects were observed even at the highest dose tested. Likewise, there were no signs of genotoxicity/mutagenicity when Oligonol® was tested using the standard battery of genotoxicity/mutagenicity assays. No adverse events were observed when Oligonol® was administered to healthy human subjects at dosages of 600 mg/day for 14 days, or 400 mg/day for 3 months, and at 200 mg/day for 60 days in subjects with pre-hypertension or hypertension. Furthermore a 90-day study designed to specifically address the potential effect on the nasal cavity indicated that unlike the green tea extract used within the NTP 14 week study, no nasal toxicity was evident at dosages up to 1,000 mg/kg body weight/day. The lack of adverse effects observed in a series of studies conducted with an Oligonol®-like product with a similar composition profile further corroborates the safety of Oligonol®.

Together, these results suggest that Oligonol® is not expected to pose any safety concerns under its intended conditions of use.

IV.H Nutritional Considerations

IV.H.1 Allergenicity

Although Oligonol® is comprised primarily of polyphenolic compounds, a small amount of protein is detected, which accounts for approximately 1 to 2% of the final product. As such, the allergenic potential of the source materials of Oligonol® (*i.e.*, lychee fruit and green tea) are discussed herein.

Currently in the U.S., lychee nut falls under the classification of “tree nuts”, and thus, any processed foods that contain the ingredient are required to have an appropriate food allergen warning label. However, allergic reactions to the lychee fruit are thought to be rare. Several cases of anaphylactic reactions, including generalized urticaria, Quincke edema, pruritus, bronchospasms, and dyspnea, have been reported following ingestion of the lychee fruit (Fäh *et al.*, 1995; Giannattasio *et al.*, 1995; Niggemann *et al.*, 2002; Saraswat and Kumar, 2005; Deswarte-Antomius, 2007; Garrido *et al.*, 2007; Raap *et al.*, 2007). It has been suggested that hypersensitivity to birch pollen has been associated with allergic reaction to lychee, as well as other fruits such as apples, hazelnut, carrots and celery, due to the cross-reactivities of specific IgE antibodies (Wellhausen *et al.*, 1996). Others have also suggested that there may be

possible cross-reactivity between allergens in the lychee fruit and latex (Niggemann *et al.*, 2002). Accordingly, the lychee fruit has been shown to contain significant amount of profilin, a panallergen that is present in plants that are both closely and distantly related (Fäh *et al.*, 1995; Song *et al.*, 2007). Moreover, various other allergens have been identified in the lychee fruit, including a 35 kDa protein that was shown to cross-react with birch pollen allergen (Wellhausen *et al.*, 1996; Song *et al.*, 2007). Hoppe *et al.* (2006) also identified a 28-kDa allergen present in the lychee fruit as triose-phosphate isomerase, an enzyme that has been described as an allergen in other plants (Hoppe *et al.*, 2006).

A small number of cases of occupationally-induced asthma have been reported in the literature among green tea factory workers (Shirai *et al.*, 1994, 1997, 2003). In these cases, the catechin EGCG was implicated in the IgE-mediated responses underlying the allergenic reaction (Shirai *et al.*, 1994, 1997, 2003). However, other studies have indicated that catechins from green tea may have anti-allergenic properties, with isolated catechins having inhibitory effects against type I allergic reactions (Shiozaki *et al.*, 1997; Sano *et al.*, 1999).

Overall, both lychee fruit and green tea have a long history of safe consumption, being widely consumed globally for thousands of years. Although there have been some cases of lychee fruit allergies reported in the literature, none of the participants consuming Oligonol[®] in the human studies conducted developed adverse effects, including symptoms of an allergic response (see Section IX.C.4). Moreover, Oligonol[®] dietary supplements have been marketed in the U.S. since 2007 without any adverse events reported, including cases of anaphylactic reactions. Therefore, the potential allergenicity of Oligonol[®], which is a highly purified polyphenolic product, is considered to be low. Although the potential for allergenicity is minimal, it would be prudent to assume that Oligonol[®] could elicit an allergic reaction among certain individual, and thus the product should have proper food labeling indicating the presence of a lychee-derived ingredient.

IV.I Summary and Basis for GRAS

Pursuant to Title 21, Section 170.30 of the CFR, Amino Up has determined that the proposed food and beverage uses of Oligonol[®] [polyphenols obtained from a 5:1 mixture of lychee fruit extract (*Litchi chinensis* Sonn.) and green tea leaf extract (*Camellia sinensis*)] as described in Table I.D-1, are GRAS on the basis of scientific procedures. Oligonol[®] is intended to be added as an ingredient in multiple food categories and use levels ranging from 12 to 192 mg per serving. Based on the proposed food uses and use levels, and using data from the 2009-2010 NHANES, the high consumer (90th percentile) all user intake of Oligonol[®] among the total U.S. population was estimated at 412 mg/person/day (7.3 mg/kg body weight/day), with a maximum level occurring in male adults at 512 mg/person/day (6.0 mg/kg body weight/day). These conservative estimates of daily intake are supported by the results of product-specific studies conducted with Oligonol[®]. In 90-day oral toxicity studies conducted in rats, no treatment-related

effects were reported at dosages up to 1,000 mg/kg body weight/day, which is more than 100-fold higher than the estimated intake of Oligonol[®] among 90th percentile consumers in the total U.S. population on an all user basis (*i.e.*, 7.3 mg/kg body weight/day). Additionally, no adverse effects were observed when Oligonol[®] was orally administered to human subjects at doses ranging from 200 to 600 mg/person/day, for durations as long as 3 months. Furthermore, Oligonol[®] was proven through scientific experimentation to be neither mutagenic or genotoxic.

While product-specific studies conducted with Oligonol[®] clearly support its use as a food ingredient in the stipulated food categories, due to the green tea extract component of the product, Amino Up conducted a close evaluation of the literature pertaining to the published adverse effects of green tea catechins so as to confirm the safety of the Oligonol[®] product. These published adverse findings include case reports of hepatotoxicity following consumption of dietary supplements containing highly concentrated, purified green tea extracts. These liver effects have subsequently been corroborated, along with the novel finding of nasal toxicity, in a 14-week oral toxicity study conducted by the NTP where a specific green tea extract preparation was administered by gavage to rodents. However, these findings are not considered relevant to the safety of Oligonol[®]. A close examination of the serum biochemistry and pathology data from the two standard 90-day toxicity studies conducted in rats revealed no adverse findings that would be suggestive of liver toxicity following administration of Oligonol[®] at doses up to 1,000 mg/kg body weight/day. Similarly, clinical chemistry data from the 3 human studies did not indicate any changes in liver function in subjects consuming Oligonol[®] at doses up to 600 mg/day. To address the potential concerns regarding nasal toxicity, it was established in another rat subchronic study that there were no histopathological changes to the nasal cavity following administration of Oligonol[®] by gavage at dosages up to 1,000 mg/body weight/day for 90 days, thereby providing confirmation that the effects noted in the 14-week toxicity studies conducted by the NTP were not relevant to Oligonol[®]. Furthermore, it was established that the intake of green tea catechins in the total population and male adults at the 90th percentile intake of Oligonol[®] equates to approximately 66 and 82 mg/person/day, respectively, which is less than the amount that would be obtained from drinking 1 cup of green tea (see also Table IV.A.3-1). Therefore, in totality these results confirm that the green tea extract component of Oligonol[®] pose no safety concern under its intended conditions of use.

Finally, the Expert Panel convened by Amino Up independently and critically evaluated all data and information presented herein and concluded that the intended food categories and use levels are safe and GRAS based on scientific precedents. The weight of the scientific evidence presented herein indicates that the intended uses of Oligonol[®] [polyphenols obtained from a 5:1 mixture of lychee fruit extract (*Litchi chinensis* Sonn.) and green tea leaf extract (*Camellia sinensis*)], meeting appropriate food-grade specifications and manufactured in-line with GMP, are GRAS. Food uses of Oligonol[®] as described herein are therefore excluded from the definition of a food additive, and thus, may be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21 of the CFR.

V REFERENCES

- Aron PM, Kennedy JA (2008). Flavan-3-ols: nature, occurrence and biological activity. *Mol Nutr Food Res* 52(1):79-104.
- Beecher GR (2003). Overview of dietary flavonoids: nomenclature, occurrence and intake. *J Nutr* 133(10):3248S-3254S.
- Bonkovsky HL (2006). Hepatotoxicity associated with supplements containing Chinese green tea (*Camellia sinensis*). *Ann Intern Med* 144(1):68-71.
- CDC (2011). *National Health and Nutrition Examination Survey (NHANES): 2009-2010*. Hyattsville (MD): Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). Available at: http://www.cdc.gov/nchs/nhanes/nhanes2009-2010/nhanes09_10.htm.
- Chan PC, Ramot Y, Malarkey DE, Blackshear P, Kissling GE, Travlos G et al. (2010). Fourteen-week toxicity study of green tea extract in rats and mice. *Toxicol Pathol* 38(7):1070-1084.
- Chow H-HS, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM et al. (2005). Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin Cancer Res* 11(12):4627-4633.
- Chun OK, Chung SJ, Song WO (2007). Estimated dietary flavonoid intake and major food sources of U.S. adults. *J Nutr* 137(5):1244-5122.
- Clifford MN (2004). Diet-derived phenols in plasma and tissues and their implications for health. *Planta Med* 70(12):1103-1114.
- DeMan JM (1999). Color [Anthocyanins and flavonoids, Tannins]. In: *Principles of Food Chemistry, 3rd edition*. Gaithersburg, (MA): Aspen Publishers, pp. 252-258.
- Déprez S, Brezillon C, Rabot S, Philippe C, Mila I, Lapierre C et al. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J Nutr* 130(11):2733-2738.
- Deswarte-Antoniou DC (2007). Litchi and mango allergy. *Rev Fr Allergol Immunol Clin* 47(Suppl. 1):S5-S7.
- Donovan JL, Crespy V, Manach C, Morand C, Besson C, Scalbert A et al. (2001). Catechin is metabolized by both the small intestine and liver of rats. *J Nutr* 131(6):1753-1757.
- Donovan JL, Manach C, Rios L, Morand C, Scalbert A, Rémésy C (2002). Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B3. *Br J Nutr* 87(4):299-306.
- Fäh J, Wüthrich B, Vieths S (1995). Anaphylactic reaction to lychee fruit: evidence for sensitization to profilin. *Clin Exp Allergy* 25(10):1018-1023.

- Francis FJ, editor (1999). Tannins. In: *Wiley Encyclopedia of Food Science and Technology: Volume 4, 2nd edition*. New York, (NY): Wiley, pp. 2285-2291.
- Fujii H, Sun B, Nishioka H, Hirose A, Aruoma OI (2007). Evaluation of the safety and toxicity of the oligomerized polyphenol Oligonol®. *Food Chem Toxicol* 45(3):378-387.
- Fujii H, Nishioka H, Wakame K, Magnuson BA., Roberts A (2008). Acute, subchronic and genotoxicity studies conducted with Oligonol, an oligomerized polyphenol formulated from lychee and green tea extracts. *Food Chem Toxicol* 46(12):3553-3562.
- Garrido S, Garcia BE, Echechipia S, Sanz ML, Ariz S, Tabar AI (2007). Anaphylaxis following the first ingestion of lychee fruit: clinical features and immunological cross-reactivity implications. *Allergy* 62(8):962-963.
- Giannattasio M, Serafini M, Guarrera P, Cannistraci C, Cristaudo A, Santucci B (1995). Contact urticaria from litchi fruit (*Litchi chinensis* sonn.). *Contact Dermatitis* 33(1):67.
- Gonthier MP, Donovan JL, Texier O, Felgines C, Remesy C, Scalbert A (2003). Metabolism of dietary procyanidins in rats. *Free Radic Biol Med* 35(8):837-844.
- Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D et al. (2004). Concentrations of proanthocyanidins in common foods and estimations of normal consumption [& suppl. data]. *J Nutr* 134(3):613-617.
- Harada M, Kan Y, Naoki H, Fukui Y, Kageyama N, Nakai M et al. (1999). Identification of the major antioxidative metabolites in biological fluids of the rat with ingested (+)-catechin and (-)-epicatechin. *Biosci Biotechnol Biochem* 1999, 63(6):973-977.
- Hoppe S, Steinhart H, Paschke A (2006). Identification of a 28 kDa lychee allergen as a triose-phosphate isomerase. *Food Agric Immunol* 17(1):9-19.
- Isbrucker RA, Edwards JA, Wolz E, Davidovich A, Bausch J (2006). Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: dermal, acute and short-term toxicity studies. *Food Chem Toxicol* 44(5):636-650.
- Kapetanovic IM, Crowell JA, Krishnaraj R, Zakharov A, Lindeblad M, Lyubimov A (2009). Exposure and toxicity of green tea polyphenols in fasted and non-fasted dogs. *Toxicology* 260(1-3):28-36.
- Kim S, Lee M-J, Hong J, Li C, Smith TJ, Yang G-Y et al. (2000). Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of green tea polyphenols. *Nutr Cancer* 37(1):41-48.
- Kitadate K, Homma K, Roberts A, Maeda T. (2013). Thirteen-week oral dose toxicity study of Oligonol containing oligomerized polyphenols extracted from lychee and green tea. *Regul Toxicol Pharmacol* [In Press – Dec. 8, 2013].
- Kühnau J (1976). The flavonoids. a class of semi-essential food components: their role in human nutrition. *World Rev Nutr Diet* 24:117-191.

- Lakenbrink C, Lapczynski S, Maiwald B, Engelhardt UH (2000). Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *J Agric Food Chem* 48(7):2848-2852.
- Leuschner JP (2011). *Repeated Dose 90-Day Oral Toxicity Study of C-SAT 100089 in Rats – According to OECD Guideline 408 and EC Guideline B.26.* (LPT Report No. 25793). Hamburg, Germany. Prepared by LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG for Düsseldorf, Germany: Cognis GmbH.
- Lewis HB, Ahern AL, Jebb SA (2012). How much should I eat? A comparison of suggested portion sizes in the UK. *Public Health Nutr* 15(11):2110-2117.
- Li C, Lee M-J, Sheng S, Meng X, Prabhu S, Winnik B et al. (2000). Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem Res Toxicol* 13(3):177-184.
- Manach C, Donovan JL (2004). Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radic Res* 38(8):771-785.
- Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004). Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 79(5):727-747.
- Manach C, Mazur A, Scalbert A (2005). Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol* 16(1):77-84.
- Martin KR, Appel CL (2010). Polyphenols as dietary supplements: A double-edged sword. *Nutr Diet Suppl* 2:1-12.
- Mazzanti G, Menniti-Ippolito F, Moro PA, Cassetti F, Raschetti R, Santuccio C et al. (2009). Hepatotoxicity from green tea: a review of the literature and two unpublished cases. *Eur J Clin Pharmacol* 65(4):331-341.
- McKay DL, Blumberg JB (2007). Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. *Nutr Rev* 65(11):490-502.
- Meng X, Sang S, Zhu N, Lu H, Sheng S, Lee M-J et al. (2002). Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. *Chem Res Toxicol* 15(8):1042-1050.
- Nakagawa K, Miyazawa T (1997). Absorption and distribution of tea catechin, (-)-epigallocatechin-3-gallate, in the rat. *J Nutr Sci Vitaminol (Tokyo)* 43(6):679-684.
- Niggemann B, Reibel S, Hipler C, Wahn U (2002). Anaphylactic reaction to lychee in a 12-year-old girl: cross-reactivity to latex? *Pediatr Allergy Immunol* 13(1):64-67.
- Okushio K, Matsumoto N, Suzuki M, Nanjo F, Hara Y (1995). Absorption of (-)-Epigallocatechin gallate into rat portal vein. *Biol Pharm Bull* 18(1):190-191.
- Okushio K, Matsumoto N, Kohri T, Suzuki M, Nanjo F, Hara Y (1996). Absorption of tea catechins into rat portal vein. *Biol Pharm Bull* 19(2):326-329.

- Piskula MZ, Terao J (1998). Accumulation of (-)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. *J Nutr* 128(7):1172-1178.
- Prior RL, Gu L (2005). Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry* 66(18):2264-2280.
- Raap U, Schaefer T, Kapp A, Wedi B (2007). Exotic food allergy: anaphylactic reaction to lychee. *J Investig Allergol Clin Immunol* 17(3):199-201.
- Rios LY, Bennett RN, Lazarus SA, Rémésy C, Scalbert A, Williamson G (2002). Cocoa procyanidins are stable during gastric transit in humans. *Am J Clin Nutr* 76(5):1106-1110.
- Rios LY, Gonthier MP, Rémésy C, Mila I, Lapierre C, Lazarus SA et al. (2003). Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am J Clin Nutr* 77(4):912-918.
- Sano M, Suzuki M, Miyase T, Yoshino K, Maeda-Yamamoto M (1999). Novel anti-allergic catechin derivatives isolated from oolong tea. *J Agric Food Chem* 47(5):1906-1910.
- Santos-Buelga C, Scalbert A (2000). Review: proanthocyanidins and tannin-like compounds — Nature, occurrence, dietary intake and effects on nutrition and health. *J Sci Food Agric* 80(7):1094-1117.
- Saraswat A, Kumar B (2005). Anaphylactic reaction to apple, banana and lychee: what is common between botanically disparate plant families? *Int J Dermatol* 44(12):996-998.
- Sarma DN, Barrett ML, Chavez ML, Gardiner P, Ko R, Mahady GB et al. (2008). Safety of green tea extracts: a systematic review by the US Pharmacopeia. *Drug Saf* 31(6):469-484.
- Shiozaki T, Sugiyama K, Nakazato K, Takeo T (1997). [Effect of tea extracts, catechin and caffeine against type-I allergic reaction]. *Yakugaku Zasshi* 117(7):448-454 [Japanese with English abstract & tables].
- Shirai T, Sato A, Hara Y (1994). Epigallocatechin gallate. The major causative agent of green tea-induced asthma. *Chest* 106(6):1801-1805.
- Shirai T, Sato A, Chida K, Hayakawa H, Akiyama J, Iwata M et al. (1997). Epigallocatechin gallate-induced histamine release in patients with green tea-induced asthma. *Ann Allergy Asthma Immunol* 79(1):65-69.
- Shirai T, Reshad K, Yoshitomi A, Chida K, Nakamura H, Taniguchi M (2003). Green tea-induced asthma: relationship between immunological reactivity, specific and non-specific bronchial responsiveness. *Clin Exp Allergy* 33(9):1252-1255.
- Song WO, Chun OK (2008). Tea is the major source of flavan-3-ol and flavonol in the U.S. diet. *J Nutr* 138(8):1543S-1547S.

- Song JJ, Zhang HY, Liu ZG, Ran PX (2007). Cloning of the panallergen profilin from lychee fruit and its cross-reactivity with birch pollen profilin Bet v 2. *Food Agric Immunol* 18(2):129-138.
- Spencer JPE, Chaudry F, Pannala AS, Srani SK, Debnam E, Rice-Evans C (2000). Decomposition of cocoa procyanidins in the gastric milieu. *Biochem Biophys Res Commun* 272(1):236-241.
- Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H (1998). Wide distribution of [(3)H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. *Carcinogenesis* 19(10):1771-1776.
- Tanaka T, Watarumi S, Matsuo Y, Kamei M, Kouno I (2003). Production of theasinensins A and D, epigallocatechin gallate dimers of black tea, by oxidation–reduction dismutation of dehydrotheasinensin A. *Tetrahedron* 59(40):7939-7947.
- Tanaka T, Yoshitake N, Zhao P, Matsuo Y, Kouno IL, Nonaka G-I (2007). Production of oligomeric proanthocyanidins by fragmentation of polymers. *Jpn J Food Chem* 14(3):134-139.
- Thilakarathna SH, Rupasinghe HP (2013). Flavonoid bioavailability and attempts for bioavailability enhancement. *Nutrients* 5(9):3367-3387.
- Tsao R (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients* 2(12):1231-1246.
- U.S. FDA (2013a). 101—Food labeling. §101.12—Reference amounts customarily consumed per eating occasion. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO), pp. 47-56. Available at: <http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR>.
- U.S. FDA (2013b). 170—Food additives. §170.30—Eligibility for classification as generally recognized as safe. (GRAS). In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO), pp. 13-15. Available at: <http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR>.
- USDA (2007). *USDA Database for the Flavonoid Content of Selected Foods Release 2.1*. Beltsville (MD): U.S. Department of Agriculture and Agricultural Research Service and Beltsville Human Nutrition Research Center and Nutrient Data Laboratory. Available at: <http://www.ars.usda.gov/SP2UserFiles/Place/12354500/Data/Flav/Flav02-1.pdf>.
- USDA (2012). *What We Eat in America: National Health and Nutrition Examination Survey (NHANES): 2009-2010*. Riverdale (MD): U.S. Department of Agriculture (USDA). Available at: <http://www.ars.usda.gov/Services/docs.htm?docid=13793#release>.
- Wellhausen A, Schöning B, Petersen A, Vieths S (1996). IgE binding to a new cross-reactive structure: a 35 kDa protein in birch pollen, exotic fruit and other plant foods. *Z Ernährungswiss* 35(4):348-355.

Williamson G, Holst B (2008). Dietary reference intake (DRI) value for dietary polyphenols: are we heading in the right direction? Br J Nutr 99(Suppl. 3):S55-S58.

Yang CS, Sang S, Lambert JD, Lee M-J (2008). Bioavailability issues in studying the health effects of plant polyphenolic compounds. Mol Nutr Food Res 52(Suppl. 1):S139-S151.

Yashin A, Nemzer B, Yashin Y (2012). Bioavailability of tea components. J Food Res 1(2):281-290.

Expert Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of Oligonol®

March 11, 2013

Amino Up Chemical Co., Ltd. (Amino Up) intends to market Oligonol®, a 5:1 mixture of lychee (*Litchi chinensis*) fruit extract and green tea (*Camellia sinensis*) leaf extract, in the United States (U.S.) for use as an ingredient in multiple food categories, including processed fruits and fruit juices, breakfast cereals, tea, dairy product analogs, granola and breakfast or meal replacement bars, flavored milk and milk products, and soups and soup mixes at levels ranging from 12 to 192 mg/serving.

At the request of Amino Up, an Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and determine whether the proposed uses of Oligonol® described in Table A-1 (see Attachment A) are safe and suitable, and considered Generally Recognized as Safe (GRAS) based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Robert J. Nicolosi, Ph.D. (University of Massachusetts Lowell); Stephen L. Taylor, Ph.D. (University of Nebraska); and John A. Thomas, Ph.D. (Indiana University School of Medicine).

The Panel, independently and collectively, critically examined a comprehensive package of scientific information and data pertinent to Amino Up's Oligonol® ingredient presented in the dossier titled "Documentation Supporting the Evaluation of Oligonol® as Generally Recognized as Safe (GRAS)". This dossier contained information on the method of manufacture and product specifications of the ingredient, supporting analytical data, the proposed use levels in specified food products, consumption estimates for all proposed uses, and a comprehensive assessment of the available scientific literature pertaining to the safety of Oligonol®, as well as its monomeric and oligomeric polyphenolic constituents.

Following independent critical evaluation of such data and information, the Panel convened on Monday, March 11, 2013. The Panel unanimously concluded that the proposed uses of Amino Up's Oligonol® ingredient, produced from a mixture of lychee (*Litchi chinensis*) fruit extract and green tea (*Camellia sinensis*) leaf extract, meeting appropriate food-grade specifications and manufactured according to Good Manufacturing Practices (GMP), is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

SUMMARY AND BASIS FOR GRAS DETERMINATION

Oligonol[®] is a reddish-brown powder comprising a mixture of polyphenolic powdered extracts from *Litchi chinensis* (lychee) fruit and *Camellia sinensis* (green tea) leaf. Oligonol[®] is produced using a novel oligomerization manufacturing process whereby the polyphenols present in the lychee and green tea leaf extracts are cleaved into lower molecular weight oligomers and monomers (Tanaka *et al.*, 2007). The compositional analysis of three non-consecutive sample lots of Oligonol[®] is presented in Table A-2 (Attachment A). Oligonol[®] consists mainly of monomeric flavan-3-ols, which are a subgroup of flavonoids that comprise simple monomers including catechin, epicatechin (EC), epicatechin 3-gallate (ECG) and epigallocatechin gallate (EGCG) (Tsang *et al.*, 2005), as well as procyanidins formed from the condensation of these monomeric units (Tsang *et al.*, 2005). Oligonol[®] contains four different flavan-3-ol monomers, (+)-catechin, (-)-epicatechin, (-)-epicatechin 3-gallate, and (-)-epigallocatechin gallate. Combined, the monomeric flavan-3-ols constitute approximately 16% of Oligonol[®] (Table A-2). In addition, 5 polyphenol dimers [procyanidin A1, procyanidin A2, procyanidin B1, procyanidin B2, and (-)-epicatechin(-)-epigallocatechin gallate] and a trimer [(-)-epicatechin-(4 β →8, 2 β →O→7)-epicatechin-(4 β →8)-epicatechin] have been identified in Oligonol[®] and quantified using high-performance liquid chromatography (HPLC). The combined dimers and trimer constitute approximately 14 and 4% of Oligonol[®], respectively (Table A-2). Longer oligomers, composed of varying combinations of monomers, also are present in Oligonol[®]; however, due to technical limitations, these cannot be readily identified or quantified. The total polyphenolic content of Oligonol[®] is measurable using the Folin-Denis assay and accounts for >80% of the total mixture (Table A-2).

To manufacture Oligonol[®], powdered lychee fruit extract and powdered green tea leaf extract are blended in a ratio of 5:1, followed by oligomerization of the polyphenols in these extracts via a de-polymerization reaction using citric acid, during which the polyphenols are cleaved into oligomers and monomers. The lychee fruit powdered extract is derived from the lychee fruit using 50% ethanol as a solvent, and contains greater than 70% polyphenol content. The green tea leaf powdered extract is obtained by extraction in 50% ethanol, and contains a minimum of 98% total polyphenols. All of the raw materials and processing aids used in the manufacture of Oligonol[®] are food grade and suitable for use in accordance with U.S. regulations. Oligonol[®] is manufactured in accordance with GMP for dietary supplements, a standard that is based on guidelines prepared by the Japan Ministry of Health, Labour and Welfare and is certified by the Japan Health and Nutrition Food Association. Analysis of 3 non-consecutive lots of Oligonol[®] demonstrates that the manufacturing process produces a consistent product meeting the defined physical, chemical, and microbiological specifications (Table A-3). In addition, stability tests conducted indicate Oligonol[®] is stable for at least one year when stored at room temperature.

Many dietary sources, including fruits, vegetables, and tea, are rich in polyphenolic compounds. Lychee fruit and green tea leaves, both of which have an extensive history of safe consumption in the diet, are used as the source of polyphenols for the manufacture of Oligonol®. Amino Up's Oligonol® ingredient is proposed for use as a food ingredient in various foods and beverages, as described in Table A-1 (Attachment A). The intake of Oligonol® from the proposed uses was estimated using food consumption data derived from dietary surveys conducted in the U.S. On an all-user basis, the mean intake of Oligonol® by the total U.S. population from all proposed food-uses was estimated to be 190 mg/person/day or 3.3 mg/kg body weight/day. The heavy consumer (90th percentile) all-user intake of Oligonol® by the total U.S. population from all proposed food-uses was estimated to be 412 mg/person/day or 7.3 mg/kg body weight/day. Male adults were determined to have the highest mean and 90th percentile all-user intakes of Oligonol® at 222 and 512 mg/person/day (2.6 and 6.0 mg/kg body weight/day, respectively), respectively. On a body weight basis, the mean and 90th percentile all-user intakes of Oligonol® were highest in infants at 9.6 and 19.7 mg/kg body weight/day, respectively. Within the total U.S. population, consumers of fruit-flavored drinks and ades made the greatest contribution to the mean and 90th percentile all-user intakes of Oligonol®. In addition, Oligonol® is approved as a New Dietary Ingredient in the U.S., with maximum recommended use levels of 200 mg/day for adults only. Therefore, under worst-case scenario where Oligonol® dietary supplements are consumed at the maximum recommended amount in addition to proposed food uses, the 90th percentile intake of Oligonol® is estimated to be 616 and 712 mg/day for female and male adults, respectively. This corresponds to intakes of approximately 8.5 and 8.3 mg/kg body weight/day for female and male adults, respectively. However, it is considered unlikely that individuals consuming foods enriched with Oligonol® will also consume Oligonol® dietary supplements, and the estimated intakes are considered as worst-case scenario (*i.e.*, where Oligonol® dietary supplements are consumed at maximum levels, in addition to proposed food-uses).

The Panel reviewed a large body of safety data during their assessment of the safety of Oligonol®. Information on the metabolic fate of orally administered Oligonol® can be extrapolated from studies conducted with its monomeric and oligomeric constituents. Animal and human studies provide evidence that monomers are absorbed to a greater extent than the oligomeric procyanidins (Manach *et al.*, 2005; Tsang *et al.*, 2005; Yang *et al.*, 2008). Monomers and procyanidins that are not absorbed in the gastrointestinal tract may be partially metabolized by the intestinal microflora to phenylvalerolactones and to a limited extent, to phenolic acids and their derivatives (Manach *et al.*, 2005). Flavonoids that are absorbed are transported to the liver *via* the portal system, where they can be conjugated through glucuronidation, sulfation, or methylation and eliminated (Manach *et al.*, 2005; Yang *et al.*, 2008).

Product-specific toxicological studies have been conducted with Oligonol®. The oral median lethal dose (LD₅₀) for Oligonol® was greater than 2,000 mg/kg in both sexes of Sprague-Dawley rats. The sub-chronic toxicity of orally administered Oligonol® has been evaluated in three

90-day gavage studies conducted in rats and one 90-day feeding study in mice. In a study conducted in accordance with Good Laboratory Practice (GLP), groups of 6 male and 6 female Sprague-Dawley rats were administered Oligonol® at doses of 0 (control), 100, 300, or 1,000 mg/kg body weight/day by gavage for 90 days (Fujii *et al.*, 2008). Administration with Oligonol® did not significantly alter body weight or food consumption compared to controls throughout the study. Hematology, clinical chemistry or urinalysis parameters was not altered by administration with Oligonol® at any dose tested in comparison to controls. All males and females in the 1,000 mg/kg body weight/day dose group had gray discoloration of the mucosa of the duodenum, as did one male in the 300 mg/kg body weight/day dose group, who also had focal red discoloration of mucosa of the jejunum. Males in the 300 mg/kg body weight/day dose group had significantly lower absolute thymus weights compared to controls. However, these changes were not considered relevant since they were not observed in males administered the 1,000 mg/kg body weight/day dose or in any of the females. Females in the 1,000 mg/kg body weight/day dose group had significantly lower absolute ovary weights than the control group. However, the relative ovary weight was not significantly different from the control and no histopathological changes were observed. Thus, these findings were not considered to be toxicologically significant. The only notable histopathological finding in animals administered Oligonol® was a dose-dependent deposition of a brown pigment in the lamina propria of the duodenum. Slight deposition was observed in all rats in the 300 mg/kg body weight/day dose group, with the severity increasing in rats in the 1,000 mg/kg body weight/day dose group. The pigment was not observed in any other tissues in the gastrointestinal tract and was not accompanied by evidence of inflammation or other changes in the tissue. The authors did not consider this finding to be toxicologically relevant, and suggested the brown pigment was probably an accumulation of oxidized phenolics, resulting in the positive staining. The authors considered the NOAEL to be 1,000 mg/kg body weight, the highest dose tested.

In one unpublished study conducted under GLP and in accordance with OECD Guidelines, CD-rats (10/sex/group) were administered Oligonol® at 0, 100, 300, or 800 mg/kg body weight/day by gavage for 90 days (Leuschner, 2011). Another set of animals in the control and high-dose group (5/sex/group) were observed for an additional 6 weeks following the end of the 90-day treatment period as a recovery group. No treatment-related changes were noted in mortality, clinical signs (*i.e.* behavior, external appearance, or feces), or functional observation tests throughout the study. No treatment-related changes were observed during the neurological screen test (*i.e.* fore- and hind limb grip strength, spontaneous motility) and ophthalmological examination conducted at the end of treatment. A slight decrease (up to 9%) in the body weight of male animals administered 800 mg/kg body weight/day of Oligonol® was observed during the first 2 weeks of treatment compared to controls, being statistically significant at week 2. However, a corresponding reduction (non-significant) in food intake was also observed in these animals during the first 2 weeks of treatment. No other treatment-related changes in body weight, or food and water consumption were reported during the study. Administration of Oligonol® did not significantly alter hematological parameters. Significant increases in plasma

total bilirubin (by 26%) and specific gravity of the urine (by 2%) were noted in females administered 800 mg/kg body weight/day of Oligonol[®] compared to control animals. However, these were not considered to be adverse given no changes in other biochemistry and urinalysis parameters were observed. Moreover, administration with Oligonol[®] was not associated with changes in organ weights, and no abnormal findings were reported following macroscopic and histopathological examination. The authors determined the no-observed-adverse-effect level (NOAEL) of Oligonol[®] to be 800 mg/kg body weight/day, the highest dose tested.

In light of the nasal toxicity observed in a 90-day study conducted by the National Toxicology Program (NTP) where green tea extract was administered to rats and mice by gavage (Chan *et al.*, 2010), an additional GLP study was conducted to evaluate the effect of Oligonol[®] administration on the nasal cavity since this tissue was not collected in the other 90-day studies described above. Sprague-Dawley rats (10/sex/group) at 5 weeks of age were administered 0 (control), 100, 300, and 1,000 mg/kg body weight/day Oligonol[®] by gavage once daily for a period of 13 weeks (Kitadate *et al.*, 2013). There were no mortalities observed, and the only treatment-related clinical sign was compound-colored stool in 2 of the males in the mid-dose group, and in all of the animals of the high-dose group. Treatment with Oligonol[®] did not affect body weight throughout the study, though males in the high-dose group had significantly higher food intake (by 7 to 13%) during weeks 5, 6, and 11 of the study compared to controls. No gross abnormalities were reported in any of the animals upon examination at necropsy. Minor microscopic changes were reported in the nasal cavity, though these occurred at similar frequency and severity in both the controls and treated animals. Thus, the findings were considered to be incidental and not toxicologically relevant. Based on these results, the authors concluded that treatment with Oligonol[®] does not produce toxicity in the nasal cavity at doses up to 1,000 mg/kg body weight/day.

In a non-GLP study, the safety of Oligonol[®] and lychee fruit extract (the starting material used to manufacture Oligonol[®]) was assessed in ddY male mice (Fujii *et al.*, 2008). The animals (5/group) were fed diets supplemented with 0 (control), 200 mg lychee fruit extract/kg body weight/day, 2 mg Oligonol[®]/kg body weight/day, 20 mg Oligonol[®]/kg body weight/day, or 200 mg Oligonol[®]/kg body weight/day for 90 days. All animals survived until the end of the study period and there were no adverse effects observed. Administration of diets containing lychee fruit extract or Oligonol[®] did not significantly alter body weight or produce changes in clinical chemistry parameters in the animals at any dose compared to controls. No significant differences in absolute and relative organ weights were reported among the animals receiving lychee fruit extract or Oligonol[®] compared to controls. The authors concluded that the lack of toxicity in this study supports the findings from the rat studies.

Oligonol[®] was not mutagenic/genotoxic when evaluated using a series of *in vitro* and *in vivo* assays (Fujii *et al.*, 2008). No evidence of mutagenicity was observed when Oligonol[®] was tested using the Ames assay at concentrations up to 5,000 µg/plate in *Salmonella typhimurium*

strains TA100, TA1535, TA98, TA1537, and *Escherichia coli* WP2uvrA, in the absence or presence of metabolic activation. Oligonol[®] was not genotoxic when tested using the chromosome aberration assay in cultured Chinese hamster lung cells, in the presence and absence of metabolic activation. Administration of Oligonol[®] at doses up to 2,000 mg/kg body weight by gavage was not clastogenic when tested using the *in vivo* micronucleus assay in male SPF mice.

Oligonol[®] has been administered to humans without adverse effects in 3 unpublished studies. Oral administration of Oligonol[®] to 6 healthy volunteers (5 men and 1 woman) at 200 mg twice a day (*i.e.*, 400 mg/day) for 3 months did not result in any significant adverse side effects or changes in blood biochemistry parameters compared to baseline. One volunteer experienced transient diarrhea for the first week, which was resolved upon cessation of treatment. In another study, Oligonol[®] was orally administered at 300 mg twice a day (*i.e.*, 600 mg/day) for 14 days to 29 subjects (16 women and 13 men). No treatment-related changes in hematology or serum biochemistry parameters were observed at the end of the study compared to baseline. A small proportion of the participants reported abdominal discomfort and bloating (3 subjects), or other minor transient symptoms (5 subjects). In a double-blind, randomized, placebo-controlled study, 76 patients with pre-hypertension or hypertension (number of each sex not provided; age 21 to 74 years old; mean age of 51.4 years) were assigned to receive either placebo or Oligonol[®] (100 or 200 mg/day) for 60 days. No adverse events were reported by the subjects treated with Oligonol[®], and no treatment-related changes in hematological and serum biochemistry parameters were observed at either dose tested. Subjects treated with Oligonol[®] (at 100 and 200 mg/day) had fasting blood glucose levels greater than 100 mg/dL following the 60 days of treatment; however, this finding was also observed during the screening visit, and may be driven by individuals who did not comply to the overnight fast prior to blood sample collection.

Following a review of the available scientific data, the Panel concluded that proposed uses of Oligonol[®] would not pose any safety concerns. Based on the lack of toxicity observed in the three 90-day oral toxicity studies conducted in rats and one 90-day feeding study in mice, the NOAEL of Oligonol[®] can be considered as 1,000 mg/kg body weight/day, the highest dose tested. The Panel concluded there was a sufficient safety margin between this NOAEL and the estimated intake levels of Oligonol[®] under the proposed uses indicated in Table A-1 (Attachment A), as well as when combined with potential supplement use. Although adverse effects were reported in a 90-day oral toxicity study conducted by the NTP for green tea extract (Chan *et al.*, 2010) and there have been case reports of hepatotoxicity from use of concentrated, purified forms of green tea in humans (Bonkovsky, 2006; Sarma *et al.*, 2008; Mazzanti *et al.*, 2009), the Panel did not consider these to be relevant to the safety of Oligonol[®]. The Panel noted that evidence of hepatotoxicity and nasal toxicity observed in a 90-day oral toxicity study conducted by the NTP for green tea extract (Chan *et al.*, 2010) were not relevant to Oligonol[®] as these effects were not observed in the product-specific 90-day toxicity studies. Moreover, the estimated intake of green

tea polyphenols, particularly EGCG, even among the high consumers of Oligonol[®] is less than the amount that would be obtained from drinking 1 cup of green tea (USDA, 2007).

CONCLUSION

We, the members of the Expert Panel, have individually and collectively critically evaluated the information summarized above and conclude that Amino Up's Oligonol[®] ingredient, which is a 5:1 mixture of lychee (*Litchi chinensis*) fruit extract and green tea (*Camellia sinensis*) leaf extract, meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practice, is Generally Recognized as Safe (GRAS) based on scientific procedures and supporting long history of safe use for its intended use within the proposed food categories described within Table A-1.

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

(b) (6)

John A. Thomas, Ph.D.
Indiana University School of Medicine

14 June 2013

Date

(b) (6)

Robert J. Nicolosi, Ph.D.
University of Massachusetts Lowell

17 June 2013

Date

(b) (6)

Stephen L. Taylor, Ph.D.
University of Nebraska

13 June 2013

Date

REFERENCES

- Bonkovsky HL (2006). Hepatotoxicity associated with supplements containing Chinese green tea (*Camellia sinensis*). *Ann Intern Med* 144(1):68-71.
- Chan PC, Ramot Y, Malarkey DE, Blackshear P, Kissling GE, Travlos G et al. (2010). Fourteen-week toxicity study of green tea extract in rats and mice. *Toxicol Pathol* 38(7):1070-1084.
- Fujii H, Nishioka H, Wakame K, Magnuson BA., Roberts A (2008). Acute, subchronic and genotoxicity studies conducted with Oligonol, an oligomerized polyphenol formulated from lychee and green tea extracts. *Food Chem Toxicol* 46(12):3553-3562.
- Kitadate K, Homma K, Roberts A, Maeda T (2013). Thirteen-week oral dose toxicity study of Oligonol containing oligomerized polyphenols extracted from lychee and green tea. Manuscript submitted for publication June, 2013.
- Leuschner (2011). *Repeated Dose 90-Day Oral Toxicity Study of C-SAT 100089 in Rats – According to OECD Guideline 408 and EC Guideline B.26*. (LPT Report No. 25793). Hamburg, Germany. Prepared by LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG for Düsseldorf, Germany: Cognis GmbH.
- Manach C, Mazur A, Scalbert A (2005). Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol* 16(1):77-84.
- Mazzanti G, Menniti-Ippolito F, Moro PA, Cassetti F, Raschetti R, Santuccio C et al. (2009). Hepatotoxicity from green tea: a review of the literature and two unpublished cases. *Eur J Clin Pharmacol* 65(4):331-341.
- Sarma DN, Barrett ML, Chavez ML, Gardiner P, Ko R, Mahady GB et al. (2008). Safety of green tea extracts: a systematic review by the US Pharmacopeia. *Drug Saf* 31(6):469-484.
- Takami S, Imai T, Hasumura M, Cho YM, Onose J, Hirose M (2008). Evaluation of toxicity of green tea catechins with 90-day dietary administration to F344 rats. *Food Chem Toxicol* 46(6):2224-2229.
- Tanaka T, Yoshitake N, Zhao P, Matsuo Y, Kouno IL, Nonaka G-I (2007). Production of oligomeric proanthocyanidins by fragmentation of polymers. *Jpn J Food Chem* 14(3):134-139.
- Tsang C, Auger C, Mullen W, Bornet A, Rouanet J-M, Crozier A et al. (2005). The absorption, metabolism and excretion of flavan-3-ols and procyanidins following the ingestion of a grape seed extract by rats. *Br J Nutr* 94(2):170-181.
- U.S. FDA (2012). Part 101—Food labeling. Section §101.12—Reference amounts customarily consumed per eating occasion. In: *U.S. Code of Federal Regulations (CFR). Title 21—Food and Drugs (Food and Drug Administration)*. Washington (DC): U.S. Government Printing Office (GPO). Available at: <http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR>.

USDA (2007). *USDA Database for the Flavonoid Content of Selected Foods Release 2.1*. Beltsville (MD): U.S. Department of Agriculture and Agricultural Research Service and Beltsville Human Nutrition Research Center and Nutrient Data Laboratory. Available at: <http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02-1.pdf>.

Yang CS, Sang S, Lambert JD, Lee M-J (2008). Bioavailability issues in studying the health effects of plant polyphenolic compounds. *Mol Nutr Food Res* 52(Suppl 1):S139-S151.

ATTACHMENT A

Table A-1 Summary of the Individual Proposed Food-Uses and Use Levels for Oligonol® in the United States (2009-2010 NHANES Data)				
Food Category	Proposed Food-Use	Oligonol® Use Level (mg/serving)	Serving Size (g/mL)*	Use Level (%)
Breakfast Cereals	Ready-to-Eat Cereals	12, 24, or 44	(Puffed) 15 (Fiber) 30 (Biscuit) 55	0.08
Coffee and Tea	Tea	100	240	0.042
Dairy Product Analogs	Soy and Imitation Milk	24	240	0.01
Grain Products and Pastas	Granola, Meal Replacement, and Breakfast Bars	32	40	0.08
Milk Products	Dairy-Based Drinks (one shot) ¹	100	100	0.10
	Flavored Milk and Milk Drinks	24	240	0.01
	Milk-Based Meal Replacement Beverages	192	240	0.08
	Yogurt	45	225	0.02
Processed Fruits and Fruit Juices	Fruit-Flavored Drinks and Aides (RTD and Powder)	100	240	0.042
	Fruit Juices	100	240	0.042
Soups and Soup Mixes	Soups with Legumes or Potatoes as Major Ingredients ²	100	245	0.041
	Tomato and Vegetable Soups ²	100	245	0.041

RTD = Ready-to-Drink

* Serving sizes based on the US FDA Reference amounts customarily consumed per eating occasion (RACCs), April 1, 2011, 21 CFR §101.12 (U.S. FDA, 2012). When a range of values is reported for a proposed food-use, particular foods within that food-use may differ with respect to their RACC.

¹ Food codes representative of one-shot dairy based drinks were not identified. Fruit smoothie drinks and milk with acidophilus will be used as surrogates for one-shot dairy-based drinks.

² Excluding meat/poultry-based soups.

Table A-2 Compositional Analysis of Three Sample Lots of Oligonol®				
Constituents	Lot Number			
	OLF0703	OLF0705	OLF0804	
Monomeric Flavan-3-ols + Procyanidins	92.5	95.6	92.7	
Moisture (%)	0.0	0.0	0.0	
Protein (%)	1.6	1.6	1.6	
Total fat (%)	0.5	0.6	1.0	
Sugars (%)	3.5	3.7	3.5	
Ash (%)	0.2	0.3	0.2	
Total (%)	98.3	101.8	99.0	
Phenolic composition				
Monomeric Flavan-3-ols (%)				
(+)-Catechin and (-)-Epicatechin	8.2	7.9	6.4	
(-)-Epicatechin-3-gallate	2.1	2.0	2.3	
(-)-Epigallocatechin gallate	6.0	6.2	6.4	
Total Monomeric Flavan-3-ols (%)	16.3	16.1	15.1	
Procyanidins (%)				
Dimers	Procyanidin A1	4.1	4.0	3.5
	Procyanidin A2	5.0	4.9	5.3
	Procyanidin B1	1.3	1.3	0.4
	Procyanidin B2	3.1	3.0	2.5
	(-)-Epicatechin(-)-epigallocatechin gallate	0.3	0.3	0.5
Trimer	(-)-Epicatechin-(4 β →8, 2 β →O→7)-epicatechin-(4 β →8)-epicatechin	3.8	3.8	1.9
Other procyanidins	58.6	62.2	63.5	
Total Procyanidins (%)	76.2	79.5	77.6	
Total Monomeric Flavan-3-ols + Procyanidins	92.5	95.6	92.7	

^a Analyses were conducted on freeze-dried samples of Oligonol®.

Table A-3 Product Specifications for Oligonol®		
Specification Parameter	Specification	Method of Analysis
Characteristic	Reddish-brown powder, characteristic rough taste	Sensory analysis
Moisture (%)	Not more than 3.0	Oven drying at 70°C for 6 h under reduced pressure
Total Procyanidin (%)	More than 70	Porter method
Monomer (%)	More than 10	HPLC method
Lead (Pb) (ppm)	Not more than 0.2	Atomic absorption spectrophotometry
Arsenic (as As ₂ O ₃) (ppm)	Not more than 1.0	Colorimetric method (arsenic limit test)
Number of bacteria (CFU/g)	Not more than 1,000	Microbial Limit test (pour plate method)
Mold and Yeast	Not detected	Microbial Limit test (spread plate method)
Coliforms (presence/absence)	Absent	Microbial Limit test (spread plate method)

As₂O₃ = arsenic oxide; CFU = colony forming units; HPLC = high-performance liquid chromatography

SUBMISSION END