Hogan Lovells US LLP
Columbia Square
555 Thirteenth Street, NW
Washington, DC 20004
T +1 202 637 5600
F +1 202 637 5910
www.hoganlovells.com

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By Federal Express

June 14, 2018

Office of Food Additive Safety (HFS–200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Re: GRAS Notice for Urolithin A

Dear Sir or Madam:

On behalf of our client Amazentis SA (Amazentis), we hereby submit the enclosed GRAS notice for urolithin A, manufactured by Amazentis and intended for use among older adults (i.e., adults aged 40 years and above) as an ingredient in select foods or for special dietary uses in meal replacement products to support general mitochondrial health. As discussed in more detail in the GRAS notice, the intended uses include powdered (reconstituted) protein shakes, beverages (ready-to-drink protein shakes, non-milk based meal replacement beverages, instant oatmeal, protein and nutrition bars, and yogurts (Greek yogurts, high-protein yogurts, and yogurt drinks) at typical use levels of 250 mg/serving or 500 mg/serving up to a maximum of 500 mg/serving or 1,000 mg/serving, with an estimated aggregate intake of 1g/person/day. The statutory basis of Amazentis' GRAS conclusion is scientific procedures.

Urolithin A is not intended for use in infant formula or meat, poultry, and egg products that would require additional regulatory review by the United States Department of Agriculture. The GRAS notice does not contain any designated confidential business information. In accordance with the agency's guidelines, we have enclosed one original copy of the GRAS notice, and one complete electronic copy of the GRAS notice on a compact disk (CD).

We are committed to cooperating with the agency and believe an open dialog is one of the most effective ways to accomplish that objective. In that regard, we met with the agency staff twice on June 1, 2017 and May 22, 2018 to discuss the GRAS notice submission. We want to thank the agency for the very helpful recommendations and input we received during these pre-submission meetings. If any questions arise in the course of your review, please contact us, preferably by telephone or e-mail, so that we can provide a prompt response.

Sincerely,

(b) (6)

Martin J. Hahn
Partner
Hogan Lovells US LLP
martin.hahn@hoganlovells.com
202 637 5926

Cc:

Renata Kolanos, Ph.D.
Consumer Safety Officer
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
E-mail: Renata.Kolanos@fda.hhs.gov

GRAS NOTICE FOR UROLITHIN A

PREPARED FOR:
Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, MD
20740

DATE:
June 8, 2018

GRAS Notice for Urolithin A

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GRAS Notice for Urolithin A

Part 1. §170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, Amazentis SA hereby informs the United States (U.S.) Food and Drug Administration (FDA) of the view that its urolithin A is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (U.S. FDA, 2016) based on its conclusion that the notified substance is Generally Recognized as Safe (GRAS) under the conditions of its intended use described in Part 1.3 below. In addition, as a responsible official of Amazentis SA, the undersigned hereby certifies that all data and information presented in this notice constitutes a complete, representative, and balanced submission, and which considered all unfavorable as well as favorable information known to Amazentis SA and pertinent to the evaluation of the safety and GRAS status of urolithin A as an ingredient for addition to food, as described herein.

Signed,

(b) (6)

June 8, 2018

William Blanco-Bose, Ph.D.
Regulatory Affairs
Amazentis, SA

Date

1.1 Name and Address of Notifier

AMAZENTIS SA
EPFL Innovation Park
Building C
1015 Lausanne
Switzerland

1.2 Common Name of Notified Substance

Urolithin A

1.3 Conditions of Use

Urolithin A manufactured by Amazentis SA is intended for use as an ingredient in select foods or for special dietary uses in meal replacement products based on its nutritive activity in supporting general mitochondrial health. As shown in Table 1.3-1, these include powdered (reconstituted) protein shakes, beverages (ready-to-drink protein shakes, non-milk based meal replacement beverages, instant oatmeal, protein and nutrition bars, and yogurts (Greek yogurts, high-protein yogurts, and yogurt drinks) at typical use levels of 250 mg/serving or 500 mg/serving up to a maximum of 500 mg/serving or 1,000

mg/serving, with an estimated aggregate intake of 1g/person/day (see Section 3.2 for a full discussion of estimated intakes).

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use Levels for Urolithin A in the U.S.

Category	Food-Uses	Urolithin A Level, as Consumed (mg/serving)	RACC[a] (g or mL)	Urolithin A Use Levels (mg/100 g or mg/100 mL)
Food Categories (21 CFR 170.3)				
Beverages and Beverage Bases	Protein shakes; meal replacement drinks	500	360	140
Breakfast Cereals	Instant oatmeals	500	240	210
Grain Products and Pastas	Protein and nutrition bars	500	40	1,250
Milk Products	Greek yogurts, high protein yogurts[b]	1,000	170	590
	Yogurt drinks[c]	500	100[d]	500
	Milk-based protein shakes	1,000	240	420

CFR = Code of Federal Regulations; na = not applicable; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2016a).

[b] No food codes were identified for yogurt drinks, but based on the high content of protein in Greek yogurts, these were deemed a suitable surrogate

[c] No food codes were identified for yogurt drinks within the NHANES dataset; however, food codes for dairy-based fruit smoothies drinks were selected as surrogates to represent the food codes in this category.

[d] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently in the U.S. market.

Older adults (*i.e.*, adults aged 40 years and above) are intended to be the primary consumers of the ingredient. Urolithin A is not intended for use in infant formula or any products that would require additional regulatory review by the United States Department of Agriculture (USDA).

1.4 Basis for GRAS

Pursuant to 21 CFR § 170.30 (a) and (b), urolithin A manufactured by Amazentis SA, has been concluded to have GRAS status for use as an ingredient for addition to specified foods as described in Part 1.3, on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be made available to the FDA either in an electronic format or on paper for review and copying upon request during customary business hours at the offices of:

AMAZENTIS SA
EPFL Innovation Park
Building C
1015 Lausanne
Switzerland

1.6 Freedom of Information Act, 5 U.S.C. 552

It is Amazentis SA's view that all data and information presented in Parts 2 through 7 of this notice do not contain any trade secret, commercial, or financial information exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

Part 2. §170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Amazentis SA produces a synthetic version of urolithin A that is identical in structure to the compound formed endogenously following consumption of ellagic acid and ellagitannins. Ellagitannins and ellagic acid are dietary polyphenols found in various fruits and berries (pomegranate, blackberries, camu-camu, strawberries, raspberries), nuts (walnuts, hazelnuts, acorns, chestnuts, pecans), muscadine grapes and oak-aged wines and spirits (Gonzalez-Sarrias *et al*., 2010; Espin *et al*., 2013).

2.1.1 Common or Usual Name

Urolithin A

2.1.2 Chemical Name

3,8-dihydroxy-6H-dibenzo(b,d)pyran-6-one; 3,8-dihydroxybenzo[c] chromen-6-one

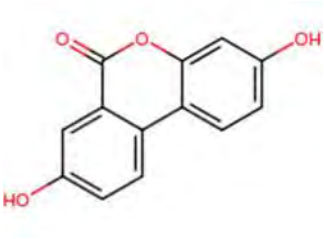
2.1.3 Chemical Abstract Service (CAS) Number

1143-70-0

2.1.4 Chemical Formula

$C_{13}H_8O_4$

2.1.5 Molecular Structure



2.1.6 Molecular Weight

228.20 g/mol

2.2 Manufacturing

2.2.1 Raw Materials and Processing Aids

The reagents involved in the synthesis of urolithin A are listed in Table 2.2.1-1. All materials are high purity ingredients and are considered safe and suitable for use in the manufacture of food ingredients. Limits have been established for the presence of residual solvents. These limits are presented in Section 2.4.1.

Table 2.2.1-1 Identity and Purity of Raw Materials and Processing Aids

Compound	CAS Number	Purity
2-Bromo-5-methoxy benzoic acid	22921-68-2	≥ 99.0%
2-Bromo-5-hydroxy benzoic acid	58380-11-3	≥ 99.0%
Resorcinol	108-46-3	≥ 99.0%
50% sodium hydroxide	1310-73-2	≥ 49.7% wt.
Copper sulfate pentahydrate	7758-99-8	≥ 98% wt.
Methanol	67-56-1	99.9% wt.
Aluminum chloride	7446-70-0	99.7%
Toluene	108-88-3	100.0%
DMSO	67-68-5	99.9%
Methanol	67-56-1	99.9%
Acetic Acid	64-19-7	≥ 99%
TBME (tert-butyl-methyl ether)	1634-04-4	99.9%

2.3 Production Details and Schematics

Urolithin A is manufactured in compliance with current Good Manufacturing Practice (cGMP), according to well-established processes. The synthesis and production processes are schematically presented in Figures 2.3-1 and 2.3-2, respectively. Urolithin A is manufactured *via* chemical syntheses using one of the two processes described here. Both processes, Process 1 and Process 2, involve an Ullmann coupling reaction, followed by a Lewis acid treatment to yield a highly purified urolithin A product.

The Ullmann reaction yields the core structure of urolithin A, while the Lewis Acid treatment accomplishes the cleavage of either the methyl-ether group (Process 1) or the "sodium oxy" group (Process 2) via the application of Lewis acids. The final product is purified by standard means of treatment in solvents, filtered, washed and dried to obtain pure urolithin A. The product is later subjected to a particle size reduction.

Step 1: Ullmann Coupling

Both Process 1 and 2 begin with a copper catalyzed cyclization between resorcinol and a corresponding 2-bromo benzoic acid in alkaline media (*i.e.,* an Ullmann reaction).

In Process 1, 2-bromo-5-methoxybenzoic acid is coupled with resorcinol to yield the intermediate “methoxy” product (Intermediate A), which is then isolated via filtration and dried for use in step 2. The solvent methanol is utilized in this step.

In Process 2, 2-bromo-5-hydroxybenzoic acid is utilized in place of the 2-bromo-5-methoxybenzoic acid used in Process 1. Ullman coupling of the 2-bromo-5-hydroxybenzoic acid and resorcinol results in a "sodium oxy" intermediate(s), which upon filtration results in crude urolithin A and its Na-adducts. Water is employed as a reaction solvent for this step in Process 2.

Step 2: Lewis Acid Treatment

In Process 1, the intermediate product, "Intermediate A", is treated with the Lewis acid, $AlCl_3$, in order to obtain a crude urolithin A. The ether cleavage of Intermediate A is accomplished by activation of the methyl ether through the addition of the Lewis acid, $AlCl_3$, in toluene. The activated species is then hydrolyzed by the addition of water. Following hydrolysis, the crude product is filtered and dried.

In Process 2, the Ullmann coupling results in a crude urolithin A or its di- or mono- Na adducts. The di- or mono- Na adduct intermediates are neutralized by Lewis Acid treatment to urolithin A via treatment with HOAc.

Purification procedures

In Process 1, crude urolithin A is dissolved in dimethyl sulfoxide (DMSO) for a polish filtration and subsequently precipitated from this DMSO solution by the addition of water. The filter cake is rinsed by water, followed by methanol. The raw urolithin A is then triturated with acetic acid (HOAc) to further purify the product and then collected by filtration. Following filtration, the purified product is rinsed with HOAc followed by tert-butyl-methyl ether (TBME), then dried to yield the final product, urolithin A.

In Process 2, the raw urolithin A is triturated with HOAc to further purify the product, which upon filtration and drying yields the final product, urolithin A.

The individual inorganic impurity AZX1 was observed in both synthesis processes. On the basis of UPLC/MS, the molecular weight of the ionized from of the substance (i.e. missing a hydrogen) was identified as 345 g/mol with the corresponding chemical formula identified as $C_{20}H_9O_6$. The impurity matches the profile of the ionized form of the dimer of urolithin A (molecular weight 346 g/mol with the corresponding chemical formula $C_{20}H_{10}O_6$). Because of the very low levels of the impurity found in the product, the exact identity of AZX1 has not been further characterized. We also find it unnecessary to characterize the substance beyond UPLC/MS because of the substantive toxicology studies that supported the safety of urolithin A with the low levels of this AZX1 impurity.

Both synthesis Process 1 and Process 2 yield the same synthesized product urolithin A, meeting the specifications described in Tables 2.4.1-1 and 2.4.2-1.

Figure 2.3-1 Process Schemes for the Synthesis of Urolithin A

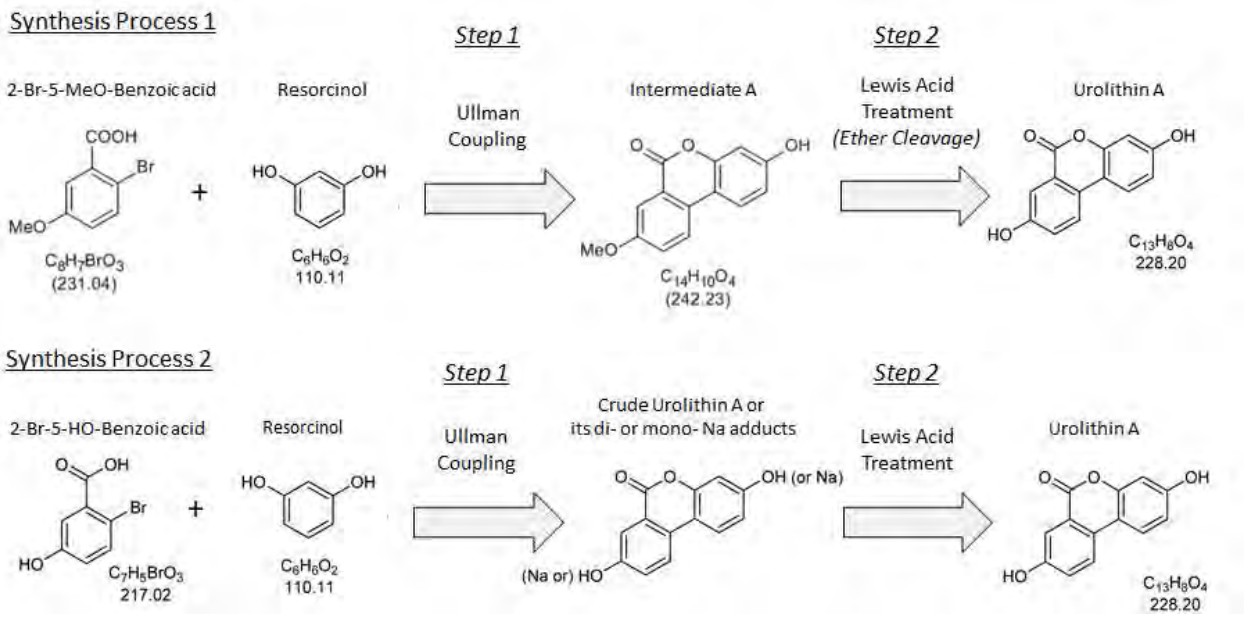
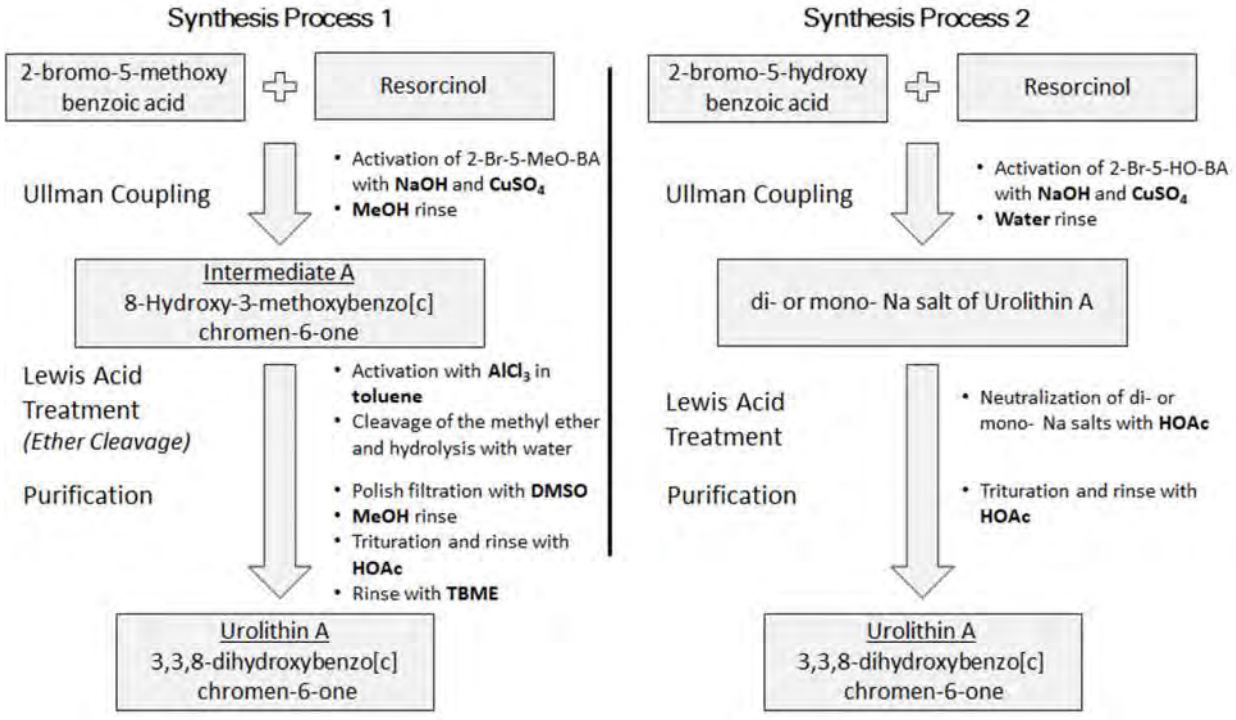


Figure 2.3-2 Schematic Overviews of the Manufacturing Process for Urolithin A



Abbreviations: $AlCl_3$ = aluminum chloride; NaOH = sodium hydroxide; $CuSO_4$ = copper sulfate; MeOH = methanol; TBME = tert-butyl-methyl ether; HOAc = acetic acid

2.4 Product Specifications and Batch Analysis

2.4.1 Chemical Specifications

The chemical specifications for urolithin A are presented in Table 2.4.1-1

Table 2.4.1-1 Chemical Specifications for Urolithin A

Specification Parameter		Specification	Method
Appearance		Solid / Powder	Visual inspection
Color		Beige to Yellow	Visual inspection
Identity (FT-IR)		Conforms to reference	FT-IR
Purity (HPLC area %)		NLT 97.0 %	HPLC
Individual Organic Impurities	Intermediate A	NMT 0.5%	
	AZX1	NMT 0.4%	
	Any other individual impurity	NMT 0.3%	
Water Content (K.F.)		Report result	Karl Fischer
Residual Solvents	Methanol	NMT 3000 ppm	GC
	TBME	NMT 1000 ppm	
	Toluene	NMT 890 ppm	
	DMSO	NMT 5000 ppm	
	Acetic Acid	NMT 5000 ppm	
Residue on Ignition		Report result	USP <281>
Inorganic Impurities	Cd	NMT 0.5 ppm	ICP-MS (USP <233>)
	Pb	NMT 0.5 ppm	
	As	NMT 1.5 ppm	
	Hg	NMT 0.10 ppm	ICP-MS
	Cu	NMT 100 ppm	
	Al	NMT 2000 ppm	
	Other heavy metals	Report each NLT 1 ppm	
Particle Size	D_{90} µm	NMT 30 µm	Laser diffraction
	D_{50} µm	NMT 20 µm	

CFU = colony forming units; HPLC = high performance liquid chromatography; GC = gas chromatography; ICP-MS = Inductively coupled plasma mass spectrometry; NLT = not less than; NMT = not more than; USP = United States Pharmacopeia

2.4.2 Microbial Specifications

The microbiological specifications for urolithin A are presented in Table 2.4.2-1.

Table 2.4.2-1 Microbiological Specifications for Urolithin A

Specification Parameter		Specification	Method
Microbial Enumeration Test	Total aerobic microbial count (TAMC)	NMT 10^3 CFU/g	USP <61>
	Total combined yeast & mold count (TYMC)	NMT 10^2 CFU/g	
Test for Microorganisms	*Escherichia coli*	Absent in 1g	USP <62>

CFU = colony forming units; NMT = not more than; USP = United States Pharmacopeia

2.4.3 Batch Analyses

Analysis of a total of 7 batches, 4 batches of urolithin A synthesized with Process 1 and 3 batches of urolithin A synthesized with Process 2, demonstrates that the manufacturing process as described in Section 2.3 produces a consistent product that meets specifications. A summary of the chemical analysis for the 7 batches of urolithin A is presented in Table 2.4.3-1.

Table 2.4.3-1 Summary of the Chemical Product Analysis for 7 Batches of Urolithin A

Parameter		Specification		Synthesis Process 1				Synthesis Process 2		
				Batch Number 071325A	**Batch Number 121508A**	**Batch Number 121511A**	**Batch Number 121516A**	**Batch Number 081708C**	**Batch Number 081717C**	**Batch Number 081722C**
Appearance		Solid / Powder		Solid	Solid	Solid	Solid	Solid	Solid	Solid
Color		Beige to Yellow		Beige	Beige	Beige	Beige	Yellow	Yellow	Yellow
Identity (FT-IR)		Conforms to reference		Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference
Purity (HPLC area %)		NLT 97.0 %		99.7%	99.6%	99.6%	99.6%	99.9%	99.8%	99.8%
Individual Organic Impurities	Intermediate A	NMT 0.5%		0.33%*	<0.10%	0.13%	< 0.10%	-**	-**	-**
	AZX1	NMT 0.4%			0.18%	0.17%	0.17%	0.09%	0.13%	0.11%
	Any other individual impurity	NMT 0.3%	(RRT 1.06)	≤ 0.1%	0.13%	≤ 0.1%	0.10%	≤ 0.1%	≤ 0.1%	0.05%
			(RRT 1.11-1.12)	≤ 0.1%	0.10%	0.10%	0.11%	0.06%	0.08%	0.09%
Residual Solvents	Methanol	NMT 3000 ppm		ND	332 ppm	253 ppm	ND	NA	NA	NA
	TBME	NMT 1,000 ppm		ND	ND	ND	ND	NA	NA	NA
	Toluene	NMT 890 ppm		185 ppm	641 ppm	202 pm	88 ppm	NA	NA	NA
	DMSO	NMT 5000 ppm		414 ppm	3390 ppm	3810 ppm	4102 ppm	NA	NA	NA
	Acetic Acid	NMT 5000 ppm		1271 ppm	3575 ppm	3750 ppm	3980 ppm	500 ppm	500 ppm	ND
Inorganic Impurities	Cd	NMT 0.5 ppm		< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	0.11 ppm	< 0.10 ppm	< 0.10 ppm
	Pb	NMT 0.5 ppm		< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm
	As	NMT 1.5 ppm		< 0.5 ppm	< 0.5 ppm	< 0.5 ppm	< 0.5 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm
	Hg	NMT 0.10 ppm		< 0.10 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm
	Cu	NMT 100 ppm		< 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm	< 1 ppm	1 ppm	1 ppm
	Al	NMT 2000 ppm		21 ppm	37 ppm	1100 ppm	930 ppm	5 ppm	9 ppm	3 ppm
	Other heavy metals	Report each NLT 1 ppm		Cr, Mg, Ni, Zn: 1 ppm; Fe: 40 ppm; Mo: 4 ppm	Fe: 3 ppm; Ti: 3 ppm; Zn: 1 ppm	Cr, Ni: 1 ppm; Fe: 7 ppm; Ti: 3 ppm; Zn: 2 ppm	Fe: 6 ppm; Ni, Zn: 1 ppm Ti: 4 ppm;	Ag, Bi, Sb, Sn: < 1 ppm; Mo: 5 ppm	Ag, Bi, Mo, Sb, Sn: < 1 ppm	Ag, Bi, Mo, Sb, Sn: < 1 ppm

* Measured value is a combination of the intermediate A and impurity AZX1 ** Intermediate A is only present in Process 1

RRT = relative retention time, ND = Not detected. Threshold for detection: Methanol: 100 ppm; TBME: 85 ppm; Acetic Acid: 230 ppm

NA = Not applicable. These solvents were not used in Process 2.

FT-IR = Fourier Transform Infrared Spectroscopy; HPLC = high performance liquid chromatography; ICP-MS = Inductively coupled plasma mass spectrometry; K.F. = Karl Fischer; NLT = not less than; NMT = not more than; TBME = tert-butyl methyl ether; USP = United States Pharmacopeia

2.4.4 Microbiological Analysis

Analysis of representative batches of urolithin A demonstrates that the product meets the microbiological specifications outlined in Section 2.4.2. A summary of the microbiological analysis for the 7 batches of urolithin A is presented in Table 2.4.4-1.

Table 2.4.4-1 Summary of the Microbiological Product Analysis for 7 Batches of Urolithin A

Parameter		Specification	Synthesis Process 1				Synthesis Process 2		
			Batch Number 071325A	Batch Number 121508A	Batch Number 121511A	Batch Number 121516A	Batch Number 081708C	Batch Number 081717C	Batch Number 081722C
Microbial Enumeration Test	Total aerobic microbial count (TAMC)	NMT 10^3 CFU/g	0 CFU/g	0 CFU/g	0 CFU/g	0 CFU/g	< 50 CFU/g	< 50 CFU/g	< 50 CFU/g
	Total combined yeast & mold count (TYMC)	NMT 10^2 CFU/g	3 CFU/g	0 CFU/g	0 CFU/g	0 CFU/g	< 50 CFU/g	< 50 CFU/g	< 50 CFU/g
Test for Microorganisms	*Escherichia coli*	Absent in 1g	Absent	Absent	Absent	Absent	Absent	Absent	Absent

CFU = colony forming units; NMT = not more than

2.4.5 Micronization of Urolithin A

Urolithin A was micronized to provide a more homogenous compound. Four independent micronizations were carried out, demonstrating Amazentis' ability to consistently meet the specifications for micronization. Micronized urolithin A was used in all the subsequent *in vivo* pre-clinical toxicology studies reported in this dossier. A summary of the particle size analysis for the 4 different micronizations of urolithin A is presented in Table 2.4.5-1. In addition, analysis of heavy metals, as well as microbiological and urolithin A purity analysis was performed on these batches following micronization.

Table 2.4.5-1 Summary of the Particle Size Analysis for 4 Batches of Micronized Urolithin A

Parameter	Specification		Micronized Batch			
			Batch Number 071325A-10814B	Batch Number 071325A-10115B	Batch Number 071325A-10616B	Batch Number 021604A-10316B
Particle Size Dimensions	D_{90} µm	NMT 30 µm	12.91 µm	13.45 µm	11.60 µm	11.82 µm
	D_{50} µm	NMT 20 µm	6.66 µm	7.08 µm	3.69 µm	4.57 µm

NMT = not more than

Table 2.4.5-2 Summary of the Chemical Product Analysis for 4 Batches of Micronized Urolithin A

Parameter		Specification	Manufacturing Batch			
			Batch Number 071325A-10814B	**Batch Number 071325A-10115B**	**Batch Number 071325A-10616B**	**Batch Number 021604A-10316B**
Appearance		Solid / Powder	Solid	Solid	Solid	Solid
Color		Beige to Yellow	Beige	Beige	Beige	Beige
Identity (FT-IR)		Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference	Conforms to reference
Purity (HPLC area %)		NLT 97.0 %	99.7%	99.7%	99.5%	99.6%
Individual Organic Impurities	Intermediate A	NMT 0.5%	0.28%*	0.27%*	0.24%	0.16%
	AZX1	NMT 0.4%			0.20%	0.17%
	Any other individual impurity	NMT 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%
Water Content (K.F.)		Report result	0.31%	0.35%	0.21%	0.31%
Residual Solvents	Methanol	NMT 3000 ppm	ND	ND	ND	177 ppm
	TBME	NMT 1000 ppm	ND	ND	ND	ND
	Toluene	NMT 890 ppm	153 ppm	155 ppm	182 pm	292 ppm
	DMSO	NMT 5000 ppm	431 ppm	444 ppm	475 ppm	3417 ppm
	Acetic Acid	NMT 5000 ppm	1237 ppm	1212 ppm	1459 ppm	3276 ppm
Residue on Ignition		Report result	< 0.1%	< 0.1%	0.1%	0.2%
Inorganic Impurities	Cd	NMT 0.5 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm	< 0.1 ppm
	Pb	NMT 0.5 ppm	0.1 ppm	0.1 ppm	< 0.1 ppm	< 0.1 ppm
	Hg	NMT 0.10 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm	< 0.10 ppm
	As	NMT 1.5 ppm	< 0.5 ppm	< 0.5 ppm	< 0.5 ppm	< 0.5 ppm
	Other heavy metals (ICP-MS screening)	Report each NLT 1 ppm	Cr: 1 ppm; Fe: 38 ppm; Mg: 1 ppm; Mo: 4 ppm; Ni: 1 ppm; Ti: 1 ppm; Zn: 1 ppm	Cr: 1 ppm; Fe: 38 ppm; Mg: 1 ppm; Mo: 4 ppm; Ni: 1 ppm; Ti: 1 ppm; Zn: 1 ppm	Cr: 1 ppm; Fe: 40 ppm; Mo: 4 ppm, Ni: 1 ppm; Ti: 1 ppm; Zn: 1 ppm	Fe: 6 ppm; Ni: 1 ppm Ti: 3 ppm; Zn: 1 ppm
	Cu	NMT 100 ppm	Not detected (< 1 ppm)	Not detected (< 1 ppm)	0.4 ppm	0.2 ppm
	Al	NMT 2000 ppm	31 ppm	31 ppm	34 ppm	770 ppm

ND = Not detected. Threshold for detection: Methanol: 100 ppm; TBME: 85 ppm. FT-IR = Fourier Transform Infrared Spectroscopy; HPLC = high performance liquid chromatography; ICP-MS = Inductively coupled plasma mass spectrometry; K.F. = Karl Fischer; NLT = not less than; NMT = not more than; TBME = tert-butyl methyl ether; USP = United States Pharmacopeia. * Measured value is a combination of the Intermediate A and AZX1

2.4.6 Stability

The stability of urolithin A was evaluated by Amazentis. Urolithin A (was evaluated under both real-time (25 ± 2°C, 60 ± 5% relative humidity) and accelerated storage conditions (40 ± 2°C, 75 ± 5% relative humidity) following ICH Q1A guidelines. The analytical stability data are presented in Table 2.4.6-1 for urolithin A (Batch 071325A-10814B). These data demonstrate that the ingredient is stable for a period of 3 years at room temperature.

Table 2.4.6-1 Stability of Urolithin A

		Storage Time	Initial	3 months		6 months		9 months	12 months	18 months	24 months	36 months
Attribute	**Method**	**Acceptance Limit**	**25 °C**	**25 °C**	**40 ° C**	**25 °C**	**40 °C**	**25 °C**	**25 °C**	**25 °C**	**25 °C**	**25 °C**
Appearance	Visual inspection	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Solid
Color	Visual inspection	Beige to Yellow	Beige	Beige (with yellow touch)	Beige (with yellow touch)	Beige	Beige	Beige	Beige	Beige	Beige	Beige
Purity (HPLC area%)	HPLC	≥ 97.0 %	99.7%	99.8%	99.8%	99.6%	99.6%	99.5%	99.5%	99.5%	99.4%	99.9%
Individual Organic Impurities: Intermediate A	HPLC	NMT 0.5%	0.22%*	0.20%*	0.21%*	0.26%*	0.28%*	0.26%*	0.25%*	0.26%*	0.24%*	< 0.10%*
Individual Organic Impurities: AZX1	HPLC	NMT 0.4%										
Individual Organic Impurities: Any other individual impurity	HPLC	NMT 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%	None ≥ 0.3%
Water content	Karl Fischer	Report	0.30%	0.28%	0.24%	0.31%	028%	0.29%	0.21%	0.29%	0.27%	0.28%
Microbial Enumeration Test	USP <61>/Ph Eur. 2.6.12 harmonized approach	TAMC ≤ 10^3 CFU/g	0 CFU/g				0 CFU/g		0 CFU/g		0 CFU/g	0 CFU/g
Microbial Enumeration Test	USP <61>/Ph Eur. 2.6.12 harmonized approach	TYMC ≤ 10^2 CFU/g	0 CFU/g				0 CFU/g		0 CFU/g		0 CFU/g	0 CFU/g
Particle size distribution	Laser Diffraction	D_{90} = NMT 30 µm	10.35 µm				17.53 µm		15.11 µm		10.61 µm	13.74 µm
Particle size distribution	Laser Diffraction	D_{50} = NMT 20 µm	5.063 µm				6.880 µm		7.475 µm		5.600 µm	6.788 µm

* Measured value is a combination of the intermediate A and AZX1

CFU = colony forming units; HPLC = high performance liquid chromatography; TAMC = Total aerobic microbial count; TYMC = Total combined yeast & mold count; USP = United States Pharmacopeia; Ph. Eur. = European Pharmacopeia

Part 3. §170.235 Dietary Exposure

3.1 History of Use in Food

Although urolithins, including urolithin A, are not directly consumed from dietary sources, a history of human exposure to urolithin compounds resulting from the digestion of food rich in ellagitannins and ellagic acid exists (Tómas-Barberán *et al.*, 2014). Dietary ellagitannins are converted into ellagic acid by the gut microbiota in the upper portion of the gastrointestinal tract and are further metabolized by the microflora in the large intestine to urolithins. Ellagitannins have poor bioavailability following oral consumption, even after consumption of very high amounts, indicating that dietary ellagitannins are broken down into other metabolites in the body (Garcia-Muñoz and Vaillant, 2014). Among these downstream metabolites, the urolithins produced by the gut microflora are absorbed, conjugated in the liver and subsequently excreted in the urine. Findings from several studies indicate that unmetabolized urolithin A, urolithin A glucuronide and urolithin A sulfate are the predominant forms of the compound detected in urine following exposure to ellagitannins (Tómas-Barberán *et al*., 2014).

Estimates of dietary intake of ellagitannins or ellagic acid are limited. In the United States, intake of ellagic acid was estimated to range from 5.3 mg/day among adults with low consumption of fruits and vegetables – the vast majority of adults – to 27.6 mg/day among adults meeting recommended levels of fruit and vegetable servings (Murphy *et al.,* 2012). These estimates were based upon publicly available data on the ellagic acid content in foods and nationally representative food consumption data (US National Health and Nutrition Examination Survey 2003-2006). The ellagic acid data reflect all sources of ellagic acid in foods reported consumed (*i.e.*, free ellagic acid, ellagitannins, and other sources).

Turchado *et al.* (2012) evaluated the fraction of dietary ellagitannins that are converted to urolithins and subsequently absorbed and excreted following consumption of strawberries and strawberry puree and reported approximately 57.5% ± 50% of the ingested ellagic acid is converted to urolithins. These data can therefore be used to provide an estimate of endogenous exposure to urolithins, including urolithin A. Assuming that all of the urolithin metabolites are urolithin A (present as the aglycone, urolithin A glucuronide or urolithin A sulfate), endogenous exposure to urolithin A from a diet, providing a range of 5.3 to 27.6 mg ellagic acid/day, would be in the range of 3.0 to 15.9 mg/day. The background intake level is insignificant when compared to the estimated intakes resulting from the proposed food uses currently proposed by Amazentis.

3.2 Estimated Intake of Urolithin A from Proposed Use in Food

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

The estimates for the intake of urolithin A were generated using the maximum use level indicated for each intended food-use, as presented in Table 1.3-1, together with food consumption data available from the 2013-2014 NHANES dataset (CDC, 2015, 2016; USDA, 2016). The full methodology and results are presented in the attached Intake Assessment Report, Appendix 1. For the sake of completeness, intakes for all population groups (*i.e.*, including children under 12 years) have been assessed and are presented in the attached report (see Appendix 1). However, the figures presented herein are representative of the population groups likely to consume the target food and beverages containing urolithin A, and as such, are most relevant from a risk assessment perspective.

A summary of the estimated daily intake of urolithin A from proposed food-uses among the population groups of interest, *i.e.,* individuals aged 12 years and older, is provided in Table 3.2-1 on an absolute basis (mg/person/day), and in Table 3.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was relatively low among all age groups evaluated in the current intake assessment; ranging from 10.6% (teenagers) to 21.6% (in older female adults and in female elderly adults) of the population groups consisted of consumers of those food products in which urolithin A is currently proposed for use (Table 1.3-1). The consumer-only intakes are more applicable to the assessment of safety as they are more likely to represent exposure in the target populations. Consequently, only the consumer-only intake results will be discussed in detail.

On an absolute basis, for the total population excluding all children (i.e., a total population age of ≥12 years), the mean and 90th percentile consumer-only intakes of urolithin A were determined to be 1,183 and 2,421 mg/person/day, respectively. Of the individual population groups, adults were determined to have the greatest mean consumer-only intakes of urolithin A on an absolute basis, at 1,528 mg/person/day, while teenagers had the greatest 90th percentile consumer-only intakes of 3,494 mg/person/day. Female elderly adults had the lowest mean and 90th percentile consumer-only intakes of 773 and 1,341 mg/person/day, respectively (Table 3.2-1).

Table 3.2-1 Summary of the Estimated Daily Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Teenagers	12 to 19	140	173	10.6	113	1,320	3,494
Adults	20 to 39	263	632	17.2	232	1,528	2,688
Older Female Adults	40 to 64	205	723	21.6	185	949	1,884
Older Male Adults	40 to 64	182	502	16.3	115	1,122	3,226
Female Elderly Adults	≥65	167	529	21.6	99	773	1,341
Male Elderly Adults	≥65	157	322*	14.1	60	1,118	2,381*
Total Population	≥12	204	538	17.2	804	1,183	2,421

NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements

On a body weight basis, the total population mean and 90th percentile consumer-only intakes were determined, excluding all children under 12 years. Among the total population excluding all children, the mean and 90th percentile consumer-only intakes of urolithin A were determined to be 15.5 and 34.1 mg/kg body weight/day, respectively. Among the individual population groups, adults were determined to have the highest mean consumer-only intakes of any population group of 19.3 mg/kg body weight/day, and teenagers had the highest 90th percentile intakes at 49.0 mg/kg body weight/day. Female elderly adults had the lowest mean and statistically reliable 90th percentile consumer-only intakes of 11.7 and 24.4 mg/kg body weight/day, respectively (Table 3.2-2).

Table 3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Teenagers	12 to 19	2.0	2.6	10.7	113	19.0	49.0
Adults	20 to 39	3.3	7.8	17.2	232	19.3	43.6
Older Female Adults	40 to 64	2.9	9.2	21.6	185	13.2	27.9
Older Male Adults	40 to 64	2.2	6.3	16.3	115	13.4	34.1
Female Elderly Adults	≥65	2.5	7.6	21.6	98	11.7	24.4
Male Elderly Adults	≥65	1.6	2.7*	11.9	59	13.6	19.1*
Total Population	≥12	2.7	6.9	17.1	802	15.5	34.1

bw = body weight; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Consumption data from the 2013-2014 NHANES dataset and information pertaining to the individual proposed food-uses of urolithin A were used to estimate the per capita and consumer-only intakes for specific demographic groups and for the total U.S. population. While younger populations were identified as the groups having higher exposures to urolithin A on a body weight basis (see Appendix 1), it should be noted that products containing urolithin A will not be targeted towards these populations. Furthermore, conservative assumptions have been included in the present assessment, which render exposure estimates that may be considered suitably conservative. For example, it was assumed that all food products within a food category would contain the ingredient at the maximum specified level of use. In reality, the levels of urolithin A added to specific foods will vary and are unlikely to have 100% market penetration.

The GRAS panel convened by Amazentis addressed the intake data and offered the conclusion, below.

"Because there are not a wide variety of target foods that could contain urolithin A, and given the premium pricing associated with food products fortified with urolithin A, it would be reasonable to assume that most consumers would eat 1 or 2 servings a day of such foods, depending on the level of urolithin A in the food. As such, ***an aggregated intake of 1,000 mg/person/day or 16.67 mg/kg bw/day is a more accurate estimate of the actual urolithin A intake."***[1]

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of urolithin A by the total U.S. population, excluding children under 12 years, from all proposed food and beverage-uses in the U.S. were estimated to be 1,183 mg/person/day (15.5 mg/kg body weight/day) and 2,421 mg/person/day (34.1 mg/kg body weight/day), respectively. Adults were determined to have the greatest mean consumer-only intakes of urolithin A on an absolute basis, at 1,528 mg/person/day (19.3 mg/kg body weight/day), while teenagers had the greatest 90th percentile consumer-only intakes of 3,494 mg/person/day (49.0 mg/kg body weight/day). The GRAS panel viewed 1,000 mg/person/day or 16.67 mg/kg bw/day as a more accurate estimate of the actual urolithin A intake.

[1] See Expert Panel Report, Appendix 2, Intended Use and Estimated Exposure, p. 3

Part 4. §170.240 Self-Limiting Levels of Use

No known self-limiting levels of use are associated with Amazentis SA's urolithin A.

Part 5. §170.245 Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. §170.250 Narrative and Safety Information

6.1 Introduction

Ellagitannins and ellagic acid are dietary polyphenols primarily found in a variety of fruits and berries (pomegranate, blackberries, camu-camu, strawberries, raspberries), nuts (walnuts, hazelnuts, acorns, chestnuts, pecans), muscadine grapes and oak-aged wines and spirits (Gonzalez-Sarrias *et al*., 2010; Espin *et al*., 2013). *In vitro* studies have reported that ellagitannins are particularly potent for vascular health (Larrosa *et al.*, 2010). However, these effects hardly translate in animal models, mostly because intestinal absorption of phenolics is highly variable, often slow, and largely incomplete (Gee and Johnson, 2001; Scalbert *et al.,* 2002). In fact, ellagitannins remain unabsorbed in the gut lumen and accumulate in the colon, where they can interact with complex intestinal bacteria. Residual ellagitannins are metabolized first into ellagic acid by microbial enzymes. Tannin-hydrolases cleave the galloyl-glucose residue from the hexahydroxydiphenoyl (HHDP) moiety of the ellagitannin and lactonases catalyze lactonization. Additional microbial enzymes catalyze the opening of the ellagic acid lactone ring and progressive dihydroxylation, resulting in a family of dibenzopyranone metabolites termed urolithins (Garcia-Muñoz and Vaillant, 2014). Urolithins and their conjugates are readily absorbed, as evidenced by their presence in the plasma and urine following consumption of ellagitannins (Doyle and Griffiths, 1980; Espin *et al.*, 2007; Mosele *et al.*, 2015).

The activity of urolithin A has been demonstrated in animal models. Ryu *et al.* (2016) characterized the biological effects of urolithins on lifespan and mitochondria in *Caenorhabditis elegans.* Feeding 50 µM concentrations of urolithin A, B, C, or D to *C. elegans* from eggs until death extended lifespan by 45.4, 36.6, 36.0, and 19.0%, respectively, as compared to treatment with the vehicle (1% DMSO). No such effect was seen with ellagic acid. The effects of urolithin A were dose-dependent over concentrations ranging from 10 to 50 µM, with a significant delay in mortality observed at advanced ages ($p \leq 0.001$). Young worms (day 1 of adulthood) treated with urolithin A presented lower mitochondrial content, when compared to control, but were able to maintain their respiratory capacity. Using gene expression data, the authors demonstrated that this effect was due to induction of selective autophagy of mitochondria (mitophagy) by urolithin A. The authors further demonstrated that as worms aged (8 to 10 days old), the long-term exposure to urolithin A triggered mitochondrial biogenesis, so that they achieved an equivalent mitochondrial content, but with an increased mitochondrial respiratory capacity when compared to untreated worms.

Likewise, urolithin A was shown to stimulate autophagy and mitophagy in mammalian muscle (C2C12 myoblasts) and intestinal cells (Mode-K intestinal cells). Administration of 50 mg/kg/day of urolithin A to 16-month-old male C57BL6J mice for 8 months prevented age-related muscle decline as measured by

grip strength and spontaneous exercise; no effects on body weight gain or fat and lean body mass were seen. In a second mouse study, urolithin A supplementation for 6 weeks resulted in a 42% increase in running endurance in aged male C57BL/6J mice (22.5 months old). As in the previous study, no effects on body weight or body composition were observed. Increases in muscle function were also observed in young male (5.5 week old) Wistar rats fed commercial diets containing urolithin A at a concentration of 25 mg/kg/day for a period of 7.5 weeks (until 13 weeks old) as measured by an average 65% greater running capacity than the control group.

The metabolism of ellagitannins and ellagic acid into urolithins is heterogenous across individuals, with individuals showing high or low conversion rates, or no conversion. Thus, the benefits of urolithin A derived from the dietary consumption of fruits and nuts are not available to all. In several human intervention trials, 25% to 80% of volunteers were found to produce only urolithin A conjugates. 10% to 50% of subjects produced isourolithin A and/or urolithin B, in addition to urolithin A. No urolithin metabolites were produced by the remaining 5% to 25% of subjects (Tómas-Barberán *et al.,* 2014). These authors also reported a higher risk of chronic illness among individuals producing isourolithin A and urolithin B compared to urolithin A producers. Several bacterial strains derived from the gut have been demonstrated to be capable of converting ellagic acids and ellagitannins into different forms of urolithins. The bacterial species, *Gordonibacter urolithinfaciens, Gordonibacter pamelaeae,* and *Ellagibacter isourolithinifaciens*, all members of the *Eggerthellaceae* family have been shown to be capable of transforming ellagic acid into urolithins M5, M6 and urolithin C, with *Ellagibacter isourolithinifaciens* also producing isourolithin A (Beltran et al., 2018; Selma et al., 2014; Selma et al., 2017). Conversion of ellagic acid into urolithins A and B has also been reported for the bacterial strain *Bifidobacterium pseudocatenulatum INIA P815* (Gaya et al., 2018).

Amazentis intends to incorporate urolithin A directly into foods as a substance offering nutritive value, based on its role as a bioactive metabolite of ellagitannins, its demonstrated ability to support mitochondrial function and metabolism in model organisms, and the potential to similarly maintain energy metabolism when consumed by humans.

The safety of urolithin A is supported by a series of published product specific studies conducted on Amazentis' urolithin A (Heilman *et al*., 2017). The absorption, distribution, metabolism, and excretion of ingested urolithin A and related compounds are reviewed in Section 6.2. Toxicological studies with urolithin A, which include a subacute, subchronic and genotoxicity studies, are presented in Section 6.3. In addition, Amazentis conducted comprehensive searches of the published scientific literature through August of 2017. The search was conducted on databases including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and Toxfile®. Although data related to the physiological role of urolithins were identified and were discussed in this section, no additional toxicity studies on urolithin A were identified. However, as ellagitannins are metabolized to urolithin A, safety reports for ellagitannins or ellagitannin-containing food products, which can be assumed to result in some lower level of *in vivo* exposure to urolithins following their formation in the gastrointestinal tract, are considered supportive of the safety of urolithin A. The results of these studies are summarized in Section 6.4. The safety and tolerability of urolithin A is further corroborated by a single-center, double-blind, placebo-controlled, randomized study that has been published in abstract form. These studies are summarized in Section 6.5.

6.2 Metabolic Fate

6.2.1 Absorption, Distribution, Metabolism, and Excretion (ADME) in Animals

Amazentis has conducted a Good Laboratory Practice (GLP)-compliant absorption, distribution, metabolism, and excretion (ADME) study to examine the absorption, distribution, metabolism and excretion of ingested urolithin A in accordance with Organization for Economic Co-operation and Development (OECD) Guideline 417 (Heilman *et al.*, 2017). The absorption, distribution, metabolism and excretion of ingested urolithin A was followed by dosing Wistar rats with ^{14}C labeled urolithin A. The study design is summarized in Table 6.2.1-1.

Table 6.2.1-1 Urolithin A Rat ADME Study Design

Group	Number of Animals		Urolithin A Dose (mg/kg bw)	Time of Euthanasia after Urolithin A Administration (h)
	Male	Female		
1: Oral, mass-balance	4	4	1,000	72
2: Oral, kinetic	8	8	1,000	24
3: Intravenous, mass-balance	4	4	2.5	72
4: Intravenous, kinetic	8	8	2.5	24
5: Oral, Tissue-distribution	4	4	1,000	0.5
	4	4		2
	4	4		8
	4	4		24
6: Intravenous, vehicle only	2	2	0	24

ADME = absorption, distribution, metabolism, and excretion; bw = body weight

As shown in the Table 6.2.1-1 above, the study included 2 groups of 4 males and 4 females for the mass-balance (ADME), 2 groups of 8 males and 8 females to determine the radioactivity in blood and plasma, 1 group of 16 males and 16 females to determine the tissue distribution at specified time points and 1 group of 2 males and 2 females to obtain a blank matrix for metabolite identification purposes. Groups 1, 2, and 5 were dosed orally at a single dose of 1,000 mg/kg, groups 3 and 4 were dosed intravenously at a single dose of 2.5 mg/kg and Group 6 was dosed intravenously with blank vehicle for metabolite identification purposes. In the mass-balance groups, urine was collected in -24h to 0h (Group 1 only), 0h to 6h, 6h to 12h, 12h to 24h, 24h to 48h and 48 to 72-hour intervals. Feces was collected in -24h to 0h (Group 1 only), 0h to 24h and 24 to 48 hours intervals.

Animals were euthanized at the end of the collection period and cage washings were collected. The carcass was stored for total [^{14}C] analysis. Total radioactivity in urine, feces, cage washings, tissues and organs was determined. In the pharmacokinetic groups, blood was sampled alternately from 4 rats per time point at 0.083, 0.167, 0.5, 1, 2, 5, 8, and 24 hours after dosing. Total radioactivity and [^{14}C] urolithin A equivalent concentrations were determined. In the tissue-distribution group, animals were euthanized 0.5, 2, 8, and 24 hours after dose administration, and several tissues and organs were collected. Total radioactivity and [^{14}C] urolithin A equivalent concentrations were determined in tissues and organs.

Following a single oral administration, the majority of radioactivity was excreted *via* the feces; at 72 hours, approximately 115% and 121% of the administered dose was excreted in the feces of males and females, respectively. The high levels of excretion in the feces corresponded with the tissue distribution findings, which demonstrated the majority of the compound to be located in the gastrointestinal tract.

In contrast, urinary excretion only accounted for 1.3% of the administered dose in males and in females after 72 hours. Plasma concentration of radiolabeled urolithin A (aglycone and metabolites) peaked around 3 hours and then again around 6 or 7 hours.

In contrast, intravenous administration resulted in the urine as the major route of excretion, accounting for 74% of the administered dose in males and 61% in females after 72 hours. Fecal excretion accounted for an additional 16% in males and 24% in females after 72 hours. These results, and the calculated fractional absorption of <2.2% (ratio of the % excreted in urine after oral administration to the % after intravenous administration) demonstrate low oral absorption.

Total recovery of radioactivity following single oral administration was 116% and 122% in males and females, respectively, and 93% and 90% in males and females, following intravenous administration, respectively. Although total recovery following oral administration was somewhat higher than 100%, no systemic factor in the analysis and no particular bias could be identified to account for this elevation in recovered material. After 72 hours, the amount of radioactivity in the blood, carcass and tissues ranged from 0.016% to 1.37%, indicating effective elimination for both exposure routes and no substantial accumulation of test article or metabolites in the body.

Tissue distribution demonstrated that the majority of the radioactivity was detected in the gastrointestinal tract. Importantly, radioactivity was detected corresponding to a radiolabeled urolithin A concentration in the bone marrow of 6.48 (± 2.87) ppm in males at the 2-hour collection time point, and 6.33 (± 2.67) ppm in females at the 2-hour collection time point, confirming that the bone marrow is exposed to urolithin A following oral gavage. These data confirm the exposure of the bone marrow to urolithin A in the *in vivo* micronucleus assays that are described in Sections 6.3.2 and 6.3.3.3.

In the labeled metabolite profiles of the urine samples, 4 peaks (M1, M2, M3, and urolithin A) were resolved. The major urinary peak M2 represented glucuronidated urolithin A. The minor urinary peak M3, identified as sulfated urolithin A, was quantifiable only for the intravenous (i.v.) group and detected but below the threshold of quantification in the orally dosed group. The minor peak M1 represented <3% of the total radioactivity in the urine of the i.v. dosed group and was not quantifiable in the orally dosed group. The presence of this minor M1 peak in the urine of both the i.v. and orally dosed groups, however, was confirmed by follow-up mass spectrometry (MS) analysis. MS screening at the retention time of the radioactive peak M1 identified 3 m/z values: m/z 245.044, m/z 421.077 and m/z 477.103, corresponding to 3 possible metabolites. The compound with *m/z* 245.044 is likely the result of the hydroxylation of urolithin A, *m/z* 421.077 is likely the result of oxidation and glucuronidation of urolithin A. The fragmentation of *m/z* 477.103 did not allow for the proposition of an exact structure, but based on fragmentation, it was deduced that this metabolite of urolithin A contains at least a glucuronide.

After oral administration of the radiolabeled urolithin A, the measured radioactivity levels in blood and plasma were low, with the majority of the measured values being within twice the background value for the pharmacokinetics arm of the ADME studies. This is consistent with the distribution data, which demonstrated low absorption and the majority of compound being present as unmetabolized parent compound in the gastrointestinal tract or feces. This low level of radioactivity in the plasma did not allow for direct metabolite identification in the plasma. To confirm the presence in plasma of the major metabolites identified in the urine (glucuronide and sulfated forms of urolithin A), bioanalytical analysis was performed on the plasma samples with standards for the glucuronidated, sulfated and aglycone forms of urolithin A. These results demonstrated the presence of the glucuronide, sulfated and aglycone

forms of urolithin A, confirming glucuronide and sulfates as the major metabolites of urolithin A in both plasma and urine (Heilman *et al.*, 2017).

6.3 Toxicological Studies

Amazentis conducted a series of safety studies on its urolithin A product. These studies included a 28-day subacute toxicity study, a 90-day subchronic toxicity study, and a battery of genotoxicity studies (*i.e.*, bacterial reverse mutation test, *in vitro* micronucleus test, and an *in vivo* micronucleus test). In addition, a repeat of the *in vivo* micronucleus assay was performed within the 90-day subchronic study. All *in* vivo studies, including the 28-day, 90-day, ADME, and in vivo micronucleus assays were performed with micronized urolithin A. Micronization of urolithin A was performed to the specifications in Section 2.4.5.

6.3.1 Subacute Studies

Amazentis has conducted a GLP-compliant 28-day dietary study of the oral toxicity of urolithin A according to OECD Guideline 407 (Heilman *et al.,* 2017). Urolithin A was administered *ad libitum* by feed admixture to SPF-bred Wistar rats (5 animals/sex/group) at a constant concentration of 0.175, 1.75, and 5.0% of the diet for a period of 28 days. These doses corresponded to average intakes of 134.7, 1,435.6, and 4,164.9 mg/kg body weight/day, respectively, in males and 149.0, 1,573.2, and 4,705.5 mg/kg body weight/day, respectively, in females. A control group was treated similarly with untreated feed only.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during the acclimatization and treatment periods. Functional observational battery, locomotor activity and grip strength were performed during week 4. At the end of the dosing period, blood samples were collected for hematology and plasma chemistry analyses. Urine samples were collected for urinalyses. All animals were sacrificed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues from all control and high-dose animals, and from all gross lesions observed in all animals.

No toxicologically significant changes were seen in any of these parameters. On this basis, the no-observed-effect level (NOEL) was considered to be 4,165 mg/kg/day for males and 4,705 mg/kg body weight/day for females, equivalent to the high dose level of 5%. These results were used to determine the doses for the 90-day oral toxicity study.

6.3.2 Subchronic Toxicity Study

Amazentis conducted a GLP-compliant 90-day dietary study of the oral toxicity of urolithin A according to OECD Guideline 408, as well as the US Food and Drug Administration, Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, Chapter IV.C.4.a, “Subchronic Toxicity Studies with Rodents”, November 2003 (OECD, 1998; U.S. FDA, 2003) (Heilman *et al*., 2017). Urolithin A was administered in the diet to Wistar rats for at least 90 days according to the study design shown in Table 6.3.2-1.

Table 6.3.2-1 Subchronic Toxicity Study Design

Group	Treatment	Level (ppm)	Number of Animals			
			Main Study		Recovery Phase	
			Male	Female	Male	Female
1	Control	0	20	20	5	5
2	Urolithin A	12,500	20	20	0	0
3	Urolithin A	25,000	20	20	0	0
4	Urolithin A	50,000	20	20	5	5

Body weights and food consumption was measured weekly. Ophthalmic examinations were conducted prior to study start, and during week 12. Animals were monitored for morbidity and mortality at least twice daily during the main study, and at least once daily during the acclimatization and recovery phases. Detailed observations in the home cage and open field were conducted weekly with particular attention to physical condition and possible indicators of neurotoxicity including such signs as convulsions, tremor, and abnormal gait or behavior. Any findings present were recorded with a grade of slight, moderate or marked. Grip strength and sensory reactivity (blinded) were assessed during week 12 of the study on 15/20 of the main study animals and all recovery phase animals.

Assessments in the functional observational battery included approach response, pinna reflex, auditory startle response, tail pinch response, and grip strength. Motor activity assessment was also conducted during week 12 of treatment in clear polycarbonate cages by beam-break method for six 10-minute intervals (60 minutes total). Testing was staggered to accommodate caging, but was conducted in a method that balanced animal numbers across groups on each day of assessment.

Similar to known dietary phytoestrogens (*e.g.*, genistein, daidzein, resveratrol, and enterolactone), molecular models suggest that urolithins could display estrogenic and/or antiestrogenic activity, and effects have such reports have been reported *in vitro* (Larrosa *et al.,* 2006). As a result, estrous cycles and estrus were assessed by daily smears taken for 14 days during week 10 and 11 of the main study treatment phase and for 7 days during week 3 of the recovery phase. Duration and regularity of cycle during the treatment period was unaffected by the treatment and all recovery animals demonstrated estrus during the recovery phase. Sperm motility, morphology and count were also assessed. Blood samples were collected from the bilingual vein following overnight fast on study weeks 2 and 7, at week 13. In addition, animals in the recovery phase group had blood collected during recovery week 4. Urine was collected in metabolic cages and assessed for the same parameters measured in the 28-day study. Rats were sacrificed by CO_2 asphyxiation and exsanguination. Necropsy was conducted and all gross lesions of animals sacrificed at necropsy or sacrificed *in extremis* were analyzed. Organ weights were measured and recorded for all organs and histopathological examination was conducted for all dose levels.

The overall mean achieved dosages during the 13-week treatment period were 834, 1,684, and 3,451 mg/kg/day for males and 896, 1,876, and 3,826 mg/kg/day for females receiving 12,500, 25,000, or 50,000 ppm, respectively. No toxicologically significant effects were observed at any of the doses tested.

There were no adverse effects and no mortality observed in the 90-day study. Minor differences in body weight gain were observed in males treated with urolithin A compared to controls, but not in females. These effects were considered unrelated to the compound, as they were not dose-dependent, only present in males, and withdrawal of the treatment did not result in any clear increase of weight gain

during the recovery period. Consequently, it was concluded that body weights and gain were unaffected by treatment with the test item urolithin A. Although sporadic effects were noted in some hematology and clinical chemistry parameters in the main study groups, these slight alterations were not considered to be of toxicologically significance as all were without dose-response, appeared in only one sex, or were within the range of historical control values for the performing laboratory. Recovery phase animals demonstrated no toxicologically relevant changes during the recovery period. Ophthalmoscopic examinations were normal, and there were no indications of neurological toxicity as indicated by the functional observational battery screen, motor activity assessment, or relevant clinical observations. Likewise, there were no effects on spermatogenesis analysis, estrus cycle analysis, or reproductive organ weights or macro- or microscopic alterations.

The no-observed-adverse-effect level (NOAEL) was determined to be 50,000 ppm, or 5% by weight of urolithin A in the rat diet, which is the highest achievable dose that does not induce dietary imbalances. This concentration corresponds to a NOAEL in male rats of 3,451 mg/kg body weight/day and in females of 3,826 mg/kg body weight/day.

6.3.3 Short-Term Tests for Genotoxicity

6.3.3.1 Ames Test

The potential of urolithin A to induce gene mutations was evaluated in a reverse mutation assay in accordance with OECD Guideline 471 (Heilman *et al.*, 2017) and GLP. The mutagenic potential of urolithin A was assessed in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 using the plate incorporation test (experiment I) and the pre-incubation test (experiment II).

No cytotoxicity was observed at concentrations of up to 5000 µg urolithin A/plate in a dose rage finding study. In each experiment, urolithin A was dissolved in dimethylsulfoxide (DMSO) and evaluated at concentrations of 0 (solvent control), 10, 33, 100, 333, 1,000, 2,500, and 5,000 µg/plate, both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.

No toxic effects, evident as a reduction in the number of revertants were observed. No substantial increase in revertant colony numbers of any of the 5 tester strains was observed following treatment with urolithin A at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies. Thus, urolithin A was not considered to be mutagenic under the conditions of this study.

6.3.3.2 In vitro Micronucleus Assay

Amazentis conducted a GLP-compliant *in vitro* micronucleus assay according to OECD Guideline 487 (Heilman *et al.*, 2017). Urolithin A, dissolved in DMSO, was assessed for its potential to induce micronuclei in human peripheral lymphocytes *in vitro* in 3 independent experiments. The study design is shown in Table 6.3.3.2-1. Following a preliminary dose range-finding cytotoxicity test, concentrations of 0.6, 1.5, 3.8, 9.4, 23.4, 58.6, 146.6, 366.4, 916.0, 2,290.0 µg/mL were selected for Experiments IA and IB, while concentrations in Experiment II (without S9 mix) of 0.3, 0.7, 1.6, 4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1,000.0 µg/mL were evaluated.

Table 6.3.3.2-1 *In vitro* Micronucleus Assay Study Design

	With S9 mix	Without S9 Mix	
	Experiment IA	**Experiment IB**	**Experiment II**
Exposure Period	4 hours	4 hours	20 hours
Recovery	16 hours	16 hours	-
Cytochalasin B exposure	20 hours	20 hours	20 hours
Preparation interval	40 hours	40 hours	40 hours
Total culture period*	88 hours	88 hours	88 hours

*Exposure started 48 hours after culture initiation

In all experiments, regardless of exposure period, cells were prepared for analysis 40 hours after the initiation of exposure. In each experimental group, 2 duplicate cultures were analyzed, and at least 1,000 binucleated cells were evaluated for cytogenetic damage per culture. The cytokinesis block proliferation index (CBPI) was determined in 500 cells per culture and cytotoxicity described as % cytostasis. Positive controls were conducted in parallel and included mitomycin pulse treatment, demecolcine continuous treatment (both for use without metabolic activation), and cyclophosphamide treatment (with metabolic activation).

The results of the assay are presented in Table 6.3.3.2-2. In the presence of S9 mix (Experiment IA), no increase in the number of micronucleated cells was observed at any concentration. In Experiments IB and II, which were both conducted in the absence of S9 mix, clear cytotoxicity was observed and used to determine the highest concentrations for evaluation (59.3% cytostasis at 58.6 µg/mL in Experiment IB, and 68.1% cytostasis at 160.0 µg/mL in Experiment II). Urolithin A demonstrated possible genotoxicity by this assay with positive results detected in some, but not all, treatment groups. In Experiment I in the absence of S9 mix, statistically significant increases in micronucleated cells (1.73 and 1.98%) slightly above the range of the laboratory historical solvent control data (0.15 to 1.40%) and the concurrent negative control data (0.85%), were observed after treatment with 23.4 and 58.6 µg/mL. In Experiment II in the absence of S9 mix after continuous treatment, statistically significant increases ($\alpha<0.05$) in micronucleated cells (3.13, 5.43, 5.03, and 3.45%) above the range of the laboratory historical solvent control data (0.05 to 1.45%) and the concurrent negative control data (1.10%) were observed after treatment with 10.2, 25.6, 64.0, and 160.0 µg/mL.

The increase in micronucleated cells did not exhibit a dose-response relationship. Furthermore, in the presence of S9 mix, no increase in the number of micronucleated cells was observed. In addition, formation of precipitate of the test item was seen at culture medium at 9.4 µg/mL and above in Experiment IA in the presence of S9 mix, in Experiment IB at 23.4 µg/mL and above in the absence of S9 mix and in Experiment II at 64.0 µg/mL and above in the absence of S9 mix at the end of treatment. Appropriate mutagens were used as positive controls and these compounds induced statistically significant ($\alpha<0.05$) increases in cells with micronuclei.

The pattern of precipitation and cytotoxicity did not account for the positive finding of genotoxic potential in this *in vitro* study. However, the increase in micronuclei with time of exposure and the absence of micronuclei formation in the presence of metabolic activation suggests that micronuclei formation may be a result of oxidative stress. This can be common to aromatic compounds such as urolithin A which has the potential to redox cycle. However, this mechanism will have a threshold and is unlikely to be relevant *in vivo*. Two additional *in vivo* micronucleus assays, discussed subsequently, were performed to provide confirmation.

Table 6.3.3.2-2 *In vitro* Micronucleus Assay Results

Exp.	Test Group	Test item concentration in µg/mL	Micronucleated Cells (%)
Exposure period 4 hrs with S9 mix			
IA	Solvent Control	1% DMSO	0.20
	CPA: Positive Control	12.5	**2.35**
	Urolithin A	3.8	0.25
		9.4[P]	0.20
		58.6[P]	0.40
		366.4[P]	0.40
		916.0[P]	0.15
Exposure period 4 hrs without S9 mix			
IB	Solvent control	1% DMSO	0.85
	MMC: Positive control	2.0	**11.35**
	Urolithin A	9.4	1.20
		23.4[P]	**1.73**[S]
		58.6[P]	**1.98**[S]
Exposure period 20 hrs without S9 mix			
II	Solvent Control	1% DMSO	1.10
	Demecolcin: Positive Control	75.0	**2.75**
	Urolithin A	10.2	**3.13**[S]
		25.6	**5.43**[S]
		64.0[P]	**5.03**[S]
		160.0[P]	**3.45**[S]

[P] Precipitation occurred at the end of treatment
[S] The number of micronucleated cells is statistically significantly higher than corresponding control values, according to Chi square test ($\alpha<0.05$)

6.3.3.3 In vivo Micronucleus Assay

Amazentis conducted a GLP *in vivo* micronucleus assay according to OECD Guideline 474 in response to the positive findings in the *in vitro* micronucleus assay (Heilman *et al*., 2017). Urolithin A was suspended in 1% carboxymethylcellulose substituted with 0.1% polysorbate 80. Bioavailability of the test material was confirmed prior to the main experiment by determination of urolithin A concentrations in plasma *via* high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). This analysis demonstrated that test material was quantifiable in plasma 1 hour and 4 hours after dosing and evidence that it reached the bone marrow. The ability of urolithin A to reach the bone marrow following gavage dosing was confirmed in the ADME study described in Section 6.2.1.

In addition, a preliminary study of acute toxicity was conducted with 2 animals/sex/dose and an observation period of up to 48 hours post-dosing. From this experiment, it was determined that there were no substantial differences between the sexes under the conditions of the study with a treatment of 2,000 mg/kg body weight, the highest recommended dose by the OECD guideline. As a result, only male Wistar rats were utilized in the main study.

For the main experiment, 7 males/group received a single oral dose of urolithin A at 500, 1,000, or 2,000 mg/kg body weight (dose volume 10 mL/kg body weight) or positive control (cyclophosphamide). Twenty-four to 48 hours after treatment, bone marrow cells were collected for micronuclei analysis; 4,000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. In addition, the ratio between polychromatic and normochromatic erythrocytes (NCEs) was determined in the same sample as a measure for cytotoxicity and reported as the number of PCEs per 500 erythrocytes.

The assay results are presented in Table 6.3.3.3-1. Urolithin A did not exert any cytotoxic effects in the bone marrow. No biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei was seen at any preparation interval after administration of the test item and with any dose level used. A substantial increase of induced micronucleus frequency was seen with the positive control (20 mg/kg body weight cyclophosphamide). Urolithin A did not induce micronuclei and it was concluded that urolithin A was not genotoxic under the conditions of this guideline assay.

Table 6.3.3.3-1 *In vivo* Micronucleus Assay Results after single oral exposure to Urolithin A

Test Group	Dose mg/kg b.w.	Sampling time (hrs)	Mean MN/4000 PCE	SD MN/4000 PCE	Range		PCE /total Ery	PCE /total Ery (% of vehicle)
					min	max		
Vehicle[a]	0	24	6.9	2.3	4	11	0.534	100.00
Urolithin A	500	24	11.1	5.5	5	21	0.540	101.10
	1000	24	8.3	6.8	0	22	0.529	99.04
	2000	24	10.9	5.4	4	20	0.572	107.09
Positive Control	20	24	**126.1***	41.7	76	175	0.476	89.11
Urolithin A	2000	48	7.7	5.6	0	17	0.542	101.47

[a]Vehicle: 1% CMC substituted with 0.1% Polysorbate 80

*Statistically significant according to the nonparametric Mann-Whitney test ($p < 0.05$).

MN = Micronuclei; PCE = Polychromatic erythrocytes; Ery = Erythrocytes; SD = Standard Deviation

6.3.3.4 Micronucleus Assay 90-Day Study

A concurrent micronucleus assay was also conducted as part of the 90-day study. The micronucleus assay was GLP and conducted according to OECD Guideline 474 (Heilman *et al.,* 2017). Five male and five female rats each from the control and all treatment groups were sampled for bone marrow from the femur, which was cleaned and prepared and analyzed for the formation of micronuclei. The results of the micronucleus assay are presented on Table 6.3.3.4-1. No micronucleus formation was observed in this study despite the repeat dose nature and longer duration of the dosing period in the 90-day study, as compared to the single gavage dose administered in the *in vivo* micronucleus assay (Section 6.3.3.2).

Table 6.3.3.4-1 *In vivo* Micronucleus Assay Results after 90-day exposure to Urolithin A

Treatment	Dosage of Urolithin A (% of diet)	Proportion of PCE, Group mean % (SD)	Group mean MPCE/4000 PCE (SD)	Group mean % MPCE
Male data				
Vehicle	0.00 %	64.2 (10.3)	9.0 (1.7)	0.23
Urolithin A	1.25 %	66.8 (6.8)	7.6 (3.7)	0.19
	2.50 %	69.2 (6.4)	5.0 (1.6)	0.13
	5.00 %	60.8 (8.8)	6.8 (2.4)	0.17
Cyclophosphamide	20 mg/kg	**46.2 (7.8)****	**78.0 (26.1)**$^{++}$	1.95
Female data				
Vehicle	0.00 %	53.3 (2.2)	5.8 (2.6)	0.15
Urolithin A	1.25 %	57.3 (7.8)	4.6 (2.6)	0.12
	2.50 %	58.8 (4.3)	5.0 (2.2)	0.13
	5.00 %	59.9 (4.7)	4.8 (2.9)	0.12
Cyclophosphamide	20 mg/kg	**42.0 (4.9)****	**82.0 (36.6)**$^{++}$	2.05

Vehicle: Basal diet
PCE: Polychromatic erythrocytes
MPCE: Number of micronucleated polychromatic erythrocytes observed per 4000 polychromatic erythrocytes examined
SD. Standard deviation
$^{**}P < 0.05$ after Wilcoxon test. $^{++}P < 0.05$ after Permutation test.

6.3.3.5 Summary of Genotoxicity Studies on Urolithin A

Urolithin A did not demonstrate any genotoxic potential except for inconsistent positive results for some of the groups in the *in vitro* micronucleus assay performed using human peripheral lymphocytes, described in Section 6.3.3.2. The *in vitro* assessment of genotoxicity is well known to have a high rate of false positive depending on the assay (Kirkland *et al.,* 2007; Walmsley and Billinton, 2010). The *in vitro* micronucleus assay in particular, while having a high level of sensitivity for detecting genotoxic compounds, has a high rate of false positives and thus identifies many non-genotoxic compounds as positive for *in vitro* genotoxicity (Kirkland *et al.,* 2005). Consequently, although positive *in vitro* data could indicate intrinsic genotoxic properties of a compound, appropriate *in vivo* data determine the biological significance of these *in vitro* signals in most cases (U.S. FDA, 2012). The increase in micronuclei with time of exposure and the absence of micronuclei formation in the *in vitro* assay in the presence of metabolic activation suggests that micronuclei formation may be a result of oxidative stress (Muller and Kasper, 2000). This can be common to aromatic compounds such as urolithin A which has the potential to redox cycle. However, this mechanism will have a threshold and is unlikely to be relevant *in vivo*. Indeed, no increase in micronuclei formation was observed *in vivo* following urolithin A treatment in two independent *in vivo* micronuclei studies in rats with short-term dosing, described in Section 6.3.3.3, and the other with 90-day dosing, described in Section 6.3.3.4. The systemic absorption of urolithin A following gavage in these *in vivo* assays was confirmed by demonstrating measurable concentrations of urolithin A and glucuronidated and sulfated metabolites in the plasma of treated animals. Furthermore, the exposure of the bone marrow (the target site for micronuclei formation) to urolithin A following gavage was confirmed in the ADME study, described in Section 6.2.1. Overall, the weight of evidence demonstrates the absence of genotoxic risk following systemic exposure to urolithin A. These findings are in agreement with the numerous *in vitro* studies that showed that urolithin A has potent antiproliferative effects in various cell lines as well as anti-inflammatory effects in either macrophages transformed cell lines or *ex vivo* cultured peripheral blood mononuclear cells collected from rodents and humans (Tómas-Barberán *et al*., 2016).

6.4 Other Published Toxicity Studies

As ellagitannins are metabolized to urolithin A, safety reports for ellagitannins or ellagitannin-containing food products, which can be assumed to result in some lower level of *in vivo* exposure to urolithins following their formation in the gastrointestinal tract, are considered supportive of the safety of urolithin A. Cerdá *et al.* (2003) evaluated the possible toxic effect of punicalagin upon repeated oral administration. Sprague-Dawley rats (10 animals/group) received either a control diet or diet containing 20% pomegranate husk extract containing an average of 6% punicalagin for a period of 37 days. Animals were monitored for effects on growth, antioxidant enzymes, hematology and clinical chemistry parameters, and pathological changes in the liver and kidney. The mean oral consumption throughout the study was reported to be 0.9 g punicalagin/day. Food intake, food utility index, and growth rate were lower in treated rats during the first 15 days. The authors noted that these findings could have been due to the decreased palatability and lower nutritional value of the punicalagin-enriched diet. A decrease in serum urea and triglyceride values were observed throughout the study. The decrease in urea was not associated with any changes in other liver parameters (*i.e.*, ALT, AST, ALP, and bilirubin were normal). Although the decrease in triglycerides reached statistical significance, values remained within the normal range. No other significant differences were found in treated rats in any hematology or clinical chemistry parameter analyzed. Likewise, no histopathological changes were seen in the liver or kidney.

Patel *et al*. (2008) examined the acute and subchronic effects of a pomegranate fruit extract standardized to 30% punicalagins. The acute oral median lethal dose (LD_{50}) of the extract in rats and mice was found to be greater than 5 g/kg body weight. In the subchronic study, Wistar strain rats (10 animals/sex/group) were administered the extract via gavage at doses of 0 (control), 60, 240, and 600 mg/kg body weight/day for 90 days. Two additional groups received 0 and 600 mg/kg/day of the extract for 90 days, followed by a 28-day recovery period. Administration of the extract did not result in any toxicologically significant treatment-related changes in clinical observations, ophthalmic examinations, body weights, body weight gains, feed consumption, urinalysis, clinical pathology evaluations and organ weights compared to controls. Although some statistical changes were seen in hematology and serum chemistry parameters that showed statistical significant changes compared to control, values remained within the normal laboratory limits and were thus considered as biological variations rather than toxic effects. No treatment-related gross or histopathological findings were reported. The NOAEL for this study was thus considered to be 600 mg/kg body weight/day, the highest dose tested.

6.5 Corroborating Safety Evidence

The safety and tolerability of urolithin A was also evaluated in a single-center, double-blind, randomized study, NCT02655393 (Singh, *et al.*, 2018). The findings from the clinical study serve as corroborative evidence that the intended use of urolithin A is safe. Specifically, this was a 2-part study with a single oral ascending dose Part A and a 4-week multiple ascending dose Part B. In Part A, 24 healthy elderly male and female volunteers [12 females and 12 males, ranging in age from 61 to 82 years (mean 68.7 ± 5.3 years) and body mass index (BMI) ranging from 20.2 to 30.4 kg/m² (mean 24.60 ± 2.72 kg/m²)] were randomized (6 subjects/group) to consume urolithin A in single ascending doses of either 250 mg, 500 mg, 1,000 mg and 2,000 mg or placebo. Each dose was separated by a washout period of 3 weeks. Similarly, in Part B, the safety and tolerability of urolithin A was evaluated following a 28-day (4 week) oral administration. Thirty-six healthy elderly male and female volunteers [12 males and 24 females, ranging in age from 61 to 78 years (mean age 66.4 ± 4.9 years) and BMI ranging from 18.8 to 30.6 kg/m² (mean of 25.02 ± 3.04 kg/m²)] were randomized (9 subjects/group) to receive 250 mg, 500 mg, or 1,000

mg of urolithin A or placebo daily for 28 days. In Part A and Part B, urolithin A was administered orally, in fasting condition, in softgel formulations with water for all dosing's. Additionally, in Part A, urolithin A was administered in high protein yogurt at doses of 500 mg and 1,000 mg. In each study, subjects underwent physical examinations and electrocardiogram (ECG) evaluations and were monitored for adverse events. Liver and kidney function [*i.e.*, creatinine, uric acid, alanine serine transferase (AST), alanine leucine transferase (ALT), gamma glutamyl transferase (GGT), and total and conjugated bilirubin] were evaluated before and after dosing's. Full laboratory tests included hematology [*i.e.*, hemoglobin, hematocrit, red blood cells (RBC), white blood cells (WBC), differential count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] and urinalysis (pH, ketone bodies, proteins, glucose, and blood).

In the single dose Part A phase there were no serious adverse events (SAE) recorded for any dosing. 5 of 24 subjects reported the occurrence of 6 non-serious adverse events, of which none were considered related to intake of urolithin A. Adverse events were distributed across all dose groups, including placebo. No clinically significant abnormal laboratory test values from study baseline were observed for any of the biochemistry tests assessing liver and kidney function, or for any of the hematology and urinalysis tests for any subjects at any of the doses during the course of the study. No abnormal and clinically significant conclusions were observed for ECG findings for any subjects taking active intervention at any of the doses during the course of the study. As a result, it was concluded that single dosing of urolithin A at the doses of 250 mg, 500 mg, 1,000 mg, or 2,000 mg was safe and well tolerated. In the multiple-dose (28 day/oral intake) Part B phase, no serious adverse events were reported. 31 non-serious adverse events were reported in 15 subjects (the majority being linked to study procedures, *i.e.* muscle biopsy), none were considered to be related to intake of urolithin A. No clinically significant changes were reported in liver and kidney function tests, hematology or urinalysis. No clinically relevant and abnormal findings were reported during physical examination. Vital signs were likewise unaffected. No significant abnormalities were reported during ECG examinations.

The results of the study support the conclusion that urolithin A is well tolerated in human and has a favourable safety profile when orally administered in single and multiple doses to elderly.

6.6 Expert Panel

Amazentis has concluded that its urolithin A, manufactured consistent with cGMP and meeting food-grade specifications, is GRAS for use as an ingredient in specified conventional food and beverage products, as described in Part 1.3, on the basis of scientific procedures. Amazentis' conclusion on the GRAS status of urolithin A under the conditions of its intended use is based on data generally available in the public domain and includes a series of product-specific toxicology studies on urolithin A.

A Panel of Experts (the Expert Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients unanimously concluded on the GRAS status of the urolithin A under conditions of its intended use. The Expert Panel consisted of the following qualified scientific experts: Dr. John Thomas (Adjunct Professor, Indiana University School of Medicine), Dr. Robert Nicolosi (Professor Emeritus, University of Massachusetts Lowell) and Dr. David Bechtel (Bechtel Consulting, Inc.)[2]

[2] The panelists participated in their individual capacities. Institutional affiliations are provided for identification purposes only.

The Expert Panel, convened by Amazentis, independently and critically evaluated all data and information presented herein and concluded that urolithin A, meeting appropriate food-grade specifications and manufactured consistent with current Good Manufacturing Practice, is safe and suitable for use as an ingredient in specified conventional food and beverage products, as described in Part 1.3, and is GRAS based on scientific procedures. A summary of data and information reviewed by the Expert Panel, and evaluation of such data as it pertains to the proposed GRAS uses of the urolithin A is presented in Appendix 2.

6.7 Conclusions

Amazentis intends to market urolithin A as an ingredient in select foods or for special dietary uses in meal replacement products based on its nutritive activity in supporting general mitochondrial health. These include powdered (reconstituted) protein shakes, beverages (ready-to-drink protein shakes, non-milk based meal replacement beverages, instant oatmeal, protein and nutrition bars, and yogurts (Greek yogurts, high-protein yogurts, and yogurt drinks) at typical use levels of 250 mg/serving or 500 mg/serving up to a maximum of 500 mg/serving or 1,000 mg/serving.

Adults aged 40 years and above are intended to be the primary consumers of the ingredient. In the general adult population (age ≥ 12 years), the mean and 90th percent all-user intake was determined to be 15.5 mg/kg bw/day and 34.1 mg/kg bw/day, respectively. The 90th percentile intake of 34.1 mg/kg bw/day or 2,046 mg/person/day greatly overestimates the actual intake of urolithin A. The target consumers will intentionally seek urolithin A and select these foods based on the labeling for its nutritional benefits in supporting mitochondria function among the elder population. Because there are not a wide variety of target foods that could contain urolithin A, and given the premium pricing associated with food products fortified with urolithin A, it would be reasonable to assume that most consumers would eat 1 or 2 servings a day of such foods, depending on the level of urolithin A in the food. As such, an aggregated intake of 1,000 mg/person/day or 16.67 mg/kg bw/day is a more accurate estimate of the actual urolithin A intake.

Toxicity studies demonstrate systemic exposure of urolithin A following oral absorption, with effective and rapid elimination of the fraction absorbed. A series of toxicity studies was performed on urolithin A to demonstrate its safety under the proposed conditions of use (Table 6.7-1). Urolithin A was non-genotoxic in the Ames assay. While micronuclei formation was observed in the absence of metabolic activation in the *in vitro* micronucleus assay, two independent *in vivo* micronuclei studies in rats, one with short-term dosing, and the other with 90-day dosing, demonstrated no increase in the frequency of formation of micronuclei.

Repeated dose 28-day and 90-day studies did not indicate any target organs, nor any specific toxic mechanisms. Dose concentrations attained were as high as could be achieved in the diet (5% of diet) without affecting food consumption and diet palatability, which could have led to decreased body weight gains and subsequent group mean body weight deficits. Treatment for up to 90 days with urolithin A did not result in any signs of reproductive or neurological toxicities in enhanced screening phases of repeated dose studies including analysis of spermatogenesis or estrus cycles, ophthalmoscopic examinations, functional observatory battery screen, and motor activity assessments. The NOAEL was the highest dose tested, 5% urolithin A by weight in the diet, or 3,451 mg/kg body weight/day in males and 3,826 mg/kg body weight/day in females (Heilman *et al*., 2017).

Table 6.7-1 Summary of Key Studies Supporting the Safety of Urolithin A

Pre-Clinical Safety Studies	**Guideline**	**Outcome**
28-Day subacute toxicity study	OECD 407	**No toxicity observed** at the highest dosing (5% of rat diet)
90-Day subchronic toxicity study	OECD 408 Redbook	**No toxicity observed** at the highest dosing (5% of rat diet) No micronucleus formation observed following chronic treatment
Genetic toxicity battery		
AMES reverse mutation assay	OECD 471	No impact on mutation rate, Urolithin A does not induce gene mutations
in vitro micronucleus assay	OECD 487	No impact on micronucleus formation with metabolic activation Micronucleus formation observed in the absence of metabolic activation
in vivo micronucleus assay	OECD 474	**No micronucleus formation observed**
Absorption, distribution, metabolism, and excretion study	OECD 417	Metabolite formation limited to glucuronide and sulfate. Pharmacokinetic & mass balance determined. Urolithin A reaches the bone marrow.
Human Clinical Safety Studies		Outcome
Phase 1 - Single ascending dose		**No serious adverse events** **No product related non-serious adverse events**
Phase 1 – Multiple ascending dose (28 days)		

The background level of urolithin A from the diet is in the range of 3.0 to 15.9 mg/day. The estimated aggregate intake level use of urolithin A as an ingredient in select foods is up to 2,046 mg/person/day (34.1 mg/kg bw/day) at 90th percentile among the general adult population (age ≥ 12) and the actual intake level is considered to be closer to 1,000 mg/person/ day or 16.67 mg/kg bw/day. The background intake level is insignificant when compared to intakes resulting from the intended uses proposed by Amazentis. There exists a margin of safety in excess of 100 from the NOAEL of 3,451 mg/kg body weight/day in the 90-day rat study.

In addition, based on the available data and information we have reviewed, Amazentis is not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status for the intended use.

Based on data and information presented herein, Amazentis has concluded that urolithin A can be determined to be GRAS for use in specified conventional food and beverage products, as described in Part 1.3, on the basis of scientific procedures using publicly available data from toxicology studies. The GRAS status of urolithin A is further supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training to evaluate the safety of food ingredients, who concluded that the intended use of urolithin A, as described herein, is GRAS.

Therefore, the intended use of urolithin A is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

Part 7. §170.255 List of Supporting Data and Information

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Appendix 1

Estimated Daily Intake of Urolithin A by the U.S. Population from Proposed Food-Uses (2013-2014 NHANES)

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ESTIMATED DAILY INTAKE OF UROLITHIN A BY THE U.S. POPULATION FROM PROPOSED FOOD-USES (2013-2014 NHANES)

PREPARED FOR:
AMAZENTIS SA
EPFL Innovation Park
Building C
1015 Lausanne
Switzerland

PREPARED BY:
Intertek Scientific & Regulatory Consultancy
Health, Environmental & Regulatory Services (HERS)
2233 Argentia Road, Suite 201
Mississauga, Ontario, Canada
L5N 2X7

DATE:
16 November 2017

Estimated Daily Intake of Urolithin A by the U.S. Population from Proposed Food-Uses (2013-2014 NHANES)

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1.0 INTRODUCTION

Urolithin A is proposed for use in the United States (U.S.) in foods, such as protein shakes, meal replacement drinks, instant oatmeals, protein bars, nutrition bars, Greek yogurts, and yogurt drinks.

Estimates for the intake of Urolithin A were based on the proposed food-uses and use levels for Urolithin A in conjunction with food consumption data included in the U.S. National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES) 2013-2014 (CDC, 2015, 2016; USDA, 2016). Calculations for the mean and 90th percentile *per capita* and consumer-only intakes were performed for all proposed food-uses of Urolithin A and the percentage of consumers were determined. Similar calculations were used to estimate the intake of Urolithin A resulting from each individual proposed food-use, including the calculations of percent consumers. In both cases, the per person and per kilogram body weight intakes were reported for the following population groups:

Infants and young children, 0 to <3;
Children, ages 3 to 11;
Teenagers, ages 12 to 19;
Adults, ages 20 to 39;
Older female adults, ages 40 to 64;
Older male adults, ages 40 to 64;
Female elderly adults, ages ≥65;
Male elderly adults, ages ≥65; and
Total population ≥12.

2.0 FOOD CONSUMPTION SURVEY DATA

2.1 Survey Description

NHANES for the years 2013-2014 are available for public use (CDC, 2015). NHANES are conducted as continuous, annual surveys, and are released in 2-year cycles. During each year of the ongoing NHANES program, individuals from the United States are sampled from up to 30 different study locations in a complex multi-stage probability design intended to ensure the data are a nationally representative sample of the U.S. population.

NHANES 2013-2014 dietary survey data were collected from individuals and households *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2) throughout all 4 seasons of the year. Day 1 data were collected in-person, and Day 2 data were collected by telephone in the following 3 to 10 days, on different days of the week, to achieve the desired degree of statistical independence. The data were collected by first selecting Primary Sampling Units (PSUs), which were counties throughout the U.S., of which 30 PSUs are visited per year. Smaller contiguous counties were combined to attain a minimum population size. These PSUs were segmented and households were chosen within each segment. One or more participants within a household were interviewed. For NHANES 2013-2014, 14,332 individuals were selected for the sample, 10,175 were interviewed (71.0%) and 9,813 were examined (68.5%).

In addition to collecting information on the types and quantities of foods being consumed, NHANES 2013-2014 collected socio-economic, physiological and demographic information from individual participants in the survey, such as sex, age, body weight, and other variables (such as height and race-ethnicity) that may be useful in characterizing consumption. The inclusion of this information allows for further assessment of food intake based on consumption by specific population groups of interest within the total population. The primary sample design for NHANES 2013-2014 includes an oversample of Non-Hispanic Asian persons, Hispanic persons, non-Hispanic black persons, older adults, and "low income whites/others", however sample weights were incorporated to allow estimates from these subgroups to be combined to obtain national estimates that reflect the relative proportions of these groups in the population as a whole (CDC, 2015).

2.2 Statistical Methods

For the intake assessment, consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of Urolithin A by the U.S. population[1]. Estimates for the daily intake of Urolithin A represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2013-2014; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. "*Per capita*" intake refers to the estimated intake of Urolithin A averaged over all individuals surveyed, regardless of whether they consumed food products in which Urolithin A is proposed for use, and therefore includes individuals with "zero" intakes (*i.e*. those who reported no intake of food products containing Urolithin A during the 2 survey days). "Consumer-only" intake refers to the estimated intake of Urolithin A by those individuals who reported consuming food products in which the use of Urolithin A is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which Urolithin A is proposed for use on either Day 1 or Day 2 of the survey.

Mean and 90th percentile intake estimates based on sample sizes of less than 30 and 80, respectively, may not be considered statistically reliable due to the limited sampling size (CDC, 2013). As such, the reliability of estimates for the intake of Urolithin A based on consumption estimates derived from individual population groups of a limited sample size should be interpreted with caution. These values are marked with an asterisk in the relevant data tables.

3.0 FOOD USAGE DATA

The individual proposed food-uses and use-levels for Urolithin A employed in the current intake analysis are summarized in Table 3-1. Food codes representative of each proposed food-use were chosen from the NHANES 2013-2014 (CDC, 2016). Food codes were grouped in food-use categories according to Title 21, Section §170.3 of the Code of Federal Regulations (CFR, 2016). All food codes included in the current intake assessment are listed in Appendix C.

[1] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2016). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

Table 3-1 Summary of the Individual Proposed Food-Uses and Use Levels for Urolithin A in the U.S.

Category	Food-Uses	Urolithin A Level, as Consumed (mg/serving)	RACC[a] (g or mL)	Urolithin A Use Levels (mg/100 g or mg/100 mL)
Food Categories (21 CFR 170.3				
Beverages and Beverage Bases	Protein shakes; meal replacement drinks	500	360	140
Breakfast Cereals	Instant oatmeals	500	240	210
Grain Products and Pastas	Protein and nutrition bars	500	40	1,250
Milk Products	Greek yogurts, high protein yogurts[b]	1,000	170	590
	Yogurt drinks[c]	500	100[d]	500
	Milk-based protein shakes	1,000	240	420

CFR = Code of Federal Regulations; na = not applicable; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2016).

[b] No food codes were identified for high protein yogurts, but based on the high content of protein in Greek yogurts, these were deemed a suitable surrogate

[c] No food codes were identified for yogurt drinks within the NHANES dataset; however, food codes for dairy-based fruit smoothies drinks were selected as surrogates to represent the food codes in this category.

[d] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently in the U.S. market.

4.0 FOOD SURVEY RESULTS

Estimates for the total daily intakes of Urolithin A from proposed food-uses are provided in Tables 4.1-1 and 4.1-2. Estimates for the daily intake of Urolithin A from individual proposed food-uses in the U.S. are summarized in Tables A-1 to A-10 and B-1 to B-10 of Appendices A and B, respectively. Tables A-1 to A-10 provide estimates for the daily intake of Urolithin A on an absolute basis (mg/person/day), whereas Tables B-1 to B-10 provide estimates for the daily intake of Urolithin A on a per kilogram body weight basis (mg/kg body weight/day).

4.1 Estimated Daily Intake of Urolithin A from All Proposed Food-Uses in the U.S.

Table 4.1-1 summarizes the estimated total intake of Urolithin A (mg/person/day) from all proposed food-uses in the U.S. population group. Table 4.1-2 presents this data on a per kilogram body weight basis (mg/kg body weight/day). The percentage of consumers was low among all age groups evaluated in the current intake assessment; 10.6 to 21.6% of the population groups consisted of consumers of food products in which Urolithin A is currently proposed for use (Table 4.1-1). The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

On an absolute basis, for the total population excluding all children (≥12 years), the mean and 90th percentile consumer-only intakes of Urolithin A were determined to be 1,183 and 2,421 mg/person/day, respectively. Of the individual population groups, adults were determined to have the greatest mean consumer-only intakes of Urolithin A on an absolute basis, at 1,528 mg/person/day, while teenagers had the greatest 90th percentile consumer-only intakes of 3,494 mg/person/day. Infants and young children were determined to have the lowest mean percentile consumer-only intakes of Urolithin A on an absolute basis

at 446 mg/person/day, while female elderly adults had the lowest statistically reliable 90th percentile consumer-only intakes of 1,341 mg/person/day, respectively (Table 4.1-1).

Table 4.1-1 Summary of the Estimated Daily Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to <3	55	173*	12.2	60	446	944*
Children	3 to 11	54	94	10.7	122	500	1,418
Teenagers	12 to 19	140	173	10.6	113	1,320	3,494
Adults	20 to 39	263	632	17.2	232	1,528	2,688
Older Female Adults	40 to 64	205	723	21.6	185	949	1,884
Older Male Adults	40 to 64	182	502	16.3	115	1,122	3,226
Female Elderly Adults	≥65	167	529	21.6	99	773	1,341
Male Elderly Adults	≥65	157	322*	14.1	60	1,118	2,381*
Total Population	≥12	204	538	17.2	804	1,183	2,421

NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

On a body weight basis, the total population mean and 90th percentile consumer-only intakes were determined for excluding all children (≥12 years). Among the total population excluding all children, the mean and 90th percentile consumer-only intakes of Urolithin A were determined to be 15.5 and 34.1 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean consumer-only intakes of any population group of 38.0 mg/kg body weight/day, while teenagers were identified as having the highest statistically reliable 90th percentile consumer-only intakes of any population group of 49.0 mg/kg body weight/day. Female elderly adults had the lowest mean and statistically reliable 90th percentile consumer-only intakes of 11.7 and 24.4 mg/kg body weight/day, respectively (Table 4.1-2).

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to <3	4.7	12.4*	12.3	60	38.0	80.4*

Table 4.1-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Children	3 to 11	2.0	3.9	10.8	122	18.2	42.7
Teenagers	12 to 19	2.0	2.6	10.7	113	19.0	49.0
Adults	20 to 39	3.3	7.8	17.2	232	19.3	43.6
Older Female Adults	40 to 64	2.9	9.2	21.6	185	13.2	27.9
Older Male Adults	40 to 64	2.2	6.3	16.3	115	13.4	34.1
Female Elderly Adults	≥65	2.5	7.6	21.6	98	11.7	24.4
Male Elderly Adults	≥65	1.6	2.7*	11.9	59	13.6	19.1*
Total Population	≥12	2.7	6.9	17.1	802	15.5	34.1

bw = body weight; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

4.2 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses in the U.S.

Estimates for the mean and 90th percentile daily intakes of Urolithin A from each individual food category are summarized in Tables A-1 to A-9 and B-1 to B-9 on a mg/day and mg/kg body weight/day basis, respectively. The total U.S. population was not identified as being significant consumers of any of the proposed food-uses; the percent consumers were highest for the food 'greek and high protein yogurts' (2.1 to 7.6% consumers), 'milk-based protein shakes' (0.3 to 7.0 % consumers) and 'instant oatmeals' (1.4 to 6.8 % consumers).

In terms of contribution to total mean intake of Urolithin A, 'milk-based protein shakes' (contributed 3.3 to 73.8% to total mean intakes), 'yogurt drinks' (contributed 1.6 to 48.7% to total mean intakes) and 'Greek and high protein yogurts' (contributed 9.7 to 41.0% to total mean intakes), and were the main sources of intake across all population groups (see Tables A-1 to A-9 and/or B-1 to B-9 for further details).

5.0 SUMMARY AND CONCLUSIONS

Consumption data and information pertaining to the individual proposed food-uses of Urolithin A were used to estimate the *per capita* and consumer-only intakes of Urolithin A for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates that may be considered suitably conservative. For example, it has been assumed in both exposure assessments that all food products within a food category contain Urolithin A at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that Urolithin A will have 100% market penetration in all identified food categories.

In summary, on consumer-only basis, the resulting mean and 90th percentile intakes of Urolithin A by the total U.S. population excluding children (≥12 years) from all proposed food-uses, were estimated to be 1,183 mg/person/day (15.5 mg/kg body weight/day) and 2,421 mg/person/day (34.1 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean consumer-only intakes of Urolithin A were determined to be 1,528 mg/person/day (19.3 mg/kg body weight/day), as identified among adults, while the highest 90th percentile consumer-only intakes of Urolithin A were determined to be 3,494 mg/person/day (49.0 mg/kg body weight/day), as identified among teenagers. Infants and young children had the lowest mean consumer-only intakes of 446 mg/person/day (38.0 mg/kg body weight/day), while female elderly adults had the lowest statistically reliable 90th percentile consumer-only intakes of 1,341 mg/person/day (24.4 mg/kg body weight/day).

When intakes were expressed on a body weight basis, infants and young children had the highest mean consumer-only intake of 80.4 mg/kg body weight/day. Although younger populations were identified as the groups having the highest exposures to Urolithin A on a body weight basis, it should be noted that products containing Urolithin A will not be targeted towards children, and estimates described herein assume *all* products, including those consumed by younger individuals, would contain the ingredient at the maximum intended use levels. In actuality, these products would, in the worst case, only be consumed incidentally and intakes described in the older populations (*i.e.*, not more than 19.3 and 49.0 mg/kg body weight/day at the mean and 90th percentile, respectively) are expected to be more accurate estimates of dietary exposure among the intended population.

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Appendix A
Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Different Population Groups Within the U.S. (2013-2014 NHANES DATA)

Table A-1 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Infants and Young Children Aged 0 to <3 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***55***	***173***	***12.2***	***60***	***446***	***944***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0	0	0	0	0	0	0
Breakfast Cereals							
Instant oatmeals	24.3	13	na	6.8	35	196	350*
Grain Products and Pastas							
Protein and nutrition bars	3.4	2*	na	0.5	3	388*	445*
Milk Products							
Greek yogurts, high protein yogurts	41.0	22*	na	4.2	14	529*	918*
Yogurt drinks	13.8	8*	na	1.9	11	393*	540*
Milk-based protein shakes	17.6	10*	na	0.6	4	1,488*	1,880*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-2 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Children Aged 3 to 11 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***54***	***94***	***10.7***	***122***	***500***	***1,418***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0	0	0	0	0	0	0
Breakfast Cereals							
Instant oatmeals	17.1	9	na	4.9	58	188	322*
Grain Products and Pastas							
Protein and nutrition bars	6.4	3*	na	0.9	11	389*	471*
Milk Products							
Greek yogurts, high protein yogurts	24.6	13*	na	2.1	17	643*	1,504*
Yogurt drinks	48.7	26	na	2.7	33	956	1,697*
Milk-based protein shakes	3.3	2*	na	0.3	7	534*	791*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-3 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***140***	***173***	***10.6***	***113***	***1,320***	***4,570***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0.9	1*	na	0.3	5	457*	524*
Breakfast Cereals							
Instant oatmeals	2.6	4*	na	1.4	23	257*	360*
Grain Products and Pastas							
Protein and nutrition bars	9.0	13	na	3.1	30	412	850*
Milk Products							
Greek yogurts, high protein yogurts	11.8	17*	na	2.8	21	581*	1,053*
Yogurt drinks	12.8	18*	na	1.8	28	1,008*	1,792*
Milk-based protein shakes	62.9	88*	na	2.9	25	3,069*	4,474*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-4 **Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Adults Aged 20 to 39 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***263***	***632***	***17.2***	***232***	***1,528***	***4,184***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	3.6	10*	na	0.6	8	1,585*	2,675*
Breakfast Cereals							
Instant oatmeals	4.4	11	na	3.4	58	335	537*
Grain Products and Pastas							
Protein and nutrition bars	8.2	21	na	4.5	51	480	850*
Milk Products							
Greek yogurts, high protein yogurts	13.2	35	na	4.7	65	731	1,420*
Yogurt drinks	15.0	39	na	3.2	47	1,221	1,958*
Milk-based protein shakes	55.7	146	na	5.3	62	2,782	5,544*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-5 **Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Older Female Adults Aged 40 to 64 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***205***	***723***	***21.6***	***185***	***949***	***1,884***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0.5	1*	na	0.5	6	211*	273*
Breakfast Cereals							
Instant oatmeals	9.7	20	na	6.2	63	320	592*
Grain Products and Pastas							
Protein and nutrition bars	5.4	11*	na	2.4	23	454*	851*
Milk Products							
Greek yogurts, high protein yogurts	30.7	63	na	7.6	52	830	1,446*
Yogurt drinks	15.8	32	na	3.3	32	981	1,541*
Milk-based protein shakes	37.9	78*	na	4.1	28	1,912*	3,997*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-6 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Older Male Adults Aged 40 to 64 Years and Over Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***182***	***502***	***16.3***	***115***	***1,122***	***4,070***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	3.8	7*	na	0.6	8	1,141*	2,563*
Breakfast Cereals							
Instant oatmeals	4.7	9*	na	2.9	29	295*	435*
Grain Products and Pastas							
Protein and nutrition bars	5.9	11*	na	3.6	21	298*	425*
Milk Products							
Greek yogurts, high protein yogurts	27.7	51	na	5.8	30	873	1,538*
Yogurt drinks	9.9	18*	na	1.7	19	1,079*	1,675*
Milk-based protein shakes	47.9	87*	na	3.1	20	2,847*	4,044*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-7 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Female Elderly Adults Aged ≥65 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***167***	***529***	***21.6***	***99***	***773***	***1,341***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	1.6	3*	na	1.0	7	270*	313*
Breakfast Cereals							
Instant oatmeals	9.7	16	na	5.8	37	282	383*
Grain Products and Pastas							
Protein and nutrition bars	1.9	3*	na	1.2	5	260*	337*
Milk Products							
Greek yogurts, high protein yogurts	23.0	39*	na	7.2	22	536*	723*
Yogurt drinks	13.5	23*	na	1.7	8	1,356*	2,160*
Milk-based protein shakes	50.2	84*	na	7.0	28	1,201*	2,734*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-8 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by Male Elderly Adults ≥65 Years Within the US Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***157***	***322***	***14.1***	***60***	***1.118***	***2,381***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	2.0	3*	na	0.4	3	801*	1,250*
Breakfast Cereals							
Instant oatmeals	12.7	20	na	5.6	35	355	627*
Grain Products and Pastas							
Protein and nutrition bars	0.2	<1*	na	0.2	1	213*	213*
Milk Products							
Greek yogurts, high protein yogurts	9.7	15*	na	3.0	9	511*	703*
Yogurt drinks	1.6	3*	na	0.4	1	709*	709*
Milk-based protein shakes	73.8	116*	na	6.5	17	1,772*	2,755*

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table A-9 Estimated Daily Intake of Urolithin A from Individual Proposed Food-Uses by the Total Population ≥12 Years Within the US Population (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***204***	***538***	***17.2***	***804***	***1,183***	***2,421***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	2.5	5	na	0.6	37	921	3,226*
Breakfast Cereals							
Instant oatmeals	6.2	13	na	4.0	245	317	537
Grain Products and Pastas							
Protein and nutrition bars	6.4	13	na	3.1	131	419	850
Milk Products							
Greek yogurts, high protein yogurts	19.6	40	na	5.4	199	746	1,420
Yogurt drinks	13.2	27	na	2.4	135	1,118	1,958
Milk-based protein shakes	52.0	106	na	4.5	180	2,331	4,166

na = not available

* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Appendix B
Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Different Population Groups Within the U.S. (2013-2014 NHANES Data)

Table B-1 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Infants and Young Children Aged 0 to <3 Year Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***4.7***	***12.4***	***12.3***	***60***	***38.0***	***80.4***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0	0	0	0	0	0	0
Breakfast Cereals							
Instant oatmeals	23.8	1.1	na	6.8	35	16.4	30.4*
Grain Products and Pastas							
Protein and nutrition bars	4.6	0.2*	na	0.5	3	45.1*	55.1*
Milk Products							
Greek yogurts, high protein yogurts	40.5	1.9*	na	4.3	14	44.5*	69.8*
Yogurt drinks	13.7	0.6*	na	1.9	11	33.2*	46.2*
Milk-based protein shakes	17.4	0.8*	na	0.7	4	125.6*	159.9*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-2 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Children Aged 3 to 11 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.0***	***3.9***	***10.8***	***122***	***18.2***	***42.7***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0	0	0	0	0	0	0
Breakfast Cereals							
Instant oatmeals	18.9	0.4	na	4.9	58	7.6	13.2*
Grain Products and Pastas							
Protein and nutrition bars	4.8	0.1*	na	0.9	11	10.6*	12.6*
Milk Products							
Greek yogurts, high protein yogurts	32.5	0.6*	na	2.1	17	30.8*	57.5*
Yogurt drinks	40.4	0.8	na	2.8	33	28.9	45.7*
Milk-based protein shakes	3.5	0.1*	na	0.3	7	21.0*	32.0*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-3 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Teenagers Aged 12 to 19 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.0***	***2.6***	***10.7***	***113***	***19.0***	***49.0***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0.6	<0.1*	na	0.3	5	4.8*	8.5*
Breakfast Cereals							
Instant oatmeals	2.6	0.1*	na	1.4	23	3.7*	5.4*
Grain Products and Pastas							
Protein and nutrition bars	9.2	0.2	na	3.1	30	6.1	9.5*
Milk Products							
Greek yogurts, high protein yogurts	13.9	0.3*	na	2.9	21	9.9*	16.1*
Yogurt drinks	14.3	0.3*	na	1.8	28	16.3*	24.9*
Milk-based protein shakes	59.2	1.2*	na	2.9	25	41.7*	57.3*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-4 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Adults Aged 20 to 39 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***3.3***	***7.8***	***17.2***	***232***	***19.3***	***43.6***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	3.5	0.1*	na	0.6	8	19.3*	32.0*
Breakfast Cereals							
Instant oatmeals	4.5	0.2	na	3.4	58	4.4	9.1*
Grain Products and Pastas							
Protein and nutrition bars	8.2	0.3	na	4.5	51	6.1	10.7*
Milk Products							
Greek yogurts, high protein yogurts	13.7	0.5	na	4.7	65	9.7	19.5*
Yogurt drinks	15.8	0.5	na	3.2	47	16.3	29.1*
Milk-based protein shakes	54.2	1.8	na	5.3	62	34.3	84.7*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-5 Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Older Female Adults Aged 40 to 64 Years Within the U.S. (2013-2014 NHANES Data)

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.9***	***9.2***	***21.6***	***185***	***13.2***	***27.9***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	0.4	<0.1*	na	0.5	6	2.2*	2.8*
Breakfast Cereals							
Instant oatmeals	9.6	0.3	na	6.2	63	4.4	8.1*
Grain Products and Pastas							
Protein and nutrition bars	5.6	0.2*	na	2.4	23	6.5*	14.8*
Milk Products							
Greek yogurts, high protein yogurts	30.7	0.9	na	7.6	52	11.6	23.7*
Yogurt drinks	15.6	0.4	na	3.3	32	13.5	22.0*
Milk-based protein shakes	38.1	1.1*	na	4.1	28	26.7*	66.4*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-6 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Older Male Adults Aged 40 to 64 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	**2.2**	**6.3**	**16.3**	**115**	**13.4**	**34.1**
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	3.0	0.1*	na	0.6	8	10.9*	24.9*
Breakfast Cereals							
Instant oatmeals	4.6	0.1*	na	2.9	29	3.4*	6.2*
Grain Products and Pastas							
Protein and nutrition bars	6.1	0.1*	na	3.6	21	3.6*	6.2*
Milk Products							
Greek yogurts, high protein yogurts	28.6	0.6	na	5.8	30	10.7	18.4*
Yogurt drinks	9.3	0.2*	na	1.7	19	12.1*	18.8*
Milk-based protein shakes	48.4	1.1*	na	3.1	20	34.3*	49.1*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-7 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Female Elderly Adults Aged ≥65 Years Within the U.S. (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.6***	***7.6***	***21.6***	***98***	***11.7***	***24.4***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	1.8	<0.1*	na	1.0	7	4.5*	5.6*
Breakfast Cereals							
Instant oatmeals	9.9	0.3	na	5.8	37	4.3	5.8*
Grain Products and Pastas							
Protein and nutrition bars	1.4	<0.1*	na	1.2	5	2.9*	3.9*
Milk Products							
Greek yogurts, high protein yogurts	20.4	0.5*	na	7.0	21	7.3*	10.3*
Yogurt drinks	13.3	0.3*	na	1.7	8	19.9*	30.5*
Milk-based protein shakes	53.2	1.3*	na	7.0	28	19.1*	48.0*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

Table B-8 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by Male Elderly Adults Aged ≥65 Years Within the US Population (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***1.6***	***2.7***	***11.9***	***59***	***13.6***	***19.1***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	2.5	<0.1*	na	0.4	3	10.0*	15.0*
Breakfast Cereals							
Instant oatmeals	16.4	0.3	na	5.8	35	4.5	8.0*
Grain Products and Pastas							
Protein and nutrition bars	0.3	<0.1*	na	0.2	1	2.5*	2.5*
Milk Products							
Greek yogurts, high protein yogurts	13.6	0.2*	na	3.1	9	7.1*	9.5*
Yogurt drinks	2.5	<0.1*	na	0.4	1	10.9*	10.9*
Milk-based protein shakes	64.7	1.0*	na	4.0	16	25.8*	57.3*

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Table B-9 **Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Individual Proposed Food-Uses by the Total Population Aged ≥12 Years Within the US Population (2013-2014 NHANES Data)**

Food-Use Category	% Contribution to Total Mean Intake	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
All	***100***	***2.7***	***6.9***	***17.7***	***802***	***15.5***	***53.7***
Beverages and Beverage Bases							
Protein shakes; meal replacement drinks	2.3	0.1	na	0.6	37	10.7	34.6*
Breakfast Cereals							
Instant oatmeals	6.4	0.2	na	4.0	245	4.2	7.4
Grain Products and Pastas							
Protein and nutrition bars	6.5	0.2	na	3.1	131	5.5	10.7
Milk Products							
Greek yogurts, high protein yogurts	20.4	0.5	na	5.4	198	10.1	19.1
Yogurt drinks	13.8	0.4	na	2.4	135	15.1	24.0
Milk-based protein shakes	50.6	1.3	na	4.4	179	30.7	71.5

bw = body weight; na = not available; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

Appendix C
Representative Food Codes for Proposed Food-Uses of Urolithin A in the U.S. (2013-2014 NHANES Data)

Representative Food Codes for Proposed Beverage-Uses of Urolithin A in the U.S. (U.S. NHANES 2013-2014)

Beverages and Beverage Bases

Protein Shakes and Meal Replacement Drinks
[Urolithin A] = 140 mg/100g

95104000	Glucerna, nutritional shake, ready-to-drink
95120050	Nutritional drink or meal replacement, liquid, soy-based

Adjusted for not being reconstituted, 16 g of powder to 240 mL of water
[Urolithin A] = 2,240 mg/100g

95201300	EAS Soy Protein Powder
95230010	Protein powder, soy based, NFS
95230020	Protein powder, light, NFS
95201600	Isopure protein powder
95201700	Kellogg's Special K20 Protein Water Mix
95201500	Herbalife, nutritional shake mix, high protein, powder

Breakfast Cereals

Instant Oatmeals
[Urolithin A] = 210 mg/100g

56202960	Oatmeal, cooked, NS as to regular, quick or instant; NS as to fat added in cooking
56203000	Oatmeal, cooked, NS as to regular, quick or instant, fat not added in cooking
56203030	Oatmeal, cooked, instant, fat not added in cooking
56203040	Oatmeal, cooked, NS as to regular, quick, or instant, fat added in cooking
56203070	Oatmeal, cooked, instant, fat added in cooking
56203080	Oatmeal, cooked, instant, NS as to fat added in cooking
56203110	Oatmeal with maple flavor, cooked
56203200	Oatmeal with fruit, cooked
56203210	Oatmeal, NS as to regular, quick, or instant, made with milk, fat not added in cooking
56203213	Oatmeal, cooked, instant, made with milk, fat not added in cooking
56203220	Oatmeal, NS as to regular, quick, or instant, made with milk, fat added in cooking
56203223	Oatmeal, cooked, instant, made with milk, fat added in cooking
56203230	Oatmeal, NS as to regular, quick, or instant, made with milk, NS as to fat added in cooking
56203233	Oatmeal, cooked, instant, made with milk, NS as to fat added in cooking

Grain Products and Pastas

Protein and Nutrition Bars
[Urolithin A] = 1,250 mg/100g

53710800	Kashi GOLEAN Chewy Bars
53710804	Kashi GOLEAN Crunchy Bars
53711002	Quaker Chewy 90 Calorie Granola Bar
53711004	Quaker Chewy 25% Less Sugar Granola Bar
53720100	Balance Original Bar

53720200	Clif Bar
53720300	PowerBar
53720400	Slim Fast Original Meal Bar
53720500	Snickers Marathon Protein bar
53720600	South Beach Living Meal Bar
53720610	South Beach Living High Protein Bar
53720700	Tiger's Milk bar
53720800	Zone Perfect Classic Crunch nutrition bar
53729000	Nutrition bar or meal replacement bar, NFS

Milk Products

Greek and High Protein Yogurts
[Urolithin A] = 590 mg/100g

11424500	Yogurt, Greek, vanilla, whole milk
11424510	Yogurt, Greek, vanilla, low fat
11424520	Yogurt, Greek, vanilla, nonfat
11428000	Yogurt, Greek, chocolate, nonfat
11434000	Yogurt, Greek, fruit, whole milk
11434010	Yogurt, Greek, fruit, low fat
11434020	Yogurt, Greek, fruit, nonfat

Yogurt Drinks
[Urolithin A] = 500 mg/100g

11553100	Fruit smoothie, NFS
11553110	Fruit smoothie, with whole fruit and dairy
11553120	Fruit smoothie, with whole fruit and dairy, added protein
11553130	Fruit smoothie juice drink, with dairy

Milk-Based Protein Shakes
[Urolithin A] = 420 mg/100g

95101000	Boost, nutritional drink, ready-to-drink
95101010	Boost Plus, nutritional drink, ready-to-drink
95103000	Ensure, nutritional shake, ready-to-drink
95103010	Ensure Plus, nutritional shake, ready-to-drink
95105000	Kellogg's Special K Protein Shake
95106000	Muscle Milk, ready-to-drink
95106010	Muscle Milk, light, ready-to-drink
95120000	Nutritional drink or meal replacement, ready-to-drink, NFS
95120010	Nutritional drink or meal replacement, high protein, ready-to-drink, NFS
95120020	Nutritional drink or meal replacement, high protein, light, ready-to-drink, NFS

Adjusted for not being reconstituted, 16 g of powder to 240 mL of water
[Urolithin A] = 6,720 mg/100g

95201200	EAS Whey Protein Powder
95220000	Nutritional drink mix or meal replacement, powder, NFS
95220010	Nutritional drink mix or meal replacement, high protein, powder, NFS
95230000	Protein powder, whey based, NFS

95230030 Protein powder, NFS

Adjusted for not being reconstituted, 50 g of powder to 454 mL of water
[Urolithin A] = 3,150 mg/100g

95202010 Muscle Milk, light, powder

Adjusted for not being reconstituted, 70 g of powder to 454 mL of water
[Urolithin A] = 4,200 mg/100g

95202000 Muscle Milk, regular, powder

Appendix 2

Expert Panel Consensus Statement

Expert Panel Report Concerning the Generally Recognized as Safe (GRAS) Status of Urolithin A for Use in Foods

November 17, 2017

INTRODUCTION

At the request of Amazentis SA, 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, under the conditions of intended use as a nutrient in traditional foods in the U.S. based on its nutritive activity in supporting general mitochondrial health, urolithin A would be "Generally Recognized as Safe" (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts: Dr. David H. Bechtel (Bechtel Consulting, Inc.), Robert J. Nicolosi (RJ Nicolosi, LLC), and Dr. John A. Thomas (Tom-Tox, LLC).

The Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on searches of the published scientific literature conducted through September 2017. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by Amazentis SA. The data evaluated by the Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of urolithin A.

Following independent, critical evaluation of such data and information, the Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, urolithin A, meeting appropriate food-grade specifications, and manufactured and used in accordance with current Good Manufacturing Practice (cGMP), is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

COMPOSITION, MANUFACTURING AND SPECIFICATIONS

Urolithin A is manufactured in compliance with cGMP, according to a well-established process. It is manufactured *via* chemical syntheses using an Ullmann coupling reaction, followed by ether cleavage and purification to yield a highly purified urolithin A product. The final product is purified by standard means of treatment in solvents, filtered, washed and dried to obtain pure urolithin A. The product is later subjected to a particle size reduction. Analysis of 4 lots of urolithin A demonstrates that the manufacturing process produces a consistent product that meets specifications. Amazentis has demonstrated that the non-micronized ingredient is stable for 3 years at room temperature. The micronized ingredient is undergoing testing up to three years and has demonstrated stability for a period of 2 years.

INTENDED USE AND ESTIMATED EXPOSURE

Amazentis intends to market urolithin A as an ingredient in select foods or for special dietary uses in meal replacement products based on its nutritive activity in supporting general mitochondrial health. As summarized in Table 1, these include powdered (reconstituted) protein shakes, beverages (ready-to-drink protein shakes, non-milk based meal replacement beverages, instant oatmeal, protein and nutrition bars, and yogurts (Greek yogurts, high-protein yogurts, and yogurt drinks) at typical use levels of 250 mg/serving or 500 mg/serving up to a maximum of 500 mg/serving or 1,000 mg/serving.

Table 1 Summary of the Individual Proposed Food-Uses and Use Levels for Urolithin A in the U.S.

Category	Food-Uses	Urolithin A Level, as Consumed (mg/serving)	RACC[a] (g or mL)	Urolithin A Use Levels (mg/100 g or mg/100 mL)
Food Categories (21 CFR 170.3				
Beverages and Beverage Bases	Protein shakes; meal replacement drinks	500	360	140
Breakfast Cereals	Instant oatmeals	500	240	210
Grain Products and Pastas	Protein and nutrition bars	500	40	1,250
Milk Products	Greek yogurts, high protein yogurts[b]	1,000	170	590
	Yogurt drinks[c]	500	100[d]	500
	Milk-based protein shakes	1,000	240	420

CFR = Code of Federal Regulations; na = not applicable; RACC = Reference Amounts Customarily Consumed per Eating Occasion; U.S. = United States.

[a] RACC based on values established in 21 CFR §101.12 (U.S. FDA, 2016a).

[b] No food codes were identified for yogurt drinks, but based on the high content of protein in Greek yogurts, these were deemed a suitable surrogate

[c] No food codes were identified for yogurt drinks within the NHANES dataset; however, food codes for dairy-based fruit smoothies drinks were selected as surrogates to represent the food codes in this category.

[d] RACC has not been established for yogurt drinks; however, an approximate serving size was established based on products currently in the U.S. market.

The estimated intake of urolithin A was generated using the maximum use level indicated for each intended food-use, together with food consumption data available from the 2013-2014 National Health and Nutrition Examination Survey (NHANES) database. A summary of the estimated daily intake is provided in Table 2 on absolute basis (g/person/day) and in Table 3 on a body weight basis (mg/kg body weight/day).

As shown in Table 2, on an absolute basis, for the total population excluding all children (≥12 years), the mean and 90th percentile consumer-only intakes of Urolithin A were determined to be 1,183 and 2,421 mg/person/day, respectively. Of the individual population groups, adults aged 20 to 39 were determined to have the greatest mean consumer-only intakes of Urolithin A on an absolute basis, at 1,528 mg/person/day, while teenagers had the greatest 90th percentile consumer-only intakes of 3,494 mg/person/day. Female elderly adults had the lowest statistically reliable 90th percentile consumer-only intakes of 1,341 mg/person/day, respectively.

Table 2 Summary of the Estimated Daily Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/ day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Teenagers	12 to 19	140	173	10.6	113	1,320	3,494
Adults	20 to 39	263	632	17.2	232	1,528	2,688
Older Female Adults	40 to 64	205	723	21.6	185	949	1,884
Older Male Adults	40 to 64	182	502	16.3	115	1,122	3,226
Female Elderly Adults	≥65	167	529	21.6	99	773	1,341
Male Elderly Adults	≥65	157	322*	14.1	60	1,118	2,381*
Total Population	≥12	204	538	17.2	804	1,183	2,421

On a body weight basis, the total population mean and 90th percentile consumer-only intakes of urolithin A were determined to be 15.5 and 34.1 mg/kg body weight/day, respectively. Among the individual population groups, adults aged 20 to 39 years were identified as having the highest mean consumer-only intake of any population group, of 19.3 mg/kg body weight/day, while teenagers were determined to have the highest consumer-only 90th percentile intake of 49.0 mg/kg body weight/day. Female elderly adults had the lowest mean and the lowest reliable 90th percentile consumer-only intakes of 11.7 and 24.4 mg/kg body weight/day, respectively (Table 3).

Table 3 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Urolithin A from Proposed Food-Uses in the U.S. by Population Group (2013-2014 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Teenagers	12 to 19	2.0	2.6	10.7	113	19.0	49.0
Adults	20 to 39	3.3	7.8	17.2	232	19.3	43.6
Older Female Adults	40 to 64	2.9	9.2	21.6	185	13.2	27.9
Older Male Adults	40 to 64	2.2	6.3	16.3	115	13.4	34.1
Female Elderly Adults	≥65	2.5	7.6	21.6	98	11.7	24.4
Male Elderly Adults	≥65	1.6	2.7*	11.9	59	13.6	19.1*
Total Population	≥12	2.7	6.9	17.1	802	15.5	34.1

bw = body weight; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.
* Indicates an intake estimate that may not be statistically reliable, as the sample size does not meet the minimum reporting requirements.

While younger populations were identified as the groups having higher exposures to urolithin A on a body weight basis, it should be noted that products containing urolithin A will not be targeted towards these populations. Furthermore, estimates described herein assume all products, including those consumed by younger individuals would contain the ingredient at the maximum intended use levels. In actuality, these products would, in the worst case, only be consumed incidentally and intakes described in the older populations are expected to be more accurate estimates of dietary exposure among the intended target consumer group.

Adults aged 40 years and above are intended to be the primary consumers of the ingredient. The target consumers will intentionally seek urolithin A and select these foods based on the labeling for its nutritional benefits in supporting mitochondria function among the elder population. Because there are not a wide variety of target foods that could contain urolithin A, and given the premium pricing associated with food products fortified with urolithin A, it would be reasonable to assume that most consumers would eat 1 or 2 servings a day of such foods, depending on the level of urolithin A in the food. As such, an aggregated intake of 1,000 mg/person/day or 16.67 mg/kg bw/day is a more accurate estimate of the actual urolithin A intake.

DATA PERTAINING TO SAFETY

The safety of urolithin A is based on a series of published product-specific safety studies, including studies of the absorption, distribution, metabolism, and excretion of urolithin A, as well as subacute, subchronic and genotoxicity studies. Finally, as ellagitannins are metabolized to urolithin A, safety reports for ellagitannins or ellagitannin-containing food products, which can be assumed to result in some lower level of *in vivo* exposure to urolithins following their formation in the gastrointestinal tract, are considered supportive of the safety of urolithin A. The safety of urolithin A also is corroborated by a human clinical study that has not yet been published but was shared with the Expert Panel.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The absorption, distribution, metabolism and excretion of ingested urolithin A was followed by dosing Wistar rats with ^{14}C labeled urolithin A. Following a single oral administration, the majority of radioactivity was excreted *via* the feces; at 72 hours, approximately 115 and 121% of the administered dose was excreted in the feces of males and females, respectively. The high levels of excretion in the feces corresponded with the tissue distribution findings, which demonstrated the majority of the compound to be located in the gastrointestinal tract. In contrast, urinary excretion only accounted for 1.3% of the administered dose in males and in females after 72 hours. Plasma concentration of radiolabeled urolithin A (aglycone and metabolites) peaked around three hours and then again around 6 or 7 hours. The glucuronide, sulfated and aglycone forms of urolithin A were major metabolites of urolithin A in both plasma and urine.

Toxicological Studies

Subacute Studies

Administration of urolithin A *ad libitum* in the feed to SPF-bred Wistar rats (5 animals/sex/group) at a constant concentration of 0, 0.175, 1.75, and 5.0% of the diet for a period of at least 28 days produced no adverse effects on body weights, food consumption, hematology, biochemistry, or urinalysis. No changes were seen on the functional observational battery, locomotor activity or grip strength. No treatment-related gross or histopathological changes were seen. On this basis, the no-observed-effect level (NOEL) was considered to be 4,165 mg/kg/day for males and 4,705 mg/kg body weight/day for females, equivalent to the high-dose level of 5% (Heilman *et al.*, 2017).

Subchronic Studies

Urolithin A (>99% purity) was administered in the diet to Wistar rats (20 animals/sex) for at least 90 days at concentrations of 0, 1.25, 2.5, or 5.0%. Five additional animals per sex received 0 or 5.0% for 90 days and were observed for an additional 4-week recovery period. The overall mean achieved dosages during the 13-week treatment period were 834, 1,684, and 3,451 mg/kg/day for males and 896, 1,876, and 3,826 mg/kg/day for females receiving 12,500, 25,000, or 50,000 ppm, respectively. No toxicologically significant effects were observed at any of the doses tested.

There were no adverse effects or mortality observed. Minor differences in body weight gain were observed in males treated with urolithin A compared to controls, but not in females. These effects were considered unrelated to the compound, as they were not dose-dependent, only present in males, and withdrawal of the treatment did not result in any clear increase of weight gain during the recovery period. Consequently, it was concluded that body weights and gain were unaffected by treatment with the test item urolithin A. Although sporadic effects were noted in some hematology and clinical chemistry parameters in the main study groups, these slight alterations were not considered to be of toxicologically significance as all were without dose-response, appeared in only one sex, or were within the range of historical control values for the performing laboratory. Recovery phase animals demonstrated no toxicologically relevant changes during the recovery period. Ophthalmoscopic examinations were normal, and there were no indications of neurological toxicity as indicated by the functional observational battery screen, motor activity assessment, or relevant clinical observations. Likewise, there were no effects on spermatogenesis analysis, estrus cycle analysis, or reproductive organ weights or macro- or microscopic alterations

The no-observed-adverse-effect level (NOAEL) was determined to be 50,000 ppm, or 5% by weight of urolithin A in the rat diet, which is the highest achievable dose that does not induce dietary imbalances. This concentration corresponds to a NOAEL in male rats of 3,451 mg/kg body weight/day and in females of 3,826 mg/kg body weight/day (Heilman *et al.*, 2017).

Genotoxicity Studies

Urolithin A was not mutagenic in the bacterial reverse mutation test in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 using the plate incorporation test or the pre-incubation test. No toxic effects, or substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with urolithin A at any dose level, either in the presence or absence of metabolic activation (S9 mix) (Heilman *et al.*, 2017).

Urolithin A, dissolved in dimethylsulfoxide (DMSO), was assessed for its potential to induce micronuclei in human peripheral lymphocytes *in vitro* in three independent experiments. Following a preliminary dose range-finding cytotoxicity test, concentrations of 0.6, 1.5, 3.8, 9.4, 23.4, 58.6, 146.6, 366.4, 916.0, 2290.0 µg/mL were selected for Experiments IA and IB (4-hour exposure), while concentrations in Experiment II (without S9 mix, 20-hour exposure) of 0.3, 0.7, 1.6, 4.1, 10.2, 25.6, 64.0, 160.0, 400.0, 1,000.0 µg/mL were evaluated. In all experiments, regardless of treatment duration, cells were prepared for analysis 40 hours after the initiation of the indicated treatment window. Urolithin A demonstrated possible genotoxicity by this assay with positive results detected in some, but not all, treatment groups. In Experiment I in the absence of S9 mix, statistically significant increases in micronucleated cells slightly above the range of the laboratory historical solvent control data and the concurrent negative control data were observed after treatment with 23.4 and 58.6 µg/mL. In Experiment II in the absence of S9 mix after continuous treatment, statistically significant increases in micronucleated cells above the range of the laboratory historical solvent control data and the concurrent

negative control data (1.10%) were observed. However, the increase in micronucleated cells did not exhibit a dose-response relationship. In the presence of S9 mix, no increase in the number of micronucleated cells was observed (Heilman *et al.*, 2017).

The pattern of precipitation and cytotoxicity observed in this study did not account for the positive finding of genotoxic potential in this *in vitro* study. However, the increase in micronuclei with time of exposure and the absence of micronuclei formation in the presence of metabolic activation suggests that micronuclei formation may be a result of oxidative stress. This can be common to aromatic compounds such as urolithin A which has the potential to redox cycle. However, this mechanism will have a threshold and is unlikely to be relevant *in vivo*. Two additional *in vivo* micronucleus assays, discussed subsequently, were performed to provide confirmation.

In the first *in vivo* micronucleus assay, no cytotoxic effects were seen following administration of a single oral dose of urolithin A at 500, 1,000, or 2,000 mg/kg body weight. Likewise, no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei was seen. In addition, a repeat of the *in vivo* micronucleus assay was performed within the 90-day study. Five male and 5 female rats each from the control and all treatment groups were sampled for bone marrow from the femur, which was cleaned and prepared and analyzed for the formation of micronuclei. No micronucleus formation was observed in this study despite the repeat dose nature and longer duration of the dosing period in the 90-day study (Heilman *et al.*, 2017).

Other Published Toxicity Studies

Cerdá *et al.* (2003) evaluated the possible toxic effect of punicalagin upon repeated oral administration. Sprague-Dawley rats (10 animals/group) received either a control diet or diet containing 20% pomegranate husk extract containing an average of 6% punicalagin. Animals were monitored for effects on growth, antioxidant enzymes, hematology and clinical chemistry parameters, and pathological changes in the liver and kidney. The mean oral consumption throughout the study was reported to be 0.9 g punicalagin/day. Food intake, food utility index, and growth rate were lower in treated rats during the first 15 days. The authors noted that these findings could have been due to the decreased palatability and lower nutritional value of the punicalagin-enriched diet. A decrease in serum urea and triglyceride values were observed throughout the study. The decrease in urea was not associated with any changes in other liver parameters (*i.e.*, ALT, AST, ALP, and bilirubin) were normal. Although the decrease in triglycerides reached statistical significance, values remained within the normal range. No other significant differences were found in treated rats in any hematology or clinical chemistry parameter analyzed. Likewise, no histopathological changes were seen in the liver or kidney.

Patel *et al*. (2008) examined the acute and subchronic effects of a pomegranate fruit extract standardized to 30% punicalagins. The acute oral median lethal dose (LD_{50}) of the extract in rats and mice was found to be greater than 5 g/kg body weight. In the subchronic study, Wistar strain rats (10 animals/sex/group) were administered the extract *via* gavage at doses of 0 (control), 60, 240, and 600 mg/kg body weight/day for 90 days. Two additional groups received 0 and 600 mg/kg/day of the extract for 90 days, followed by a 28-day recovery period. Administration of the extract did not result in any toxicologically significant treatment-related changes in clinical observations, ophthalmic examinations, body weights, body weight gains, feed consumption, urinalysis, clinical pathology evaluations and organ weights compared to controls. Although some statistical changes were seen in hematology and serum chemistry parameters that showed statistical significant changes compared to control, values remained within the normal laboratory limits and were thus considered as biological variations rather than toxic effects. No treatment-related gross or histopathological findings were reported. The NOAEL for this study was thus considered to be 600 mg/kg body weight/day, the

highest dose tested.

Ryu *et al.* (2016) characterized the biological effects of urolithins, on lifespan and mitochondria in *Caenorhabditis elegans.* Feeding 50 µM urolithin A to *C. elegans* from eggs until death extended lifespan by 45.4% as compared to treatment with the vehicle (1% DMSO). No such effect was seen with ellagic acid. The effects of urolithin A were dose-dependent over concentrations ranging from 10 to 50 µM, with significant delay in the mortality observed at advanced ages ($p \leq 0.001$). Young worms (day 1 of adulthood) treated with urolithin A presented lower mitochondrial content when compared to control, but were able to maintain their respiratory capacity due to induction of selective autophagy of mitochondria (mitophagy) by urolithin A. This triggered mitochondrial biogenesis at later age, since 8- to 10-day-old worms exposed to urolithin A had an equivalent mitochondrial content, but higher mitochondrial respiratory capacity when compared to untreated worms. Likewise, urolithin A was shown to stimulate autophagy and mitophagy in mammalian muscle and intestinal cells. Administration of 50 mg/kg/day of urolithin A to 16-month-old male C57BL6J mice for 8 months prevented age-related muscle decline as measured by grip strength and spontaneous exercise. In a second mouse study, urolithin A supplementation for 6 weeks resulted in a 42% increase in running endurance in aged male C57BL/6J mice (22.5 months old). Increases in muscle function were also observed in young male (5.5 weeks old) Wistar rats fed commercial diets containing urolithin A at a concentration of 25 mg/kg/day as measured by a 65% greater running capacity than the control group.

Corroborating Safety Evidence

The Expert Panel relied on the published safety studies summarized above to support their GRAS position. Amazentis also shared the following summary results of a clinical study with the Expert Panel as corroborative evidence that the intended use of urolithin A is safe.[1]

The safety and tolerability of urolithin A was also evaluated in a single-center, double-blind, randomized study (NCT02655393). Specifically, this was a 2-part study with a single oral ascending dose Part A and a 4-week multiple ascending dose Part B. In Part A, 24 healthy elderly male and female volunteers [12 females and 12 males, ranging in age from 61 to 82 years (mean 68.7 ± 5.3 years) and body mass index (BMI) ranging from 20.2 to 30.4 kg/m² (mean 24.60 ± 2.72 kg/m²)] were randomized (6 subjects/group) to consume urolithin A in single ascending doses of either 250 mg, 500 mg, 1,000 mg and 2,000 mg or placebo. Each dose was separated by a washout period of 3 weeks. Similarly, in Part B, the safety and tolerability of urolithin A was evaluated following a 28-day (4 week) oral administration. 36 healthy elderly male and female volunteers [12 males and 24 females, ranging in age from 61 to 78 years (mean age 66.4 ± 4.9 years) and BMI ranging from 18.8 to 30.6 kg/m² (mean of 25.02 ± 3.04 kg/m²)] were randomized (9 subjects/group) to receive 250 mg, 500 mg, or 1,000 mg of urolithin A or placebo daily for 28 days. In Part A and Part B, urolithin A was administered orally, in fasting condition, in soft-gel formulations with water for all dosing's. Additionally, in Part A, urolithin A was administered in high protein yogurt at doses of 500 mg and 1,000 mg. In each study, subjects underwent physical examinations and electrocardiogram (ECG) evaluations and were monitored for adverse events. Liver and kidney function [i.e., creatinine, uric acid, alanine serine transferase (AST), alanine leucine transferase (ALT), gamma glutamyl transferase (GGT), and total and conjugated bilirubin] were evaluated before and after dosing's. Full laboratory tests included hematology [*i.e.*, hemoglobin, hematocrit, red blood cells (RBC), white blood cells (WBC), differential count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)] and urinalysis (pH, ketone bodies, proteins, glucose, and blood).

In the single dose Part A phase there were no serious adverse events (SAE) recorded for any dosing. 5 of 24 subjects reported the occurrence of 6 non-serious adverse events, of which none were considered related to intake of urolithin A. Adverse events were distributed across all dose groups, including placebo. No clinically

[1]Publication of the abstract of the clinical study is pending.

significant abnormal laboratory test values from study baseline were observed for any of the biochemistry tests assessing liver and kidney function, or for any of the hematology and urinalysis tests for any subjects at any of the doses during the course of the study. No abnormal and clinically significant conclusions were observed for ECG findings for any subjects taking active intervention at any of the doses during the course of the study. As a result, it was concluded that single dosing of urolithin A at the doses of 250 mg, 500 mg, 1,000 mg, or 2,000 mg was safe and well tolerated. In the multiple-dose (28 day/oral intake) Part B phase, no serious adverse events were reported. 31 non-serious adverse events were reported in 15 subjects (the majority being linked to study procedures, i.e. muscle biopsy), none were considered to be related to intake of urolithin A. No clinically significant changes were reported in liver and kidney function tests, hematology or urinalysis. No clinically relevant and abnormal findings were reported during physical examination. Vital signs were likewise unaffected. No significant abnormalities were reported during ECG examinations.

The results of the study support the conclusion that urolithin A is well tolerated and has a favorable safety profile when orally administered in single and multiple doses to elderly.

CONCLUSION

Having considered all the relevant information, it is our opinion as qualified experts that there is reasonable certainty that no harm will result from the intended use of Amazentis SA's urolithin A meeting appropriate food-grade specifications and manufactured in accordance with current Good Manufacturing Practices (cGMP), for use in the U.S. based on its nutritive activity in supporting general mitochondrial health. These include powdered (reconstituted) protein shakes, beverages (ready-to-drink protein shakes, non-milk based meal replacement beverages, instant oatmeal, protein and nutrition bars, and yogurts (Greek yogurts, high-protein yogurts, and yogurt drinks) at typical use levels of 250 mg/serving or 500 mg/serving up to a maximum of 500 mg/serving or 1,000 mg/serving.

These uses would result in mean and 90th percentile consumer-only intakes of 15.5 and 34.1 mg/kg body weight/day, respectively. Thus, there exists a margin of safety of at least 100 over the NOAEL (3451 mg/kg/bw/day) from the 90-day study. Furthermore, because there are not a wide variety of target foods that could contain urolithin A, and given the premium pricing associated with food products fortified with urolithin A, an aggregate intake of 1,000 mg/person/day or 16.67 mg/kg bw/day is considered to be a more accurate estimate of the actual urolithin A intake.

Such use would be considered Generally Recognized as Safe (GRAS) through scientific procedures, making Amazentis SA's urolithin A exempt from the premarket approval requirements outlined in section 201(s) of the Federal Food, Drug, and Cosmetic Act (U.S. FDA, 2016b).

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11/21/17

John A. Thomas, Ph.D., F.A.T.S., D.A.T.S
Adjunct Professor, Department of Pharmacology & Toxicology
Indiana University School of Medicine
Indianapolis, IN

Date

Robert J. Nicolosi, Ph.D., C.N.S.
Professor Emeritus, Department of Clinical Laboratory
and Nutritional Sciences
University of Massachusetts Lowell, Lowell MA

Date

David H. Bechtel, Ph.D., DABT
President,
Bechtel Consulting, Inc.
Monroe, NJ

Date

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John A. Thomas, Ph.D., F.A.T.S., D.A.T.S
Adjunct Professor, Department of Pharmacology & Toxicology
Indiana University School of Medicine
Indianapolis, IN

Date

(b) (6)

Robert J. Nicolosi, Ph.D., C.N.S.
Professor Emeritus, Department of Clinical Laboratory
and Nutritional Sciences
University of Massachusetts Lowell, Lowell MA

20, Nov/2017
Date

David H. Bechtel, Ph.D., DABT
President,
Bechtel Consulting, Inc.
Monroe, NJ

Date

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John A. Thomas, Ph.D., F.A.T.S., D.A.T.S
Adjunct Professor, Department of Pharmacology & Toxicology
Indiana University School of Medicine
Indianapolis, IN

Date

Robert J. Nicolosi, Ph.D., C.N.S.
Professor Emeritus, Department of Clinical Laboratory
and Nutritional Sciences
University of Massachusetts Lowell, Lowell MA

Date

(b) (6)

David H. Bechtel, Ph.D., DABT
President,
Bechtel Consulting, Inc.
Monroe, NJ

11-20-2017

Date

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