



Notice to US Food and Drug Administration of the Conclusion that the Intended Use of Guayusa Leaf Aqueous Extract (RUNA® Concentrate) is Generally Recognized as Safe

Submitted by the Notifier:

All Market Inc.
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Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc.
2800 E. Madison St., Ste. 202
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August 19, 2019



AIBMR Life Sciences, Inc.

August 19, 2019

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

Dear Dr. Carlson:

In accordance with regulation 21 CFR Part 170 Subpart E (Generally Recognized as Safe (GRAS) Notice), on behalf of All Market Inc. (the notifier), the undersigned, Amy Clewell, submits, for FDA review, the enclosed notice that Guayusa leaf aqueous extract (RUNA® Concentrate) is GRAS for use in foods.

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

Sincerely,

Amy Clewell, ND, DABT (agent of the notifier)
VP, Scientific and Regulatory Affairs
AIBMR Life Sciences, Inc. ("AIBMR")



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Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

All Market Inc. (the notifier) is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that guayusa leaf aqueous extract (RUNA® Concentrate) is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act.

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

Notifier

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1.3 Name of the Substance

Guayusa leaf aqueous extract (RUNA® Concentrate)

1.4 Intended Conditions of Use

RUNA® Concentrate is intended to be used as ingredient in carbonated and still energy beverages at an addition level of 150 mg caffeine/serving, equivalent to an average addition level of 5000 mg of RUNA® Concentrate per serving. RUNA®



Concentrate is not intended for use in foods where standards of identity would preclude such use, infant formula, or any products that would require additional regulatory review by USDA. It is also not intended to be used in beverages containing alcohol, or in beverages intentionally marketed to children.

1.5 Statutory Basis for GRAS Conclusion

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

1.6 Not Subject to Premarket approval

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

1.7 Data and Information Availability Statement

The data and information that serve as the basis for this GRAS conclusion will be available for review and copying during customary business hours at the office of All Market Inc. 250 Park Ave South (at 20th St) 7th Floor, New York, NY 10003 USA, or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

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



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1.9 Certification of Completion

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

August 20, 2019

Aytunc Atabek
Sr. Director of Quality and R&D
Notifier

Date

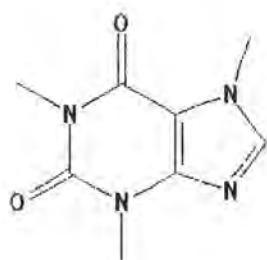


Part 2: Identity, Manufacture, Specifications, and Physical or Technical Effect

2.1 Identification

RUNA® Concentrate is manufactured from the leaves of the *Ilex guayusa* plant found in the Amazon region. *I. guayusa* is a small shrub or tree with smooth bark, growing at low elevations from southern Colombia to northern Peru.^{1, 2} It is a cultivated plant, and its scientific classification is as follows: kingdom Plantae, order Aquifoliales, family Aquifoliaceae, genus *Ilex* (which contains ~600 species), and species *Ilex guayusa*.²⁻⁶ The guayusa fruit is a drupe of 6–7 mm in diameter and is green when immature and dark red when ripe, and the morphology of the fruit suggests suitability for bird dispersal.² *I. guayusa* plant constituents include the methylxanthines caffeine and theobromine, phenols, tannins, reducing sugars, steroids, terpenes, carotenoids, flavonoids, and quinones.^{2, 7-10}

The caffeine content of guayusa tea (hot water extract) has been found to be similar to that of *Camellia sinensis* tea (2.9–3.2% in guayusa tea versus 2.6–3.1% in *C. sinensis* tea).⁹ RUNA® Concentrate is an aqueous extract of raw guayusa leaves, with a caffeine concentration of 2.7–3.7%. Caffeine (CAS #58-08-2; synonyms include 1,3,7-trimethylxanthine and methyltheobromine) is a white crystalline bitter water-soluble xanthine alkaloid, with the molecular formula C₈H₁₀N₄O₂ and a molecular mass of 194.19 g/mol.¹¹ It occurs naturally in more than 60 plant species around the world, including *Coffea* spp. (source of coffee), *C. sinensis* (source of tea), *Theobroma cacao* (source of chocolate), *Cola* spp. (source of kola nuts), *Ilex paraguariensis* (source of yerba maté) and *Paullinia cupana* (guarana). It is a component of foods and beverages made from these plants, most of which have been consumed for centuries. Coffee is the most common source of caffeine in the U.S. diet when all age groups are considered,¹²⁻¹⁵ and chemical analyses of coffee beverages have demonstrated wide ranges of caffeine content (e.g., 107–194 mg per 12 oz. serving for coffee, 48–322 mg per espresso serving).¹⁶⁻¹⁹



**Figure 1. Chemical Structure of Caffeine²⁰**

Chlorogenic acids (CAs, hydroxycinnamic acid derivatives) are phenolic compounds found in relatively high levels in guayusa leaves.⁷ Phenolic compounds are secondary plant metabolites known to exhibit antioxidant activities²¹⁻²³ and have been associated with a host of beneficial effects largely attributed to their inherent antioxidant potentials.²⁴⁻²⁶ Analysis of RUNA® Concentrate, as shown in section 2.3.4 of this report, suggests that the ingredient is approximately 5.2% chlorogenic acids.

Hydroxycinnamic acid derivatives are the major subclass of plant phenolic acid compounds.²⁷⁻²⁹ The most common hydroxycinnamic acids include *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid; these compounds are ubiquitous in nature and largely exist as quinic acid and glucose ester derivatives.³⁰ Among these phenolic esters, CAs are recognized as the most abundant hydroxycinnamic acid derivatives found in fruits and vegetables and are notably at high levels in coffee beans.³¹⁻³³

In its classical singular form, CA refers to 5-*O*-caffeoylequinic acid (5-CQA) although it is still often called 3-caffeoylequinic acid or 3-CQA, its pre-IUPAC numbering identification, which has caused much confusion in the literature. The complex nomenclature of cyclitols, including quinic acids and the acyl-quinic acids commonly known as CAs, has been reviewed in the literature.^{34,35} Confusion arises in part from the use of trivial names (fully explained in the supplementary information to these reviews) but primarily from the availability of two numbering systems for the cyclohexane ring and the failure of authors to define which system is being used. C2 and C3 in one system become C6 and C5, respectively, in the other (e.g., 5-CQA (IUPAC) and 3-CQA (IUPAC) are regioisomers, while 5-CQA (IUPAC) and 3-CQA (non-IUPAC) are the same compound). The confusion is confounded when both systems are used arbitrarily in the same publication.^{19,34-36} Even when not stated explicitly, it is possible in most cases to determine which system of numbering has been used, and in this document any non-IUPAC numbering has been changed to IUPAC (1976) numbering and the change noted explicitly. Similarly, where it is impossible to define which system has been used, no change was made, and this also is noted explicitly. For the purposes of presentation and comparisons made later in this document, compositional data for certain CA isomers (e.g., 3-CQA and 5-CQA) are combined in various summary tables herein.

The CQAs are comprised of caffeic acid and quinic acid covalently bonded via an ester linkage^{26,29,37}; the IUPAC isomers include 5-CQA (chlorogenic acid), 4-CQA (cryptochlorogenic acid), and 3-CQA (neochlorogenic acid). In its plural form, CAs (often written as singular “chlorogenic acid” in the literature) collectively refer to a group of closely related isomers and derivatives.^{29,38} These include dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), diferuloylquinic acids (diFQA), *p*-



coumaroylquinic acids (pCoQA), caffeoylferuloylquinic acids (CFQA), dimethoxycinnamoyl-caffeoylequinic acids (dimCQAs) and others.³⁹ The major CAs in green/roasted coffee beans are 5-CQA, 3-CQA and 4-CQA (all three have a molecular formula of C₁₆H₁₈O₉ and a molecular weight of 354.311 g/mol), with lower amounts of FQAs and diCQAs (see Figure 2 below). As discussed below, the same CAs are present in guayusa extracts. The composition of the individual CAs with regard to R-group substitutions are also shown for clarity in Table 1 below.

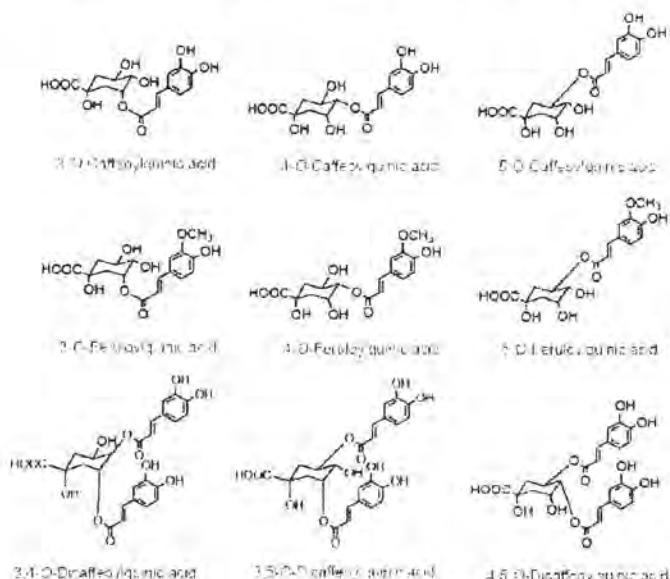


Figure 2. Chemical Structures (IUPAC nomenclature) of Major Chlorogenic Acids in Green Coffee Beans (borrowed in part with permission from del Rio et al., 2010)⁴⁰

Table 1. R-group Substitutions of Quinic Acid in Chlorogenic Acids (table borrowed in part from Kreml et al., 2016; Structure of (-)Quinic Acid Shown in Top Row)³⁶

Compound Abbreviation (IUPAC)	Identity of R3	Identity of R4	Identity of R5
3-CQA	Caffeic acid	Hydrogen	Hydrogen
4-CQA	Hydrogen	Caffeic acid	Hydrogen
5-CQA	Hydrogen	Hydrogen	Caffeic acid
3-FQA	Ferulic acid	Hydrogen	Hydrogen
4-FQA	Hydrogen	Ferulic acid	Hydrogen
5-FQA	Hydrogen	Hydrogen	Ferulic acid



3,4-dicQA	Caffeic acid	Caffeic acid	Hydrogen
3,5-dicQA	Caffeic acid	Hydrogen	Caffeic acid
4,5-dicQA	Hydrogen	Caffeic acid	Caffeic acid

Garcia-Ruiz et al. (2017) characterized the polyphenols found in guayusa.⁷ Leaves of fresh (stored at -20°C until freeze-dried) and processed (blanched and fermented before freeze-dried) guayusa were extracted using a methanol/water mixture. Polyphenols were identified by HPLC-DAD-ESI-MS methodology, as well as by a more traditional method using Folin-Ciocalteu reagent. A total of 14 phenolic compounds were detected, of which nine corresponded to hydroxycinnamic acids and related derivatives (the leaves were especially rich in the IUPAC-named compounds 5-CQA, 3-CQA, and 3,5-diCQA), and five were flavonols (the most abundant being quercetin-3-O-hexose). 5-CQA (IUPAC) stood out as the most abundant phenolic compound (at 24.10 mg/g), and the authors stated that the concentration was similar or higher than that found in maté (21–28 mg/g) and black/green tea (0.2–0.5 mg/g) and lower than that found in green coffee (50–120 mg/g). The flavonol concentration was 11 mg/g, which was higher than that previously described for maté and other *Ilex* species (0.5–5 mg/g). Quercetin-3-O-hexose was the most abundant flavonol glycoside in the guayusa extracts. The authors explained that quercetin is also the most abundant flavonol glycoside in tea varieties, although flavonol concentrations are reportedly lower in tea (e.g., 0.4 mg/g). Carotenoid content was 287–469 µg/g (consisting of α- and β-carotene, lutein, violaxanthin and neoxanthin). Antioxidant capacity was also evaluated and was found by the authors to be in line with other beverages with high antioxidant capacity such as maté and green teas and was found to decrease following leaf fermentation.

Villacís-Chiriboga et al.⁸ also found that 5-CQA, 3-CQA, and 3,5-diCQA were the major phenolic compounds in the leaves of the guayusa plant and that leaf age has diminishing effects on phenolic content and antioxidant capacity. Several scientific publications have also highlighted the antioxidant activity of guayusa plant material.^{9,41}

Catechins are the major polyphenol group of green tea (*Camilla sinensis*) and are also found in *Ilex* species.^{10,42} Section 2.3.4 discusses that RUNA® Concentrate contains approximately 0.36% catechins. Catechins are flavan-3-ols, which are a subcategory of flavonoids. The major catechin in green tea is (-)-epigallocatechin gallate (EGCG), with lesser amounts of catechin (C), (-)-epicatechin (EC), galloycatechin (GC), galloycatechin gallate (GCG) and (-)-epicatechin gallate (ECG).^{42,43} A daily intake of 3–5 cups per day of green tea is estimated to provide at least 250 mg/day of catechins.⁴² These same compounds have also been found at low levels in guayusa extracts.¹⁰ Catechins are known antioxidants, and



consumption of tea has been linked to various health benefits, which are usually attributed to the catechin content.⁴⁴⁻⁴⁶

Theobromine (3,7-dimethylxanthine) is a naturally occurring methylxanthine that is found in cocoa, chocolate products and tea, and is also found in various *Ilex* species.^{3, 47-49} It can be present at low levels in guayusa extracts,¹⁰ and additional analysis of RUNA® Concentrate discussed in Part 2.3.4 of this report suggest a theobromine level of approximately 0.03%.

Theophylline is another naturally occurring methylxanthine that can occur in plants with caffeine and theobromine.^{48, 50-52} Testing of the guayusa leaves used to make RUNA® Concentrate shows that it contains <50 ppm of theophylline, and testing of the RUNA® Concentrate extract itself shows that it contains <5 ppm theophylline.

Isoflavones are naturally occurring in a number of plants, especially in soybeans, red clover and kudzu root. Major isoflavones include genistein, daidzein, glycinein, formononetin, biochanin A and puerarin, and their chemical structures are related to 17 β -estradiol.⁵³ Additional analysis of RUNA® Concentrate discussed in Part 2.3.4 of this report suggest an isoflavone concentration of approximately 0.08%.

2.2 Manufacturing

2.2.1 Manufacturing Overview

The manufacturing process for RUNA® Concentrate begins with the harvesting of fresh guayusa leaves from growers in Central America. When the leaves reach the factory, they are placed in pre-drying area where over the course of several days, the moisture content is reduced by 40%. They are then placed in drying ovens, which reduces moisture content below 6%. The dried leaves are then milled and sorted into three different sizes. Microbiological testing is performed on one 50-gram sample from one batch of leaves produced each week. The processed guayusa leaves are packed in 4-ply tea sacks, each containing 45 to 90 pounds of dried milled guayusa.

RUNA® concentrate is an aqueous extract of the dried guayusa leaves, and its manufacturing process is comparable to brewing tea on a large scale. The extract is concentrated by a gentle evaporation phase.

2.2.2 Good Manufacturing Practice

Production of RUNA® Concentrate complies with US and European Pharmacopoeias, Food Chemicals Codex, Hazard Analysis and Critical Control Point systems, and WHO, as well as laws and governmental regulations of the US FDA and the European Community and their member states. These apply to production unit operations, biotechnological processing aids, utilities, and quality



control and assurance procedures. Independent, third party auditors are used to assess the Food Safety Programs at the production facility and laboratories on an annual basis. Production standards include traceability in regard to raw materials, packaging materials, and finished goods. All products are produced in accordance with finished product specifications and are manufactured, verified, packed, stored and shipped under cGMP regulations. The most recent third-party inspection took place in May of 2018.

2.2.3 Raw Materials

Raw materials used in the production of RUNA® Concentrate are of appropriate food grade. No material of human or animal origin is used in the manufacturing process. RUNA® Concentrate is not manufactured from genetically modified plant material and is not produced using irradiation or ethylene oxide treatments.

2.3 Specifications

The specifications for the food-grade RUNA® Concentrate product, along with the specification methods, are listed in Table 2 below.

Table 2. RUNA® Concentrate Specifications

Test Items	Specification	Method
Caffeine	2.7–3.7%	AOAC 980.14
Taste/Odor	Characteristic of guayusa	ISO 10399:2004
Appearance	Dark brown viscous liquid	ISO 10399:2004
Flash Point	>212°F (closed cup)	ASTM D6450
Brix	Report*	AOAC 932.12
Solubility	Water soluble	
Solids	As Is*	AOAC 925.19
pH @ 25°C	As Is*	AOAC 973.41
Moisture	As Is*	AOAC 925.19
Heavy Metals		
Arsenic	<1.0 ppm	AOAC 2013.06 (ICP-MS)
Cadmium	<1.0 ppm	AOAC 2013.06 (ICP-MS)
Lead	<1.0 ppm	AOAC 2013.06 (ICP-MS)
Mercury	<1.0 ppm	AOAC 2013.06 (ICP-MS)
Microbiological Tests		
Total Aerobic Plate Count	<10,000 CFU/mL	AOAC 966.23
Total Yeast & Mold	<1000 CFU/mL	FDA BAM CH 18
<i>Escherichia coli</i>	<0.3 MPN/mL	AOAC 966.24
<i>Salmonella</i>	Negative	AOAC 2013.01
<i>Staphylococcus aureus</i>	< 1 CFU/mL	FDA BAM CH 12
Pesticide Residue		
Pesticides	Not detected	AOAC 2007.01 (GC-MSMS)



*While these parameters are tested on every batch, they do not have true specification limits.

Abbreviations: ISO=International Organization of Standardization; ASTM=American Society for Testing and Materials; ICP-MS=Inductively coupled plasma mass spectrometry; FDA BAM=Food and Drug Administration's Bacteriological Analytical Manual; GC-MSMS=Gas Chromatography-Tandem Mass Spectrometry.

2.3.1 Batch Analysis

Production conformity and consistency of RUNA® Concentrate is tested in production lots. Batch analyses of three non-consecutive lots are shown below in Table 3 and are reasonably consistent and met the product specifications for the marker compounds, physical/chemical composition, product content/identity, manufacturing impurities, heavy metals, microbial analyses and residual solvents.

Table 3. RUNA® Concentrate Batch Analyses

Test Items	Specification	Lot# GUY415-15244	Lot# GUY415-15245	Lot# GUY415-15360
Caffeine	2.7-3.7%	3.0%	2.7%	2.8%
Taste/Odor	Characteristic of guayusa	Conforms	Conforms	Conforms
Appearance	Dark brown viscous liquid	Conforms	Conforms	Conforms
Flash Point (closed cup)	>212°F	>212°F	>212°F	>212°F
Brix	42°Bx-45°Bx	42.7°Bx	42.1°Bx	42.6°Bx
Solubility	Water soluble	Conforms	Conforms	Conforms
Solids	AS IS*	36.81%	34.82%	38.80%
pH @ 25°C	AS IS*	5.24	5.17	5.01
Moisture	AS IS*	63.19%	65.18%	61.20%
Heavy Metals				
Arsenic	<1.0 ppm	<0.02 ppm	<0.02 ppm	<0.02 ppm
Cadmium	<1.0 ppm	0.12 ppm	0.1 ppm	0.1 ppm
Lead	<1.0 ppm	<0.02 ppm	<0.02 ppm	<0.02 ppm
Mercury	<1.0 ppm	<0.01 ppm	<0.01 ppm	<0.01 ppm
Microbiological Tests				
Total Aerobic Plate Count	<10,000 CFU/mL	<1 CFU/mL	<1 CFU/mL	<1 CFU/mL
Total Yeast & Mold	<1000 CFU/mL	<1 CFU/mL	<1 CFU/mL	<1 CFU/mL
E. coli	Negative	< 0.3 MPN/mL	<0.3 MPN/mL	<0.3 MPN/mL
Salmonella	Negative	Not detected/25g	Not detected/25g	Not detected/25g



<i>Staphylococcus aureus</i>	Negative	<1 CFU/mL	<1 CFU/mL	<1 CFU/mL
Pesticide Residue				
Pesticides	Not detected	Not detected	Not detected	Not detected

*While these parameters are tested on every batch, they do not have true specification limits.

2.3.2 Residual Solvent Analysis

The only solvent used in the production of RUNA® Concentrate is water; thus, residual solvent testing is not performed on the product.

2.3.3 Residual Pesticide Analysis

In accordance with standard operating procedures, All Market Inc. performs 3rd party testing of pesticide residues on every batch of leaves used as the raw material for RUNA® Concentrate.

2.3.4 Additional Product Analysis

Several lots of RUNA® Concentrate have been analyzed for levels of additional chemical constituents; the analyses as published by Kapp et al. are shown in Table 4 and 5 below.

Table 4. Additional Analysis of RUNA® Concentrate*

Analyte	Results	
	mg/mL	%
Caffeine	36	3.6
Theobromine	0.3	0.03
Chlorogenic acids	52	5.2
Total polyphenols	10	1.0
Catechin (C)	2	0.2
Isoflavones	0.8	0.08
Epicatechin (EC)	0.179	0.0179
Epicatechin gallate (ECG)	0.199	0.0199
Epigallocatechin gallate (EGCG)	0.0876	0.00876
Epigallocatechin (EGC)	1.11	0.111
Kaempferol	Trace	Trace
Naringin	Trace	Trace

* Table borrowed from Kapp et al., 2016¹⁰

**Table 5. Proximate Analysis of RUNA® Concentrate***

Analyte	Units	Results	Standard deviation
Moisture	%	66.41	0.011
Ash	%	4.9	0.5
Protein	%	7.0	0.7
Total sugars	%	3.5	—
Total fat	%	0.39	—
Dietary fiber	%	3.8	1.0
Cholesterol	mg/100 g	Not determined**	—

* Table borrowed from Kapp et al., 2016¹⁰

** Reporting limit: 1.0 mg/100 g

Additionally, as published in Kapp et al., 2016,¹⁰ no detectable levels of apigenin, b-sitosterol, campesterol, cholesterol, cyanadins, delphinidins, genistein, hesperidin, kuromanin, luteolin, malvidins, naringenin, ononin, peonidins, petunidins, pterostilbene, puerarin, resveratrol, rutin, sissotrin, stigmastanol, stigmasterol, theanine, theophylline, or vitexin were found from several lots tested.

2.4 Physical or Technical Effect

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



Part 3: Dietary Exposure

3.1 Intended Uses

RUNA® Concentrate, manufactured in accordance with GMP, is intended to be used as an ingredient in carbonated and still energy beverages, at addition levels based on maximum caffeine concentrations of 150 mg per serving. The extract is not intended for use in infant formula, meat, poultry, egg products, catfish, or any products that would require additional regulatory review by USDA. It is also not intended to be used in beverages containing alcohol, or in beverages intentionally marketed to children. A summary of the intended uses is shown in Table 6 below:

Table 6. Intended Use Categories and Addition Levels for RUNA® Concentrate in terms of Total Extract and Caffeine

Beverage category	Caffeine concentrations and serving sizes				Average RUNA® Concentrate addition level needed to achieve maximum caffeine concentrations based on caffeine specification of 2.7–3.7% as mg/serving (mg/mL)
	Maximum caffeine as mg/mL	Maximum caffeine as ppm	Maximum caffeine as mg/serving	Serving size	
Energy-carbonated	0.45	451	150	12 oz. can (355 mL)	5000 (14.1)
Energy-still	0.39	387	150	14 oz. bottle (414 mL)	5000 (12.1)

Energy beverages are ubiquitous in the current marketplace. They are generally formulated with the intention of increasing mental alertness and/or physical performance and contain synthetic caffeine and/or caffeine from natural sources such as green coffee beans, guarana, kola nuts and yerba maté. Guayusa is another source option for naturally occurring caffeine. The intended addition level of RUNA® Concentrate to energy beverages will give a maximum delivery of 150 mg of caffeine per serving, or a maximum concentration of 451 ppm caffeine. This amount of caffeine per serving, as well as the intended serving sizes are within the typical range in energy drinks currently on the market as cited by Somogyi et al.⁵⁴ For example, the AMP beverages, Full Throttle, Go Girl, Java Monster, Inko's White Tea Energy, Monster Energy, Mana Energy, Rockstar, Rockstar Energy Cola, Rockstar Juiced, Rumba Energy Juice, Starbucks Double-shot Coffee, and SoBeNo Fear all contain between 142 and 184 mg of caffeine per serving (note that there are also many examples of beverages with higher caffeine levels per serving), and servings sizes range from 12 to 16 oz.⁵⁴ For comparison, note this maximum intended caffeine level is also significantly less than that found in a Starbuck's Tall



roasted brewed coffee, which according to caffeineinformer.com contains approximately 280 mg per 12 fl. oz.

As further discussed below, the intended use of RUNA® Concentrate in energy beverages will also give an approximate delivery (at the 90th lifetime percentile) of 211 mg CA per serving (a level typically found in a serving of coffee), 26.3 mg catechins (much less than the typical 50–100 mg found in a cup of green/black tea),^{55, 56} 2.2 mg theobromine (minimal compared to the approximate 450–1394 mg found in a chocolate bar as discussed below),⁵⁷ and 5.8 mg isoflavones per serving (much less than found in servings of soy foods; for example soy milk can contain ~10 mg isoflavones/100 g and tofu and soy yogurt can contain up to 48 mg and 84 isoflavones/100 g, respectively, as discussed below⁵³).

3.2 Exposure to Guayusa

3.2.1 History of Exposure to Guayusa Leaves

Guayusa is cultivated in the Amazon region, and decoctions of the leaves have a long history of consumption by the people of Peru, Ecuador, Columbia and Bolivia in the form of a morning stimulant and general tea, with additional traditional uses dating back to 500 B.C.^{1, 2, 4, 5, 58-60} Guayusa was found carefully packaged in a 5th century tomb of what is thought to be a Tiahuanacoid Culture medicine man in highland Bolivia, signifying its importance dating back centuries.^{2, 58, 61} Consumption of decoctions made from guayusa leaves is a daily ritual in many cultures due to its energy and stimulating properties; it is consumed in a manner similar to the way that Americans consume coffee or green/black tea.

There is extensive use among ethnic groups in the cultivation regions, such as by the Kichwa, Shuar, Achuar, Cofán, *Tsa'chi* as well as mestizo and white populations.² For example, Kichwa prepare guayusa leaves as an infusion, which is sometimes consumed in combination with ginger, lime juice, *chuchuwasu* and/or cane sugar liquor. The drink plays a central role in daily sociality, and is considered the most commonly used plant species in the culture.⁵⁹ Dueñas-Serrano et al. explain in their article that it is generally considered the responsibility of women in the culture to wake up early to heat guayusa tea and serve gourds full of the drink to all family members and any visitors. The tea is drunk while individuals participate in activities like weaving, playing music and telling stories.² The Mestizos brew guayusa, leave it to cool and mix it with lemon juice and unrefined sugar to serve cold during the hot midday hours, similar to the consumption of the yerba maté drink tereré.⁴ Human use of guayusa leaves in Bolivia has occurred for at least 1500 years and the plant's distribution among different ethnic groups and across ethnic lines provides evidence of prolonged trading practices of guayusa.⁴



Families in the cultivation regions often have several personal guayusa plants growing near their homes for easy access to the leaves for their morning beverage, and guayusa is also served in Amazonian *peñas* (similar to bars or cafés).^{2, 59} Interestingly, a Jivaro Indian ritual is described in the guayusa literature; it involves drinking large amounts of leaf decoctions before daybreak followed by forced vomiting (which reduces caffeine intake to a level that doesn't induce unpleasant side effects).¹ The vomiting is a learned behavior by this tribe for this specific ritual. Cultivars with caffeine levels ranging from 1.5–3.5% are used for the ritual; cultivars with higher caffeine levels are avoided because their consumption at high levels leads to unsettling symptoms, typical of high caffeine intake.¹ Researchers observed guayusa consumption by one man over 45 minutes during the ritual to be equivalent to the amount of caffeine found in approximately 5.5 cups of coffee (470 mg). The individual then eliminated approximately half of it through forced emesis.¹ Transformation of caffeine from guayusa to dimethylxanthines was approximately 40% in this individual over 55 minutes. The guayusa plant was analyzed and did not contain emetine or other ipecacuanha compounds that would cause an emetic effect, and the plant is not known to otherwise cause emesis on its own outside of this learned ritual.^{1, 2}

Several other species of *Ilex* are consumed by humans in the form of herbal teas in various parts of the world.⁶² *I. paraguariensis* is consumed as yerba maté tea.³ Guayusa tea preparations and drink consumption patterns resemble that of yerba maté,⁴ although *I. paraguariensis* has a comparably lower concentration of caffeine (0.78–1.25%).³ *I. ambigua* is also known to contain caffeine.^{1, 2} *I. vomitoria*, native to North America, was also consumed as yaupon tea by Native Americans and European colonists,⁶²⁻⁶⁴ and *I. kudingcha*, *I. latifolia*, *I. cornuta* and *I. pentagona*, are consumed as Chinese Kudingcha tea.^{6, 9, 62, 65, 66} The leaves of various species of *Ilex* are also known to contain CAs and/or caffeic acid, including *I. guayusa*, *I. paraguariensis*, *I. aquifolium* and *I. integra*.^{3, 9}

3.2.2 Exposure Estimates for RUNA® Concentrate

Exposure to RUNA® Concentrate from the intended use categories were estimated for the U.S population using food consumption data from the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Surveys (NHANES). The most recent NHANES data available to us (2015–2016) was analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). This data was obtained from 7027 individuals that underwent two non-consecutive 24-hour dietary recall interviews (the first was collected in-person, the second by phone 3–10 days later). WWEIA food codes that were considered most similar to the intended use categories (energy beverages) were utilized in the assessment and were assigned the maximum intended use concentration (i.e., 14.1 mg/mL).



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

The relative standard error (RSE; calculated by dividing the standard error of the estimate by the estimate itself and multiplying by 100) is a statistical criterion that can be used to determine the reliability of estimates as pertains to the population (the larger the RSE the less reliable the estimate).⁶⁷ RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.^{67, 68} For the purpose of this safety assessment, an RSE value of greater than 25% was used to indicate that the estimated value was unreliable with regard to representing the population. RSE values are shown in the tables below for the 90th percentile values only, as the 90th percentile values are the most pertinent for the exposure estimates.

Data estimated directly from the NHANES short 2-day survey do not necessarily adequately represent individual usual long-term intake due to the large amount of random error. This is because it may not correctly capture infrequent consumers. It assumes that subjects who consumed a product on a survey day consume it every day of the year, and it does not adjust for potential day-to-day variation in intake (i.e., intra-individual variation over time is not accounted for). Thus estimation of "usual" or "lifetime" exposure was also added to the model based on methodologies developed by Nusser et al., 1996, at Iowa State University.⁶⁹ This lifetime data is considered the most relevant data, as food/food ingredient exposure estimates should be based on expected regular exposure over the lifespan. The technique of estimating usual/lifetime intakes relies on the ability to transform the input daily average data (from food consumers) into normality, which is tested using the Anderson-Darling test statistic within the Creme Global software. Occasionally the Creme software determined that lifetime intake estimates required warnings or were not possible due to issues with the original data set; such issues are noted with



asterisks and are explained below the tables. If lifetime intake estimate calculations failed then they were replaced by the original daily average data results.

Results of the Creme assessment are shown in Tables 7 and 8 below. It should be noted that these estimates are extremely conservative, as they assume that 100% of the surveyed energy drinks in the marketplace will contain RUNA® Concentrate.

Table 7. Total Absolute Exposure to RUNA® Concentrate by Energy Drink Consumers Using NHANES 2015–16 data (mg/day)

Population Group	Age in yrs	N (% of total)	Absolute RUNA® Concentrate consumption Daily Average (mg/day)				90 th % RSE Value	Lifetime 90 th % Exposure Estimates (mg/day)
			Mean	Mean std err	90 th %	90 th % std err		
Children	2–12	1 (0.02)	1093	*	*	*	*	*
Adolescents	13–18	10 (1.5)	4772	793	7614	1132	14.9	6120**
Adults	19+	111 (2.8)	4624	462	10304	1094	10.6	7271
Women of Reproductive Age	14–44	33 (3.2)	3554	532	5563	1566	28.2 [§]	5524
Total Population	2+	122 (2.3)	4628	450	10265	1026	10.0	7307

Creme run #415

*Only one child (age 2–12) reported consuming Energy Beverages in the NHANES survey, thus standard error and 90th percentile data cannot be calculated

**Creme Warning -8 “Fewer than 4 people with multiple observations”; data can still be used.

[§]RSE value > 25, data considered potentially unreliable

Table 8. Total Exposure to RUNA® Concentrate by Energy Drink Consumers Relative to Body Weight Using NHANES 2015–16 data (mg/kg bw/day)

Population Group	Age in yrs	RUNA® Concentrate consumption relative to body weight Daily Average (mg/kg/bw/day)					90 th % RSE Value	Lifetime 90 th % Exposure Estimates (mg/kg bw/day)
		N (% of total)	Mean	Mean std err	90 th %	90 th % std err		
Children	2–12	1 (0.02)	67.5	*	*	*	*	*
Adolescents	13–18	10 (1.5)	65.5	11.9	103.3	15.5	15.0	109.8**
Adults	19+	111 (2.8)	54.6	4.6	87.3	12.5	14.3	88.4***
Women of Reproductive Age	14–44	33 (3.2)	52.9	10.7	69.9	40.3	57.7 [§]	86.0***
Total	2+	122 (2.3)	55.2	4.6	90.2	12.3	13.6	90.1***

Creme run #415



*Only one child (age 2–12) reported consuming Energy Beverages in the NHANES survey, thus standard error and 90th percentile data cannot be calculated

**Creme Warning -8 "Fewer than 4 people with multiple observations"; data can still be used.

***Creme Warning -32 "Fourth moment of Usual intakes less than 3.0"; data can still be used.

*§RSE value > 25, data considered potentially unreliable

The exposure analysis suggests that a relatively small percent of the total population (2.3%) is exposed to energy beverages and, thus, is expected to be exposed to RUNA® Concentrate from use in energy beverages. The 90th percentile lifetime RUNA® Concentrate exposure estimates for the total population were approximately 7307 mg/day (absolute) and 90.1 mg/kg bw/day (relative to body weight). As a reminder, RUNA® Concentrate is an aqueous guayusa leaf extract that is comparable to a brewed tea, and will often be the major ingredient in the intended use products. Hence the exposure levels are relatively high; in other words, the extracts may in some cases be consumed more like a food than like a food additive as the extract will make up the majority of the beverage.

The above estimates are considered extremely conservative as they assume that ALL energy beverages in the marketplace contain the maximum addition level of RUNA® Concentrate. Thus, these are considered maximum scenarios for exposure to the population.

3.2.3 Summary of Guayusa Leaf Aqueous Extract Exposure

In summary, exposure estimates to RUNA® Concentrate based on use in energy beverages were evaluated using Creme Global software. The lifetime exposure at the 90th percentile was estimated at approximately 7307 mg/day (absolute) and 90.1 mg/kg bw/day (relative to body weight). Additionally, there is a long history of daily use of guayusa leaf decoctions as caffeinated beverages in the Amazon region. Decoctions of several other related *Ilex* species are consumed in other regions with similar consumption patterns (e.g., *Ilex paraguariensis* is consumed as yerba maté tea, and several *Ilex* species are consumed as teas in China).

3.3 Caffeine Dietary Exposure Estimates

3.3.1 Caffeine Exposure Estimates based on Background plus Intended Uses

Somogyi et al. determined that 97% of caffeine consumption by American teens and adults, and 95% by American children comes from beverage sources (as opposed to food sources).⁵⁴ RUNA® Concentrate contains up to 3.7% caffeine by weight and is intended to be used as an ingredient in energy beverages.



Caffeine consumption by the U.S. population has remained relatively consistent over the years despite the introduction of various new caffeinated food and beverage products into the marketplace.^{13–15, 70–72} The energy beverages that will contain caffeine from RUNA® Concentrate are expected to replace consumption of other similar caffeinated energy beverages available in the marketplace. Thus, exposure to caffeine from products containing RUNA® Concentrate is expected to be substitutive (as opposed to additive) in the population. In other words, the caffeine consumed from the proposed food categories is expected to take the place of caffeine intake from other similar caffeinated beverage products on the market.

The most common source of caffeine consumed by adults is coffee.^{12, 73, 74} An 8 oz. cup of coffee contains 65–200 mg caffeine depending on the brand, type of coffee (roasted vs. instant) and/or the method of preparation (drip, brewed or percolated to a particular strength).^{16, 75–79} Alternative sources of caffeine include black tea (30–80 mg caffeine per 8 fl. oz. serving); soda beverages (25–70 mg caffeine per 12 fl. oz. serving); coffee-flavored ice cream and yogurt (20–30 mg caffeine per 4 oz. serving); and dark chocolate candy bars (4–20 mg caffeine per serving).⁸⁰ Caffeine is also commonly added to energy beverages, weight loss pills/supplements, and certain drugs (e.g., Excedrin).^{54, 76}

To determine caffeine exposure from background and RUNA® Concentrate, caffeine concentrations were assigned to all relevant NHANES food codes using composition data from the United States Department of Agriculture (USDA)'s Food and Nutrient Database for Dietary Studies (FNDDS), except for energy drink food codes to which the maximum RUNA® Concentrate caffeine concentration of 0.45 mg/mL was assigned. The most recent FNDDS database available to us (2014–15) provides information on the amount of approximately 60 food constituents (including caffeine) per 100 g of each NHANES food code and accounts for both naturally occurring and added caffeine levels in food. The caffeine exposure data was then derived using analysis by Creme software and is shown in the tables below. It is expected to cover both background and intended use exposure to caffeine, since RUNA® Concentrate intended uses are expected to be substitutive. Tables 9 and 10 show caffeine exposure by the U.S. population (absolute and relative to body weight, respectively).

Table 9. Total Absolute Exposure to Caffeine from Background and Intended Use in Energy Beverages by Caffeine Consumers Using NHANES 2014–15 data (mg/day)

Population Group	Age in yrs	N (% of total)	Absolute caffeine consumption Daily Average (mg/day)				90 th % RSE Value	Lifetime 90 th % Exposure Estimates (mg/day)
			Mean	Mean std err	90 th %	90 th % std err		
Children	2–12	1229	13.7	0.9	34.1	3.4	10.0	27.0



		(78.5)						
Adolescents	13–18	705 (84.5)	56.8	5.4	128.7	27.2	21.1	115.4
Adults	19+	4213 (92.6)	166.5	3.9	360.0	9.8	2.7	348.3
Women of Reproductive Age	14–44	1281 (88.0)	109.3	5.7	249.0	16.2	6.5	224.6
Total Population	2+	6147 (89.9)	138.7	3.5	323.4	9.0	2.8	302.1*

Creme run #416

*Creme Warning -2048: "Number of days per person should be constant for a Foods calculation" (result may still be used)

Table 10. Total Exposure to Caffeine from Background and Intended Use in Energy Beverages by Caffeine Consumers Relative to Body Weight Using NHANES 2014–15 data (mg/kg bw/day)

Population Group	Age in yrs	Caffeine consumption relative to body weight Daily Average (mg/kg/bw/day)					90 th % RSE Value	Lifetime 90 th % Exposure Estimates (mg/kg bw/day)
		N (% of total)	Mean	Mean std err	90 th %	90 th % std err		
Children	2–12	1229 (78.5)	0.5	0.03	1.2	0.1	8.3	0.8
Adolescents	13–18	705 (84.5)	0.8	0.07	1.9	0.19	10.0	1.7
Adults	19+	4213 (92.6)	2.1	0.05	4.6	0.13	2.8	4.4
Women of Reproductive Age	14–44	1281 (88.0)	1.5	0.07	3.6	0.3	8.3	3.2
Total Population	2+	6147 (89.9)	1.8	0.04	4.2	0.1	2.4	3.8*

Creme run #416

*Creme Warning -2048: "Number of days per person should be constant for a Foods calculation" (result may still be used)

The caffeine exposure estimates from all sources using the NHANES data show that approximately 90% of the population consume caffeine-containing products. Consumption of caffeine from RUNA® Concentrate energy beverages was estimated within this assessment and is also expected to be substitutive for other energy drinks in the population. The lifetime 90th percentile caffeine exposure estimates for the total population were 302.1 mg/day (3.8 mg/kg bw/day). Consumption estimates for the children and adolescent subgroups were 27 mg/day (0.8 mg/kg bw/day) and 115.4 mg/day (1.7 mg/kg bw/day) at the lifetime 90th percentile. Reproductive aged women (defined here as women aged 14–44 years) were estimated to consume approximately 224.6 mg/day (3.2 mg/kg bw/day) at the lifetime 90th percentile. Note that all maximum lifetime 90th percentile exposure



levels for total caffeine intake (background plus intended use) are below those that are considered safe, as discussed in Part 6 below (400 mg/day for adults, 300 mg/day for pregnant women, and 2.5 mg/kg bw/day for children).

3.3.2 Published Caffeine Exposure Estimates

In addition to the background exposure estimates based on NHANES data above, a number of studies on caffeine intake in the U.S. have been published in recent years. Mitchell et al. published a study in 2014 on caffeine intake by the U.S. population based on a comprehensive nationally representative caffeinated beverage survey—the Beverage Consumption Panel conducted by the Kantar Worldpanel (KWP).¹² Respondents in the survey completed an online beverage diary once a day for seven consecutive days between October 2010 and September 2011. A total of 37,602 individuals aged 2 years and older reported consuming at least one caffeinated beverage during the days studied.

The study concluded that 85% of the population consumes at least one caffeinated beverage per day. The mean daily caffeine intake from all beverages for the total population was 165 mg per day. Consumption of caffeine was highest in the 50–64 year age subgroup, with a mean of 226 mg per day. Mean consumption in children and adolescents was 1.5 mg/kg bw/day or lower, depending upon the specific age group. Intake at the 90th percentile was approximately 380 mg/day for the total population, and was highest for adults aged 50–64, at 467 mg/day. In children and adolescents, caffeine exposure at the 90th percentile ranged from 2.9–3.7 mg/kg bw/day. However, the sample sizes for consumption of some beverage categories in these measurements was too low to accurately estimate a 90th percentile value; as such, the reliability of these 90th percentile exposure estimates is unclear (the authors discussed that the sample size for some of the children's age groups were not robust enough to obtain a reliable estimate of caffeine intake, and they recommended that more focused studies with larger sample sizes in children may provide better estimations for this subgroup). Consumption of coffee accounted for the majority of total caffeine intake in the overall study, while tea, carbonated beverages and energy drinks contributed much less (less than 10% of those surveyed were energy drink consumers). At the 90th percentile, exposure to caffeine from energy drinks did not exceed 160 mg/day, and exposure to caffeine from teas did not exceed 154 mg/day in any age range studied.

While the data were not shown, the authors reported that women aged 18–34 (considered reproductive age) consumed less than the 300 mg per day maximum recommended by many scientific and/or regulatory organizations during pregnancy (although data on pregnancy status was not available in this study).¹² At the 90th percentile, women aged 18–24 consumed 228 mg of caffeine per day, and women



aged 25–34 consumed 284 mg. The authors unfortunately did not report the data for women aged 35–44, which can still be considered childbearing age.

The Somogyi report showed single-day data from the U.S. NHANES WWEIA 2005–2006 survey in which women of childbearing age (12–59 years) consumed mean levels of 46.6–225.3 mg (depending on the age subgroup) of caffeine per day.⁵⁴ In a survey of 10,712 individuals, Knight et al. reported that pregnant women consumed about half the amount of caffeine from caffeinated beverages than did general women of reproductive age (20–34 years); 90th percentile consumption levels during pregnancy were 157 mg/day versus 229–247 mg/day in reproductive aged non-pregnant women. Mean consumption by pregnant women was 58 mg/day.⁸¹

While the age groups assessed were different, the 90th percentile results were lower in our NHANES 2013–2014 Creme analysis (tables above) as compared to Mitchell et al.¹² (note that Mitchell et al. used data collected in 2011 and 2012). The reason for the discrepancy is unknown; it may be the age group differences or that individuals consumed less caffeine in 2013–2014 than during 2011–2012, or it could be that the lengths and number of subjects in the surveys (7-day, 37,602 individuals for Mitchell and 2-day, 7,574 individuals for NHANES 2013–2014) play a role in the differences. Finally, it could be that the USDA concentration assignments for caffeine in various beverages differ slightly from those utilized in the Mitchell et al. methods.

In 2015, Mitchell et al. published a comparison of the data from the 2014 Mitchell study cited above (which was considered to have used a brand-specific approach to assigning caffeine levels to specific beverages)¹² to data collected using a method that assigned caffeine values to beverages using a more general category-specific methodology.⁷¹ They found that regardless of the method used for assigning caffeine values, the population estimates for caffeine exposure were relatively similar. Some small differences observed suggested that detailed brand-specific data might provide more accurate estimates of caffeine exposure for some age groups.

Ahluwalia et al. (2014) published a study using 2001–2010 NHANES data from children/adolescents aged 2–19 years of age.⁷⁰ The authors compared caffeine consumption from the five different 2-year NHANES data sets that fell between the years 2001 and 2010. They found that approximately 71% of those aged 2–19 consumed caffeine on a given day. In the more recent 2009–2010 NHANES data set, caffeine intake for all children who were caffeine consumers was 12.4 mg/day at the median and 116.6 mg/day at the 90th percentile. With regard to intake relative to body weight, the total population of children consumed 0.4 mg/kg bw/day at the median and 2.27 mg/kg bw/day at the 90th percentile. When broken down into smaller population groups, children aged 2–5, 6–11 and 12–19 consumed 4.7, 9.1 and 40.6 mg/day at the median and 20.9, 58.5 and 186.3 mg/day at the 90th



percentile, respectively. With regard to intake relative to body weight, the exposures for these subgroups were 0.29, 0.30 and 0.64 mg/kg bw/day at the median, and 1.34, 1.80 and 2.66 mg/kg bw/day at the 90th percentile, respectively. When the authors analyzed NHANES data from the other four surveys over the 10-year study period, they noted a small decline in caffeine intake in all children over time (when expressed as either mg/day or mg/kg bw/day). However, the decrease in caffeine intake was only significant in those younger than 12 years of age, indicating that caffeine intake in adolescents (aged 12–19) remained relatively stable over the decade studied.

The 90th percentile caffeine intake results from the Mitchell¹² and Ahluwalia⁷⁰ studies as well as the NHANES 2013–2014 Creme analysis shown in the above tables are again somewhat difficult to compare because they looked at slightly different age group populations. The 2–5 age group designation was identical in both of the published studies. In that age group, the results from the Mitchell study were over twice that of the Ahluwalia study at the 90th percentile (57.8 mg caffeine per day and 3.7 mg/kg bw/day in the Mitchell study compared to 20.9 mg/day and 1.34 mg/kg bw/day in the Ahluwalia study). The Creme NHANES assessment found children aged 2–12 consumed 34 mg/day and 1.2 mg/kg bw/day. With regard to other age groups in children, Mitchell looked at the 6–12 year-old bracket, and Ahluwalia looked at 6–11 year-olds; while they cannot be directly compared because they were slightly different, the Mitchell results were higher again at the 90th percentile (94 mg/day and 2.7 mg/kg bw/day compared to 58.5 mg/day and 0.8 mg/kg bw/day).

The results for the teenage age ranges were more similar at the 90th percentile, even though the age groupings were again different (13–17 year-olds in the Mitchell study consumed 182.9 mg/day and 2.9 mg/kg bw/day, while 12–19 year-olds in the Ahluwalia study consumed 186.3 mg/day and 2.66 mg/kg bw/day). The NHANES 2013–2014 Daily Average Creme results at the 90th percentile were lower, at 128.7 mg/day and 1.9 mg/kg bw/day for ages 13–18.

Branum et al. (2014) conducted a similar study on caffeine consumption in the 2–22 year old population using NHANES data from 1999–2010.⁷³ These authors found that 73% of this population consumed caffeine, and also noted (as did Ahluwalia et al.) that caffeine consumption generally decreased over the time period in children 2–11 years of age. Caffeine consumption from soda decreased from 62% to 38% over the time period studied while consumption from coffee increased from 10% to 24%. Intake from tea remained relatively stable while intake from energy drinks rose from 0% to 6%. Intake levels remained stable among adolescents and young adults over the 11-year time period. The authors only reported mean intake levels (versus 90th percentile intakes); hence, the specific results are not detailed here.



Fulgoni et al. (2015) looked at caffeine intake in adults (aged 19 and older) also using NHANES data from the years 2001 to 2010.⁷² The authors found that 89% of adult men and women in the United States consume caffeine. They found that caffeine intake among consumers remained remarkably similar over the decade studied, including for the total population of adults as well as all age and gender sub-population groups of adults studied. The 90th percentile caffeine consumption level by all caffeine-consuming adults was 436 mg/day. The 90th percentile levels for the age groups of 19–30, 31–50, 51–70 and 71+ years were 292, 492, 484 and 336 mg/day, respectively. Because the age group populations were different than those in other published studies and the NHANES Creme data in Tables 9 and 10 above, it is again difficult to compare the results directly; overall, the Fulgoni caffeine exposure results appear to be slightly higher for some populations but fell within a generally similar range to those in the Mitchell, 2014 study.

In 2015, Ahluwalia et al. reviewed the findings from national quantitative studies published since the year 2000 specifically related to caffeine intake among U.S. children and adolescents.¹³ The authors concluded that intake of caffeine by teenagers has remained relatively stable over the period examined (early 2000s to 2010), and a slight decline in caffeine intake by younger children was noted. Over half of children aged 2–5 and approximately 75% of children over the age of five consumed caffeine. Soda, coffee, tea and flavored milk were the main sources of intake. Overall, at the 90th percentile, children over the age of 12 years slightly exceeded the recommended maximum Health Canada guidelines of 2.5 mg/kg bw/day, and 10–25% of this age group may be consuming more than the recommended amount on a given day.

Bailey et al. (2014) reviewed sales data, data from federal sources and reports from the Drug Abuse Warning Network to characterize the use of energy drink products in the United States.⁸² They found that general use of these products remains low overall in the U.S. population (2.7% of the population using NHANES 2007–2010 data). The highest usage was by males aged 19–30 years.

Similar to many of the above investigations, Tran et al. (2016) studied caffeine intake in teens, young adults and adults using NHANES data (2003–2012).¹⁴ Eighty-five percent consumed caffeine (84% via beverages). The percentage remained constant despite new caffeine sources being added to the market. Less than 7.1% consumed energy drinks, and the majority was consumed from coffee and tea. Mean caffeine intake was found to have decreased in teens (age 13–17 years) over the time period examined (from 62 to 55 mg/day). Mean intake per consumption occasion was equivalent between coffee and energy drinks for teenagers and young adults, and the authors found an inverse relationship between caffeine intake from energy drinks compared to intake from coffee, tea and soda, which together supports the concept that caffeine intake from various beverages is substitutive. For children 12 years and under, caffeine exposure estimates were



either at or exceeded the recommended maximum consumption levels of 2.5 mg/kg bw/day suggested by Health Canada and 3 mg/kg bw/day suggested by EFSA; however, the authors noted that the daily average approach that they used often overestimates consumption. The authors also suggested that the 400 mg/day safe consumption level for adults is not necessarily appropriate for light weight adolescents but may be appropriate for heavier adolescents. The 90th percentile estimates for young and older adults for total caffeine intake were below 400 mg/day.

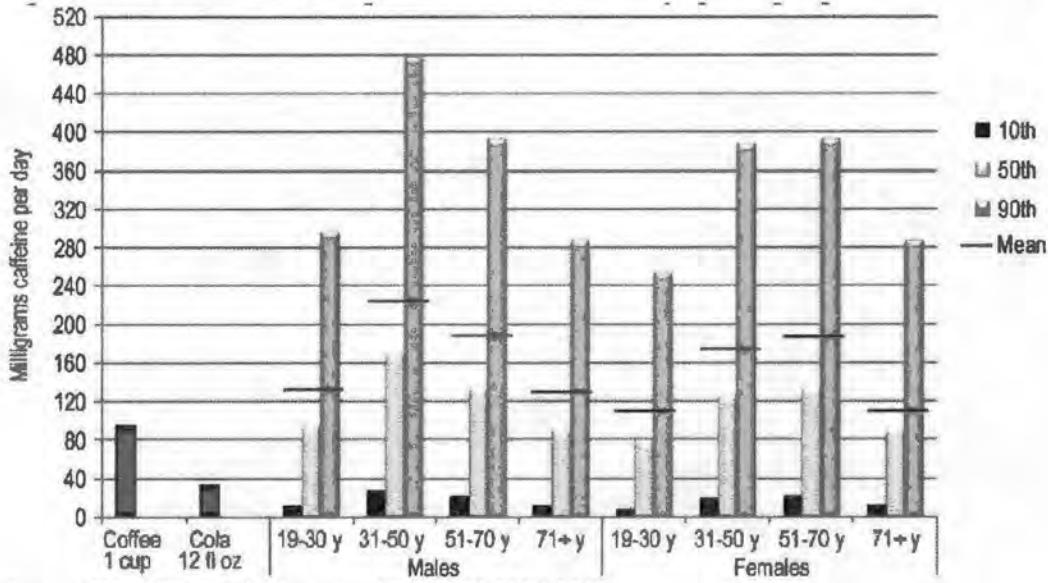
Drewnowski and Rehm (2016) reviewed NHANES data from 2011–2012 and compared it to the previous 14 years to look for trends in caffeine consumption.¹⁵ They found that coffee and tea remain the principle drivers of caffeine intake despite various new sources of caffeine being introduced into the U.S. food supply (for example, only 2% came from energy drinks). Among both children and adults combined, they found caffeine intake declined from 175 mg/day in the 1999–2000 data to 142 mg/day in the 2011–2012 data, mainly due to a drop in soda consumption. Mean consumption level for children was low at 15 mg/day for ages 4–8 and 26 mg/day for ages 9–13.

Chen et al. (2014) reported on pre-pregnancy caffeine consumption and changes during pregnancy, based on data from the National Birth Defects Prevention Study (October 1997–December 2007).⁸³ Of the 8,488 control women analyzed (controls in this large study were mothers of babies without birth defects—this particular analysis did not include mothers of babies with birth defects), 97% reported caffeine consumption prior to pregnancy, with a mean intake of 129.9 mg/day. Caffeine intake of over 300 mg/day was associated with unplanned pregnancies, smoking and alcohol drinking during pregnancy. While pregnant, 78.9% decreased or stopped consumption of caffeinated beverages, 13.7% continued their pre-pregnancy consumption habits, and only 3.6% increased their consumption of caffeinated beverages. Forbes et al. (2018) studied women's dietary changes during pregnancy, and confirmed that the majority of women decrease their caffeine intake during pregnancy, with the most common reason being an awareness of pregnancy recommendations and related concern for their baby's health.⁸⁴

The scientific report of the 2015 U.S. Dietary Guidelines Advisory Committee (DGAC) assessed caffeine consumption from all sources using NHANES 2007–2010 data, and published Figures 3 and 4 below (which were directly borrowed from the report).⁸⁵ Caffeine intake in adults was found to peak between the ages of 31–70 years, and younger adults (19–30 years) and older adults (71 years and older) had lower intakes comparatively. Relatively few individuals (less than 10 percent) had intakes above 400 mg/day. In children, caffeine intake increased with age, with mean intakes remaining below 100 mg/day in adolescents (14–18 years). Recommended intakes from Health Canada⁷⁴ of no more than 2.5 mg/kg/day were not shown to be exceeded by most children and adolescents (although the authors

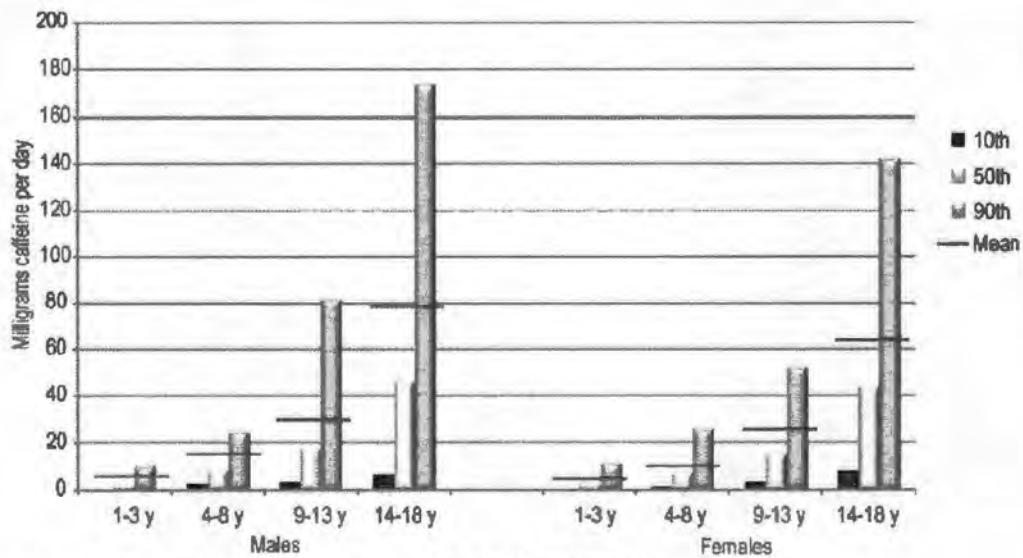


cite Ahluwalia et al. in stating that as many as ten percent of 12–19 year-olds may exceed this intake level).



Source: What We Eat in America, NHANES 2007-2010

Figure 3. Mean and Percentiles of Usual Caffeine Intake by Age/Sex Groups; Adults (graph borrowed from DGAC report)⁸⁵



Source: What We Eat in America, NHANES 2007-2010

Figure 4. Mean and Percentiles of Usual Caffeine Intake by Age/Sex Groups; Children and Adolescents (graph borrowed from DGAC report)⁸⁵



3.3.3 Summary of Caffeine Dietary Exposure Estimates

In summary, caffeine exposure estimates for the U.S. population from the background diet plus using the energy beverage intended use concentration for caffeine from Runa® Concentrate were performed using Creme analysis of NHANES 2013–2014 data. In addition, background diet caffeine exposure estimates from published studies were also summarized.

The results from our Creme NHANES exposure analyses suggest that caffeine exposure for the total population and subgroups is expected to remain below levels considered safe for these populations (400 mg/day for adults, 2.5 mg/kg bw/day for children, and 300 mg/day for pregnant women).^{74, 86} Results from other recently published caffeine exposure estimates showed similar results to those from our Creme analyses, in that the majority of individuals in the U.S. consume less caffeine than the levels that are considered safe for various population groups, although certain subpopulations may exceed these safe levels at the 90th percentile (e.g., men age 31–50 were estimated to consume over 400 mg/day⁸⁵). Women of childbearing age were found to consume less than the estimated safe 300 mg/day level, and consumption levels drop by most women during pregnancy. The combined data shows that 85% or more of adults and 70% or more of children consume caffeinated products (mainly beverages) on a given day, and importantly, data from a number of recent exposure studies show that caffeine intake has remained relatively stable over the past 10+ years despite the addition of many new caffeinated beverage categories to the marketplace, and consumption of caffeine by children has actually decreased in recent times. This emphasizes the expectation that energy beverages containing RUNA® Concentrate are expected to replace consumption of similar beverages in the marketplace with comparable caffeine levels. RUNA® Concentrate is not expected to lead to additional caffeine consumption due to both the substitutive nature of its application, and the substitutive nature of caffeinated beverage consumption in general.

3.4 Exposure to Chlorogenic Acid, Catechins, Theobromine, and Isoflavones

RUNA® Concentrate is not standardized to CAs, catechins, theobromine or isoflavones. Regardless, general exposure to these constituents from the extract were derived using the exposure analysis of RUNA® Concentrate. These constituents are all substances found in various other foods and beverages in U.S. diet, as is discussed below.



3.4.1 Chlorogenic Acid Exposures

CAs comprise approximately 5.2% of RUNA® Concentrate. Using exposure to total RUNA® Concentrate from Tables 7 and 8 above, mean exposure to CAs from use in energy beverages is calculated to be approximately 241 mg/day (2.9 mg/kg bw/day), while 90th percentile lifetime exposure is calculated to be approximately 380 mg/day (4.7 mg/kg bw/day) for the total population, ages 2 and older. The per serving exposure would be approximately 211 mg CAs/serving (based on the 150 mg/serving limit for caffeine).

While numerous foods consumed by humans contain CAs, coffee beans are especially rich.³¹⁻³³ Instant roasted coffee (caffeinated and decaffeinated) have been reported to have approximately 30–40 mg of CAs per gram.^{87, 88} A single cup of brewed coffee contains anywhere from 15 mg to 675 mg CAs.^{19, 75, 89-91} Espresso beverages from various locations were recently analyzed and found to contain 24–422 mg of CAs per single serving.¹⁸ Daily intake of CAs by coffee drinkers is considered to be in the range 500–1000 mg.^{29, 30, 87, 92, 93}

CAs are also widely prevalent in other fruits and vegetables at much lower levels compared to coffee beans⁹⁴⁻⁹⁸ although, as in coffee beans, the CQAs, especially 5-and/or 3-CQA, are generally the most dominant conjugate forms, depending on the specific plant.⁹⁹ CAs are found in potatoes (up to 4.6 g/kg dry weight (DW)), apples (up to 1.2 g/kg DW or 62–385 mg/kg in whole apples), peaches (up to 1.6 g/kg DW), tomatoes (up to 0.4 g/kg dry weight), carrots (up to 18.8 g/kg DW), eggplant (up to 28 g/kg DW) and sunflower seeds (up to 45.5 g/kg DW).^{29, 94, 100} CAs are also present in whole grain flours such as corn and barley (0.08 g/kg DW).¹⁰¹

A publication on the dietary intake of polyphenols by French adults found mean hydroxycinnamic acids intake from supplements, vitamins and main food sources for the 4922 participants was 599 ± 426 mg/day.¹⁰² The dietary intake values for the three main CAs (IUPAC) were as follows: 216 ± 142 mg/day for 5-CQA, 141 ± 117 mg/day for 3-CQA and 131 ± 104 mg/day for 4-CQA (approximately 488 mg total CAs/day). The main dietary sources for the CAs were coffee (76–99%), potatoes (10%), apples (4%), and artichokes (3%) with minor contributions from plums, prunes, tomatoes, carrots and tea.

A study on the intake of polyphenols in a Polish population found the mean intake was 1756.5 ± 695.8 mg/day in 10,477 randomly sampled individuals who completed a validated food frequency questionnaire.¹⁰³ The average individual CA (IUPAC) intakes were 224.6 ± 112.7 mg/day for 5-CQA (mainly from coffee (73%), apples and potatoes); 149.1 ± 124.8 mg/day for 4-CQA (mainly from coffee (94%), tea and apples); and 128.2 ± 111.6 mg/day for 3-CQA (mainly from coffee (96%), plums and tea).¹⁰³ Thus approximately 74.6 mg/day of the 502 mg/day CQAs shown above came from dietary sources other than coffee.



Similar results were noted in several other studies. Hydroxycinnamic acid consumption in 6661 Polish individuals was determined to be 492 mg/day, 71% of which came from coffee consumption (and thus approximately 143 mg came from other food sources in the diet).¹⁰⁴ Average caffeic acid derivative intake (including CAs) was found to be 417 ± 325 mg/day in Finnish adults¹⁰⁵; coffee accounted for 67.9% followed by breads and cereals (12.3%) and tea (9.7%) with minor contributions from fruits and vegetables. A study of polyphenol consumption in 620 elderly Brazilians found that average intake was approximately 1200 mg/day, with approximately 46% derived from coffee.¹⁰⁶ The individual phenolic compounds with the highest intake were CAs. Mean phenolic acid consumption by individuals in São Paulo, Brazil was determined to be approximately 284.8 mg/day with nearly all being from hydroxycinnamic acids.¹⁰⁷ Again, coffee was the major contributor at 70.5% of total phenolics and 92.4% of phenolic acids. Mediterranean countries were found to consume a mean total phenolic acid intake of 304 mg/day, derived using data from the PREDIMED (Primary Prevention of Cardiovascular Disease with a Mediterranean Diet) study.¹⁰⁸ Hydroxycinnamic acids was the phenolic group with the highest consumption, and 5-CQA was the most abundantly ingested individual polyphenol. Again, coffee was the major phenolic contributor.

A recent study on intake of CAs from consumption of traditional maté (as chimarrão and terere) by 450 residents of Brazil found that depending upon the method of preparation, beverages contained 65.6–575.4 mg/100 mL and 105.3–460.2 mg/100 mL of CQAs and diCQAs, respectively. Daily consumption of CAs from the maté beverages ranged from 512.5–1779.7 mg/day.¹⁰⁹

In summary, published data suggests that mean daily intake of CAs is approximately 500 mg/day in various populations around the globe, with the vast majority coming from coffee consumption. FDA recognizes that consumption at the 90th percentile is usually approximately 2 times the mean¹¹⁰, thus the mean data from the published studies suggests 90th percentile intakes maybe approximately 500–1000 mg/day. The mean exposure to CAs from RUNA® Concentrate is estimated at approximately 241 mg/day, while 90th percentile exposure is estimated as 380 mg/day. Due to the substitutive nature of caffeinated beverages for each other by consumers, it is expected that some of the CAs from RUNA® Concentrate will be substitutive for CAs from coffee beverages.

3.4.2 Catechin Exposures

Catechins comprise approximately 0.36% of RUNA® Concentrate. Using exposure to total RUNA® Concentrate from Tables 7 and 8 above, mean exposure to catechins from use in energy beverages is calculated to be approximately 16.7 mg/day (0.2 mg/kg bw/day), while 90th percentile lifetime exposure is calculated to be approximately 26.3 mg/day (0.32 mg/kg bw/day) for the total population, ages 2



and older. The per serving exposure would be approximately 15 mg catechins/serving (based on the 150 mg/serving limit for caffeine).

Catechins are widely distributed in foods, for example, catechin concentrations are especially high in certain beans, grapes, apricots and strawberries; epicatechin concentrations are especially high in apples, blackberries, cherries, certain beans and grapes, pears, raspberries and chocolate; and gallates/gallocatechins are especially concentrated in green tea.¹¹¹ Brewed black tea infusions have been shown to contain 50–370 mg/cup of total catechins, while green tea infusions contain 50–540 mg/cup.^{43, 55, 56} Dark chocolate bars were found to contain 29.8–269.7 mg of epicatechin and 15.9–205.7 mg catechin per 100 g of product.⁵⁷

Catechin consumption has been estimated in various populations.^{102, 103, 112, 113} The intake of catechins by 4942 participants in a French study was 99 ± 116 mg/day, mainly from tea, red wine, apples, and cocoa products.¹⁰² Exposure levels were slightly higher for women when the results were divided by gender; 114 ± 133 mg/day). In a study of 10,477 individuals from Krakow, Poland, average catechin intake was 50 mg per day, mainly from tea and cocoa.¹⁰³ Average intakes for individual catechin compounds were as follows: GCG, 73.6 ± 64.8 mg/day (from tea); EGCG, 48.1 ± 39.5 mg/day (from tea); EC, 45.9 ± 34.2 mg/day (from tea, apples and chocolate); ECG, 38.6 ± 22 mg/day (from tea); EGC, 38.0 ± 21.2 mg/day (from tea); and CG, 24.9 ± 12.3 mg/day (from tea).¹⁰³ Bai et al. (2014) found that U.S. adults consume approximately 179 mg of catechins per day.¹¹² Catechin was the most abundantly consumed, followed by epicatechin, epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate and gallocatechin. Consumption was mainly from tea, beer, apples, wine and peaches.¹¹² EFSA determined that daily catechins exposure from traditional green tea infusions ranges from 186.4–931.4 mg/day, based on mean and high exposures.¹¹³

In summary, total aggregate exposure to catechins from RUNA® Concentrate is potentially much less than can be found in a single cup of green/black tea. As caffeinated beverages are often substituted for each other with regard to consumption it is estimated that catechin content from energy drinks with RUNA® Concentrate will likely be substitutive for that from green or black tea consumption. Overall RUNA® Concentrate is not expected to significantly increase catechin consumption in the population.

3.4.3 Theobromine Exposures

Theobromine comprises approximately 0.03% of RUNA® Concentrate. Using exposure to total RUNA® Concentrate from Tables 7 and 8 above, mean exposure to theobromine from use in energy beverages is calculated to be approximately 1.4 mg/day (0.02 mg/kg bw/day), while 90th percentile lifetime exposure is calculated to be approximately 2.2 mg per day (0.03 mg/kg bw/day) for the total population,



ages 2 and older. The per serving exposure would be approximately 1.2 mg theobromine/serving (based on the 150 mg/serving limit for caffeine).

Theobromine is found in various food sources, especially cocoa beans. Langer et al. (2011) measured levels in 12 dark chocolate bar products and found that they contained 0.53–1.64% theobromine by weight. This is equivalent to approximately 450–1394 mg of theobromine in an 85 g bar.⁵⁷ Hot cocoa beverages have been reported to contain an average of 65 mg of theobromine per serving.^{114, 115} Hence the total aggregate exposure estimates from RUNA® Concentrate are considered very minimal compared to intake from other dietary sources, especially chocolate.

3.4.4 Isoflavone Exposures

Isoflavones comprise approximately 0.08% of RUNA® Concentrate. Using exposure to total RUNA® Concentrate from Tables 7 and 8 above, mean exposure to isoflavones from use in energy beverages is calculated to be approximately 3.7 mg/day (0.04 mg/kg bw/day), while 90th percentile lifetime exposure is calculated to be approximately 5.8 mg per day (0.07 mg/kg bw/day) for the total population, ages 2 and older. The per serving exposure would be approximately 3.2 mg isoflavones/serving (based on the 150 mg/serving limit for caffeine).

Isoflavones are best known for their presence in soy bean products. According to EFSA's 2015 risk assessment of isoflavones for peri- and post-menopausal women taking food supplements containing isolated isoflavones, soy milk can contain ~10 mg isoflavones/100 g and tofu and soy yogurt can contain up to 48 mg and 84 isoflavones/100 g, respectively.⁵³ EFSA estimated isoflavone intakes of approximately 20 mg/day among vegetarians and consumers of soy products. The 75th percentile of isoflavone intake has also been reported to be as high as 65 mg/day in some Asian populations.¹¹⁶

The USDA Database on the isoflavone content of selected foods shows that Natto contains approximately 82.29 mg/100 g, Kellogg's Smart Start cereal contains approximately 93.9 mg/100 g, soy cheese contains up to 25.72 mg/100 g, soy yogurt contains approximately 33.17 mg/100 g, fried tempeh contains approximately 72.80 mg/100 g, and fried tofu contains approximately 34.78 mg/100 g. Compared to these levels of isoflavones in soy products, the total lifetime daily exposure from RUNA® Concentrate's intended uses is considered relatively low.



AIBMR Life Sciences, Inc.

Part 4: Self-limiting Levels of Use

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



AIBMR Life Sciences, Inc.

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

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



Part 6: Narrative

6.1 Guayusa Safety

The major safety conclusion of this subpart is comprised of:

1. The bioequivalence of caffeine pharmacokinetics between a guayusa extract, coffee bean extract, and synthetic caffeine;
2. A published bacterial reverse mutation test and in vitro mammalian chromosomal aberration assay on RUNA® Concentrate, suggesting no genotoxic effects;
3. A published acute oral toxicity up and down study, 14-day range finding study, and 90-day repeated dose oral gavage study in rats, suggesting no toxic effects other than those also displayed in the caffeine control group, and a NOAEL for the 90-day study of the highest dose tested (aside from caffeine effects) of 5000 mg/kg bw/day;
4. Authorized European novel food status for aqueous extracts of dried leaves of *Ilex guayusa*.

6.1.1 Pharmacokinetics of Guayusa

Guayusa is a complex plant; pharmacokinetic studies have been performed on some of its constituents, as are discussed further below in appropriate subsections. Additionally, Krieger et al. (2016) conducted randomized, double-blind, three-period crossover clinical trial that investigated both the safety and pharmacokinetics of a guayusa leaf hydroethanolic extract (AmaTea®) and a green coffee extract (JAVA.g) in 12 healthy adult males ages 21–34.¹¹⁷ At each visit, subjects received one of three caffeine sources: AmaTea (20% caffeine and 30% polyphenols by weight), JAVA.g (30% caffeine and 40% polyphenols by weight), or synthetic caffeine. The test articles were administered in liquid form, each containing 200 mg caffeine per 4 fluid ounces (2.5 mg/kg bw on average), and subjects were required to drink them in 5 minutes or less. Serum caffeine was measured at baseline, 30, 60, 120, 180, and 240 minutes post-dose. Serum levels of caffeine differed significantly from baseline in the subjects after consumption of each caffeine source. At the end of the four-hour period, levels of caffeine were still present in the body at an average of 2.50 µg/mL for AmaTea®, 2.54 µg/mL for JAVA.g and 2.36 µg/mL for synthetic caffeine, above baseline levels. The average C_{max} was 4.13 µg/mL for AmaTea®, 3.95 µg/mL for JAVA.g and 4.12 µg/mL for the synthetic control. The average T_{max} was 47.50 minutes for AmaTea, 60 minutes for JAVA.g and 72.50 min for the synthetic caffeine control. In summary, significant absorption of caffeine occurred over the 4-hour time period in all groups, and maximum levels of serum caffeine



were comparable to that found in other published studies. The ratios of caffeine C_{max}, AUC₀₋₄, and AUC_{0-∞} were bioequivalent for each test article.¹¹⁷

6.1.2 Toxicology Studies on RUNA® Concentrate

A set of toxicology studies on RUNA® Concentrate (described as an aqueous guayusa extract, provided by RUNA, LLC), performed according to Good Laboratory Practice (GLP) and Organization for Economic Cooperation Development (OECD) guidelines, was published by Kapp et al. in 2016.¹⁰ The published studies are summarized in the sections below. The test article in the studies was referred to in the publication as “Guayusa Concentrate” (GC), prepared by adding dried guayusa leaves to purified water (1.3–1.6:1), followed by brewing for 2–4 hours, cooling and storage (i.e., RUNA® Concentrate). Chemical analysis of the GC test article, as stated in the paper, is shown in Tables 4 and 5 in section 2.3.4 of this report.

6.1.2.1 Bacterial Reverse Mutation Test

A bacterial reverse mutation test was performed to investigate the potential of GC to induce genetic mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2uvrA.¹⁰ It followed US FDA GLP regulations, and was based on ICH¹¹⁸ and US FDA Redbook guidelines,¹¹⁹ in the presence and absence of a metabolic activation system (S9 mix). Sterile water served as the negative control and the positive controls were sodium azide, ICR 191 acridine, daunomycin, methyl methane-sulfate and 2-aminoanthracene. Plates were prepared in triplicate. GC concentrations were 1.58, 5.0, 15.8, 50, 158, 500, 1580, and 5000 µg/plate. After incubation, the number of revertant colonies was counted and the mutation factor (MF) was calculated by dividing the mean revertant colony count by the mean revertant colony count for the corresponding vehicle control group. Results were considered positive when the MF was increased by at least a factor of 2 for strains TA98, TA100 and WP2 uvrA or by at least a factor of 3 for strains TA1535 and TA1537. To be positive, any increases had to be dose-related and/or reproducible.

No toxic effects or precipitation of the test material were observed in any strain at any concentration of the test material. The mean number of revertant colonies was less than twice that of negative control values at all test article concentrations. There was an increase in revertant colony counts in strain TA100 at the highest dose level without metabolic activation using the pre-incubation method only. When the preincubation test was repeated using six replicate plates (versus three), an increase in revertant colonies was not seen. Thus, the observed increase was attributed to normal experimental variation rather than mutagenicity. No increase in the number of revertant colonies was observed in the remaining strains, in either the absence or the presence of S9 and using either the plate incorporation or the pre-incubation



method. Therefore, GC was considered negative for mutagenicity in the bacterial reverse mutation test.

6.1.2.2 In Vitro Mammalian Chromosomal Aberration Assay

A chromosomal aberration assay was performed to evaluate the clastogenic potential of GC.¹⁰ The assay was performed according to US FDA Redbook¹²⁰ and OECD 473¹²¹ guidelines using human peripheral blood lymphocytes (HPBL). Sterile water was used as the vehicle for test article preparation and as the vehicle control. Cyclophosphamide and mitomycin C were positive controls for treatment with and without S9 metabolic activation, respectively. Caffeine was also included as an internal control at doses equivalent to those found in the GC groups. Cells were treated for 4 hours in the S9-activated test system and for 4 and 20 hours in the non-activated test system. All cells were harvested 20 hours after treatment initiation. Based on preliminary cytotoxicity assays, the concentrations chosen for the chromosomal aberration assay ranged from 0.5–5% vol/vol for the non-activated and activated 4-hour exposure groups and from 0.01–0.5% vol/vol for the non-activated 20-hour exposure group.

Results revealed no significant or dose-dependent increases in structural or numerical aberrations in either the GC or caffeine control groups with or without S9. GC and the equivalent concentrations of caffeine control were negative for the induction of chromosomal aberrations in this assay.

6.1.2.3 Acute Oral Toxicity Up and Down Study in Rats

An acute oral toxicity study was performed on GC according to OECD 425 guidelines,¹²² to determine the potential of GC to produce toxicity following a single oral dosing in rats.¹⁰ Female Sprague Dawley albino rats 8 to 9 weeks of age (191–204 g) were utilized for the study (females were selected for the test because they are frequently more sensitive to the toxicity of test compounds than males). The test substance was administered at an initial limit dose of 5000 mg/kg of GC to one healthy female rat by gavage. Due to the absence of mortality in this animal, two additional females received the same dose level simultaneously. Since these animals survived, no additional animals were tested. All animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days after dosing. A battery of clinical observations was made, and body weights were recorded prior to administration and again on days 7 and 14 following dosing. On day 14, all animals were sacrificed, and gross necropsies were performed. Tissues and organs of the thoracic and abdominal cavities were examined.

All animals survived test substance administration through to study termination and gained body weight during the study. Immediately following administration, the



animals were hypoactive and exhibited oral discharge, abnormal respiration, hunched posture, reduced fecal volume, and/or soft feces. However, the animals recovered from these symptoms by day three and appeared active and healthy for the remainder of the study. No gross abnormalities were noted in any of the animals when necropsied at the conclusion of the 14-day observation period. The LD₅₀ of the test substance was considered >5000 mg/kg bw in female rats. The authors noted that this dose is equivalent to (>)150 mg caffeine/kg, and this was compared to previously reported rat oral caffeine LD₅₀ values ranging from 200–400 mg/kg.¹²³

6.1.2.4 Fourteen-Day Range Finding Study in Rats

A 14-day range finding study that generally followed OECD 407¹²⁴ and FDA Redbook¹²⁵ guidelines for the purpose of setting dose levels for the 90-day study.¹⁰ Seven groups of five males and five females each (vehicle control group, three GC dose groups (1200, 2500, and 5000 mg/kg/day); and three equivalent caffeine reference control groups (36, 75, and 150 mg/kg/day)) were utilized. The caffeine doses mirrored the amount of caffeine in the GC dose levels, given a GC caffeine concentration of 3%. Rats were dosed daily via gavage for 14 days.

Animals were observed daily for viability, signs of gross toxicity, and behavioral changes and were observed in more detail once weekly (detailed clinical observations). Body weights were recorded two times during the acclimation period (including prior to dosing on day 1) and on days 3, 7, 11, and 14. Individual food consumption was also recorded to coincide with body weight measurements. The animals were sacrificed on Day 15 and samples were evaluated for any macroscopic changes (the authors did not report measuring hematology/clinical chemistry, organ weights or performing histopathological examinations).

There were no mortalities in this study. Animals treated with GC at 5000 mg/kg/day had evidence of salivation and hypoactivity. Dose-dependent hypoactivity was also observed in the intermediate (75 mg/kg/day) and high-dose (150 mg/kg/day) caffeine groups. Statistically significant dose-dependent reductions in body weights were noted in both sexes in both the GC and caffeine groups; however, they were more pronounced in males. In addition, initial reductions in body weight gain, food consumption, and food efficiency were observed in both males and females in test substance and caffeine-treated groups. Although residual decreases in food efficiency were considered test substance related, they did not adversely affect the animals as indicated by their steady weight gain following initial reductions. There were no macroscopic observations at necropsy in male or female rats attributable to the administration of either GC or caffeine.

6.1.2.5 90-day Repeated Dose Oral Gavage Study in Rats

The purpose of the 90-day study was to evaluate the potential subchronic toxicity of GC in male and female rats and to determine a no-observed-adverse-effect level



(NOAEL).¹⁰ The study was performed according to OECD 408¹²⁶ and US FDA Redbook 2000, IV.C.4¹²⁵ guidelines, and was approved by the Institutional Animal Care and Use Committee of the laboratory.

One hundred healthy 8-week old CRL Sprague-Dawley CD IGS rats (50/sex) were selected and equally divided into five groups (10/sex/group). Doses of 0, 1200, 2500, and 5000 mg/kg bw/day for GC, and 150 mg/kg bw/day for the caffeine control (equivalent to the amount of caffeine in the 5000 mg/kg/day GC group) were given by gavage based on the results of the 14-day range finding study described above. Test and reference control substances were found to be stable and the dosing solutions homogeneous over the course of the study. Based on stability and concentration verification testing, it was concluded that the animals received the targeted dose levels of GC and the caffeine reference substance.

Animals were maintained in a temperature- and humidity-controlled room at 19–23 °C and 41–95% RH, respectively, under a 12-hour light-dark cycle, and were fed a standard Harlan Teklad Global 16% protein rodent diet and given filtered tap water ad libitum. At least once daily during the study, animals were observed for viability, signs of gross toxicity, and behavior changes and were examined weekly for detailed clinical observations. Rats underwent eye examination (focal illumination and indirect ophthalmoscopy) prior to the start of the study and again on day 81. Body weights were recorded twice during acclimation, then weekly thereafter, and prior to terminal sacrifice. Individual food consumption was recorded with body weight measurements, and food efficiency was calculated. Urine and fasting blood samples were collected on Days 86 for males and 87 for females for urinalysis, hematology, and clinical chemistry analysis. Coagulation assessments were performed at study termination (on Days 94 for males and 95 for females) prior to necropsy. Gross necropsies were performed on all decedent and surviving study animals, which included examination of the external surface of the body, all orifices, and the thoracic, abdominal, and cranial cavities and their contents. The following tissues were weighed wet as soon as possible after dissection to avoid drying: adrenals (combined), kidneys (combined), spleen, brain, liver, thymus, epididymides (combined), ovaries (combined), uterus, oviducts, heart, retroperitoneal fat, and testes (combined). A more extensive list of organs and tissues were preserved for histopathological examination. Histological examination was performed on the preserved organs and tissues of the animals from the vehicle control, high dose, and reference control groups. Tissues from other dose groups were examined if any changes were otherwise noted that potentially indicated an effect from GC. Selected organs and tissues from all animals of the vehicle control, high dose, and caffeine control groups were evaluated histologically, along with tissues from other specific dose group animals that showed gross changes that could potentially indicate an effect from GC.



Results

Mortality

There were no GC-related mortalities in the study. Three animals were found dead during the course of the study: one male from the 2500 mg/kg bw/day dose group was found dead on day 84, and two caffeine reference control animals were found dead on day 47. The cause of death could not be determined for these three animals. One male from the 5000 mg/kg bw/day group was additionally sacrificed after finding it in a moribund condition on Day 81. It had displayed a decline in general health associated with reduced food consumption and body weight after sustaining a malocclusion prior to being sacrificed. Examination of this animal revealed a small thymus, enlarged adrenal glands, distended small and large intestines and malocclusion of the upper incisors. These signs correlated with microscopic findings of moderate atrophy of the thymus and a moderate abscess within the maxillary teeth and surrounding bones respectively. There were no microscopic correlations with the gross findings observed in the adrenal glands and intestines of this animal. The tooth abscess was considered the cause of morbidity and was considered unrelated to GC intake.

Necropsy findings for the 2500 mg/kg bw/day male found on Day 84 were distended large intestines, red discolored lungs, fluid in the thoracic cavity and dark thymus. Microscopic evaluation revealed moderate acute inflammation of the thymus and slight diffuse pleuritis. The cause of death could not be determined.

The two remaining mortalities were in the caffeine control group. Both animals were found dead on Day 47. One animal presented with red discolored lungs, small intestines and kidneys, a dark thymus and mottled liver. Microscopically, there was diffuse slight congestion of the lungs. The other animal presented with enlarged adrenal glands, small intestines filled with a soft, green substance, a distended, fluid-filled stomach, and red/dark discolored liver, lungs, ovaries uterus, oviducts, thymus and kidneys. Microscopically, there was minimal to moderate hemorrhage present in the adrenal cortex, liver and thymus. A definitive cause of death could not be determined.

Clinical Observations

Clinical observations directly attributed to GC administration for decedents and surviving animals included salivation in most animals of the 5000 mg/kg bw/day group males and females and the caffeine reference control males and females. Sporadic hypoactivity was observed in one male in the 2500 mg/kg bw/day group and four males in the 5000 mg/kg bw/day group as well as four males in the caffeine control animals.



Ophthalmological examination findings revealed no significant differences in males and females receiving GC or in the caffeine control group compared to controls.

Body Weight and Food Consumption

Statistically significant body weight and body weight gain reductions occurred in males in all treated groups. The weight gain reduction was increased in severity in males of the caffeine reference control group. Mean weekly body weights for males in the 1200 mg/kg bw/day group were comparable to vehicle control males from Days 1–64. Statistically significant body weight decreases in males in the treated groups occurred in the 1200 mg/kg bw/day group on Days 71–92 and in 2500 and 5000 mg/kg bw/day dose groups on Days 22–92, and in the caffeine reference control group on Days 15–92. Females in the test groups and the caffeine reference group showed no statistically significant differences in body weight or body weight gain compared to controls.

There were no significant changes in food consumption in males or females in the study. However, there were some statistically significant, dose-dependent decreases in food efficiency in the GC groups and in the caffeine control group. The decreases in food efficiency corresponded to decreases in body weight gain for males of the 2500 and 5000 mg/kg bw/day dose groups over the course of the study as well as in males of the caffeine reference control group.

Urinalysis

There were no GC-related changes in urinalysis parameters in male rats. Urinary parameters were within normal ranges for females with the exception of decreased urinary protein concentration in the 5000 mg/kg bw/day dose group and in the caffeine control group.

Hematology

There were no GC-related red blood cell changes in male animals. Changes observed in 5000 mg/kg bw/day group females consisted of increased hemoglobin concentration (HG), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red blood cell distribution width (RDW) (the latter was also observed in the caffeine reference controls). Dose-dependent increases in neutrophil and basophil counts were observed in females in the 2500 and 5000 mg/kg bw/day groups as well as the caffeine reference control. Absolute monocytes increased in the 5000 mg/kg bw/day female group, increased WBC, lymphocytes, and large unstained cell counts in the 2500 and 5000 mg/kg bw/day females and in the caffeine control females. Eosinophil counts were decreased in the 5000 mg/kg bw/day group males and caffeine control group males.



Prothrombin Times and Activated Partial Thromboplastin Times

There were no significant changes in coagulation patterns in females. There were statistically significant decreases in Prothrombin Times (PT) and Activated Partial Thromboplastin Times (aPTT) in all male GC and caffeine groups.

Clinical Chemistry

Various statistically significant changes in clinical chemistry measures were observed in male and female rats and are shown in Table 34. Statistically significant increases were observed in males in the 5000 mg/kg bw/day group and caffeine control group for aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and phosphorus, in the 5000 mg/kg bw/day dose group for bilirubin, and in the caffeine control group for albumin. Statistically significant increases were also observed in females in the 2500 mg/kg bw/day and 5000 mg/kg bw/day groups and in the caffeine control for AST, phosphorus and potassium, in all treatment groups and caffeine controls for ALT, and in the 5000 mg/kg bw/day dose group for alkaline phosphatase (ALP).

With regard to lipid metabolism, all test groups and caffeine controls showed significantly decreased triglyceride levels. Males in the 2500 and 5000 mg/kg bw/day groups, females in the 1200 mg/kg bw/day group and males in the caffeine control group showed significantly increased cholesterol.

Table 11. Summary of Mean Clinical Chemistry Values—90-day Guayusa Concentrate Study (borrowed from Kapp et al.)¹⁰

Parameter (unit)	Test substance dose levels, mg/kg/d								Caffeine dose level, mg/kg/d	
	0		1,200		2,500		5,000		150	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
AST (IU/L)	66 ± 7	66 ± 7	71 ± 12	86 ± 35	77 ± 10	95 ± 40 ^a	99 ± 19 ^a	98 ± 14 ^a	110 ± 23 ^a	96 ± 14 ^a
ALT (IU/L)	39 ± 5	35 ± 3	42 ± 5	49 ± 20 ^a	45 ± 7	61 ± 47 ^a	52 ± 13 ^a	54 ± 12 ^a	55 ± 8 ^a	56 ± 8 ^a
SDH (IU/L)	11.2 ± 2.6	10.9 ± 3.2	10.3 ± 2.6	10.9 ± 6.3	10.5 ± 3.4	11.0 ± 6.0	8.5 ± 2.1	8.5 ± 2.4	10.7 ± 4.7	9.7 ± 3.6
ALKP (IU/L)	101 ± 21	60 ± 15	101 ± 24	59 ± 17	92 ± 17	72 ± 20	114 ± 27	101 ± 50 ^a	101 ± 23	79 ± 22
BILJ (mg/dL)	0.15 ± 0.03	0.18 ± 0.02	0.17 ± 0.03	0.17 ± 0.03	0.17 ± 0.02	0.17 ± 0.03	0.22 ± 0.04 ^a	0.23 ± 0.09	0.19 ± 0.03	0.20 ± 0.03
BUN (mg/dL)	12 ± 1	15 ± 3	13 ± 2	13 ± 3	13 ± 2	15 ± 3	14 ± 2	17 ± 4	13 ± 2	14 ± 4
CREA (mg/dL)	0.24 ± 0.02	0.32 ± 0.04	0.25 ± 0.03	0.33 ± 0.04	0.27 ± 0.03	0.32 ± 0.04	0.29 ± 0.04 ^a	0.34 ± 0.04	0.33 ± 0.03 ^a	0.34 ± 0.06
CHOL (mg/dL)	71 ± 12	87 ± 18	90 ± 21	116 ± 24 ^a	95 ± 11 ^a	111 ± 22	96 ± 23 ^a	105 ± 30	102 ± 18 ^a	98 ± 13
TRIG (mg/dL)	104 ± 38	79 ± 25	68 ± 20 ^a	47 ± 8 ^a	65 ± 20 ^a	53 ± 15 ^a	54 ± 13 ^a	44 ± 13 ^a	51 ± 11 ^a	39 ± 9 ^a
GLUC (mg/dL)	130 ± 12	124 ± 14	134 ± 17	143 ± 20	140 ± 15	135 ± 19	130 ± 17	126 ± 13	127 ± 13	126 ± 19
TP (g/dL)	6.2 ± 0.3	6.9 ± 0.5	6.2 ± 0.4	7.1 ± 0.6	6.5 ± 0.3	6.8 ± 0.4	6.3 ± 0.1	6.6 ± 0.5	6.5 ± 0.3	6.5 ± 0.5
ALB (g/dL)	3.3 ± 0.1	3.9 ± 0.2	3.3 ± 0.3	3.9 ± 0.3	3.4 ± 0.2	3.8 ± 0.3	3.4 ± 0.1	3.7 ± 0.3	3.5 ± 0.2	3.7 ± 0.3
GLOB (g/dL)	3.0 ± 0.3	3.0 ± 0.3	2.9 ± 0.3	3.2 ± 0.4	3.1 ± 0.2	3.1 ± 0.2	2.9 ± 0.1	2.9 ± 0.2	3.0 ± 0.3	2.9 ± 0.4
CALC (mg/dL)	10.5 ± 0.2	10.7 ± 0.3	10.6 ± 0.4	10.9 ± 0.4	10.8 ± 0.3	10.8 ± 0.3	10.7 ± 0.1	10.7 ± 0.6	10.8 ± 0.3	10.6 ± 0.5
IPHS (mg/dL)	6.7 ± 0.4	5.7 ± 0.7	7.3 ± 0.5 ^a	5.6 ± 0.8	7.1 ± 0.5	6.6 ± 0.7 ^a	8.0 ± 0.7 ^a	7.4 ± 0.5 ^a	7.9 ± 0.5 ^a	6.9 ± 0.6 ^a
NA (mmol/L)	139.5 ± 2.7	139.4 ± 4.5	141.5 ± 6.2	138.7 ± 6.5	140.4 ± 3.4	139.8 ± 5.8	141.9 ± 5.7	137.7 ± 3.6	142.0 ± 4.9	138.7 ± 4.4
K (mmol/L)	5.26 ± 0.50	4.48 ± 0.35	5.55 ± 0.82	4.89 ± 0.43	5.51 ± 0.22	5.03 ± 0.36	5.69 ± 0.66	5.11 ± 0.32	5.41 ± 0.34	5.19 ± 0.41
CL (mmol/L)	101.3 ± 1.7	108.9 ± 3.9	102.0 ± 4.3	86 ± 35	101.6 ± 2.9	101.6 ± 3.9	101.1 ± 3.7	99.2 ± 4.0	101.2 ± 3.0	101.0 ± 3.2

Abbreviations: ALB, albumin; ALKP, alkaline phosphatase; ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; BILJ, total bilirubin; BUN, urea nitrogen; CALC, calcium; CHOL, total cholesterol; CL, chloride; CREA, blood creatinine; GLOB, globulin; GLUC, fasting glucose; IPHS, inorganic phosphorus; K, potassium; NA, sodium; SDH, sorbitol dehydrogenase; TP, total serum protein; TRIG, triglycerides.

^aP < 0.05.



Macroscopic Examination

Individual macroscopic findings included a small thymus with associated reduced organ weight and without microscopic correlates in one caffeine reference group male. Enlarged adrenal glands were observed in one 5000 mg/kg bw/day group female and one caffeine reference control group female. Only the 5000 mg/kg bw/day group female presented with correlated slight cortical hypertrophy.

Organ Weights

Statistically significant reductions in gonadal and retroperitoneal absolute and relative fat pad weights compared with vehicle control were observed in all males and females in the GC treated groups and caffeine control groups. Statistically significant decreases also occurred in the 5000 mg/kg bw/day groups and caffeine control group for brain, epididymides (males only), liver, spleen, and thymus weights. These changes were slightly more severe in females. Other changes in mean organ weights and mean organ weight ratios were noted; however, they were considered to be secondary to proportional reductions in overall body weight and/or decreased animal health status.

Microscopic Examination

Microscopic examination revealed minimal to marked hypertrophy in the salivary glands of animals in all treatment groups as well as the caffeine control group. The incidence and severity of the changes in the salivary glands were largely dose dependent with a greater impact seen in females. Submandibular and sublingual salivary glands were affected at all dose levels. Changes in the parotid glands were only observed in the intermediate- and high-dose levels. Salivary gland hypertrophy in high-dose females was similar to that of females in the caffeine control group.

Slight hypertrophy was also observed in the adrenal glands of one high-dose female and one caffeine control female. Other microscopic findings were considered incidental as they occurred sporadically or at a similar incidence to control and other test-treated groups and were generally the type commonly seen in rats of this strain and age.

Discussion

Table 12 is a composite summary of the relevant significant findings in the 90-day study by treatment group with historical control ranges presented when available. Several changes appear to be related to treatment with GC; the most prominent dose-related effects were decreased body weight gain, salivary gland hypertrophy, reduced serum TGs and reduced weight of gonadal and retroperitoneal fat pads. The vast majority of the findings in the GC groups mimicked those seen in the caffeine



150 mg control group, and thus it is presumed they were caused by the caffeine in GC.

Table 12. Summary of Effects of Guayusa Concentrate and Caffeine, from Kapp et al.¹⁰

Quantitative effects	Test substance dose levels, mg/kg/d										Caffeine dose level, mg/kg/d	
	Laboratory historical control range		0		1,200		2,500		5,000			
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Decreased terminal BW (g)	-	-	556	316	501 ^a	299	493 ^b	300	489 ^b	305	456 ^c	296
Decreased food efficiency (weeks 1-13)	-	-	0.118	0.065	0.103	0.054 ^a	0.100 ^a	0.056	0.097 ^a	0.057	0.079 ^c	0.049 ^c
Hypoadrenia	-	-	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Increased salivation	-	-	0/10	0/10	0/10	0/10	0/10	0/10	8/10	7/10	8/9	7/9
Sublingual salivary gland hypertrophy	-	-	0/10	0/10	0/10	2/10	2/9	8/10	2/9	6/10	2/9	7/9
Adrenal cortex hypertrophy	-	-	0/10	0/10	1/10	2/10	3/9	2/10	3/9	1/10	4/9	1/9
Decrease in eosinophils ($10^3/\mu\text{L}$)	0.88	0.04-0.35	0.18	0.15	0.15	0.12	0.14	0.16	0.09 ^a	0.12	0.10 ^a	0.10
Increase in MCHC (g/dL)	30.1-35.3	32.5-36.4	33.7	34	33.7	34	33.9	34.1	34	34.1	34.3 ^a	34
ARET concentration ($10^3/\mu\text{L}$)	0.29-1.55	91.3-247.1	212	170	216	173	198	172	225	189	226	211 ^a
Increased WBC ($10^3/\mu\text{L}$)	7.6-42.09	14.1-14.79	11.37	8.01	10.77	9.68	12.35	13.40 ^a	11.82	12.99 ^a	11.62	12.93 ^a
Decreased PT (seconds)	9.5-11.5	9.2-10.4	10.9	10.0	10.6 ^a	10.0	10.6 ^a	10.0	10.5 ^a	10.1	10.4 ^a	10.0
Decreased APTT (seconds)	13.5-33.4	13.2-40.6	19.6	18.1	17.1 ^a	17.3	16.7 ^a	17.3	17.3 ^a	17.6	17.6 ^a	17.1
Increased AST (U/L)	56-345	47-249	66	69	71	86	77	95 ^a	99 ^a	98 ^a	110 ^a	96 ^a
Increased ALT (U/L)	23-221	17-144	39	35	42	49 ^a	45	61 ^a	52 ^a	54 ^a	55 ^a	56 ^a
Increased ALKP (U/L)	55-183	21-179	101	60	101	59	92	72	114	101 ^a	101	79
Increased BIL (mg/dL)	0.10-0.28	0.10-0.26	0.15	0.18	0.17	0.17	0.17	0.17	0.22 ^a	0.23	0.19	0.20
Increased CREA (mg/dL)	0.20-0.48	0.23-0.53	0.24	0.32	0.25	0.33	0.27	0.32	0.29 ^a	0.34	0.33 ^a	0.34
Increased CHOL (mg/dL)	34-145	42-225	71	87	90	116 ^a	95 ^a	111	96 ^a	105	102 ^a	98
Decreased TG (mg/dL)	18-226	16-265	104	79	68 ^a	47 ^a	65 ^a	53 ^a	54 ^a	44 ^a	51 ^a	39 ^a
Increased ALB (g/dL)	2.9-3.9	2.9-5.0	33	3.9	3.3	3.9	3.4	3.8	3.4	3.7	3.5 ^a	3.7
Increased IPHS (mg/dL)	4.9-9.0	2.6-7.5	6.7	5.7	7.3 ^a	5.6	7.1	6.6 ^a	8.0 ^a	7.4 ^a	7.9 ^a	6.9 ^a
Increased K (mmol/L)	4.26-8.46	3.60-7.07	5.26	4.48	5.55	4.89	5.51	5.03 ^a	5.69	5.11 ^a	5.41	5.19 ^a
Increased urine volume (mL)	0.4-30	0.2-21.4	11.6	5.1	11.5	5.4	14.5	7.4	12.6	16.2	23.3	23.2 ^a
Decreased urinary protein (mg/dL)	44-1330	11-620	143	78	139	57	94	65	199	35 ^a	117	20 ^a
Decrease urinary pH	5.5-8.5	5-8.5	6.6	6.8	6.5	6.2 ^a	6.9	6.7	6.6	6.7	6.8	7.0
Decreased gonadal fat pad weight (g)	-	-	11.15	8.15	8.87 ^a	3.72 ^a	6.18 ^a	4.05 ^a	5.09 ^a	2.91 ^a	4.84 ^a	2.70 ^a
Decreased RETP fat pad weight (g)	-	-	15.0	8.06	8.87 ^a	3.30 ^a	6.62 ^a	3.48 ^a	4.78 ^a	2.44 ^a	5.11 ^a	1.97 ^a

Abbreviations: ALB, albumin; ALKP, alkaline phosphatase; ALT, serum transaminase; APTT, active partial thromboplastin time; ARET, absolute reticulocyte count; AST, serum aspartate aminotransferase; BIL, total bilirubin; BW, body weight; CHOL, cholesterol; CREA, blood creatinine; IPHS, inorganic phosphate; K, potassium; MCHC, mean corpuscular hemoglobin concentration; PT, prothrombin time; RETP, retroperitoneal; SD, standard deviation; TG, triglycerides; WBC, total white blood cell; -, data not applicable.

^aP < 0.05.

^bP < 0.01.

There were four premature deaths that occurred during the study; for reasons discussed above, none were considered related to test article administration. In surviving animals, the body weight, body weight gain and feed efficiency reductions seen in male animals were also noted in the caffeine control group and were interpreted to be associated with the caffeine content of GC. Numerous studies have identified decreased body weight in rodents as an effect of caffeine ingestion.^{123, 127-131} For example, rats given between 20 and 287 mg/kg bw/day in drinking water for 90-days showed decreased body weight gain in all groups; the effect was statistically significant only at the highest dose, and slightly more pronounced in males versus females (reduction of 26% in males, 20% in females).¹²³

When administering 0.5% caffeine in the diet (approximately 250 mg/kg bw/day using the Lehman method¹³²) to male rats for 7-8 weeks, decreased food intake and decreased body weight gain in males as well as decreased thymus gland weights



were observed.¹²⁸ The higher caffeine dose may be the reason for the food intake decrease that was noted in the Gans study, although decreased food intake was not noted along with the body weight decreases in the current (gavage) experiment.

Clinical observations attributed to administration of GC were slight-moderate increased salivation and hypoactivity in the high dose and caffeine control animals of both sexes. Similar hypoactivity in rats has been noted after caffeine exposure in other studies. For example, it was noted after 30 mg/kg bw intraperitoneal injection of caffeine to rats,¹³³ and after gavage of 1 mg/kg and 9 mg/kg caffeine in rat pups.¹³⁴

The increased salivation was correlated microscopically (in both the high dose and caffeine control animals) with salivary gland hypertrophy in the submandibular, sublingual and parotid salivary glands, and was more severe in females. The effect of caffeine and other phosphodiesterase inhibitors on salivary glands is well documented.^{123, 135-137} In the National Toxicology Program study on caffeine in Fischer rats, a dose-dependent effect on cellular enlargement in salivary glands was observed and considered to be adaptive.¹²³ Such sympathomimetic effects of caffeine on the salivary glands are known to be reversible.¹²³ Adaptive and reversible changes of the salivary glands have also been observed in response to substances such as tannic acid and grape skin extract (both are bitter/astringent taste components, which may increase production and excretion of saliva and modify the components of saliva).¹³⁸ The astringent nature of GC may have contributed to the salivary gland effects, which were considered adaptive and not toxicologically relevant.

The decreased urinary protein concentration in high-dose and caffeine control group females remained within the historical control range of the performing laboratory and were unaccompanied by any other corresponding clinical or histopathological changes. The finding is also in the opposite direction of that usually seen with kidney toxicity and was considered to be secondary to caffeine intake and non-adverse.

The increased HG, MCV, and MCH observed in high dose females (but not in the caffeine control group) and the increased RDW (noted in both high dose and caffeine control females) were generally within the laboratory's historical control ranges, were of very low magnitude and were not associated with other hematological, histopathological, or clinical findings and, thus, were not considered adverse. The dose-dependent increases in neutrophil and basophil counts in mid-dose, high-dose and caffeine control females were interpreted to be potentially associated with the caffeine content of the test article (no information was provided about whether or not the values fell within historical control ranges). Other hematological differences related to WBC counts, including monocytes, lymphocytes and large unstained cell counts, were not dose-dependent, were of very low magnitude and were within historical ranges; thus, they were interpreted to be



unrelated to treatment. The increased eosinophil counts in males of the high-dose and caffeine control groups also fell within historical control ranges and were not associated with other hematology changes.

Although there were statistically significant decreases in PTs and aPTT in all male GC and caffeine control groups, the effects remained within the historical control range of the performing laboratory and revealed no correlating clinical or pathological findings. Thus, the findings were not considered adverse and not related to the administration of GC other than as relates to caffeine.

Clinical chemistry changes were observed in male rats at all treatment levels and in females of the mid-dose and high-dose groups. The changes in liver enzymes, including AST, ALT and ALP, in both males and females remained within the historical control ranges. Because the slight significant increases in AST and ALT occurred in the caffeine control group at similar magnitudes to the high dose group, the findings were considered most likely due to the caffeine content of GC. The increases may be related to adaptive processes associated with caffeine metabolism, which occurs in the liver.^{139, 140} While a significant increase was not seen in the female caffeine control group for ALP as it was in the high dose female group, the increase in the high dose females was of relatively low magnitude, falling well within the historical control range as mentioned previously.

Several animal studies using energy drinks as the test article resulted in significant increases in AST, ALT and/or ALP compared to controls.¹⁴¹⁻¹⁴⁴ While the energy drinks contained other ingredients such as B-vitamins, taurine and herbs, caffeine is generally considered the major active ingredient. These drinks contained from 24 to 141 mg of caffeine per serving (about 8 ounces) and were given to rats at various doses up to total substitution of drinking water for several weeks. The NTP's 90-day study administered caffeine to Fischer rats at doses of 19.7, 42, 85.4, 151, 272 mg/kg bw/day (males) and 23, 51, 104, 174 and 287 mg/kg bw/day (females). The results showed significant changes in AST and ALT values, but they were not considered by the authors to be adverse since the changes were not considered to have a dose-response, and the NOAEL was considered to be 151 mg caffeine/kg bw/day for males and 174 mg caffeine/kg bw/day for females.¹²³ Slight but significant increases in AST and ALT have been noted in humans with regard to coffee consumption, but coffee/caffeine consumption has also been associated with protective effects with regard to liver enzyme increases (e.g., ALT) and liver protection in general.¹⁴⁵⁻¹⁴⁹ Ruhl and Everhart found that in adults at high risk for liver injury, consumption of coffee and especially caffeine was associated with lower risk of elevated ALT.¹⁴⁷ A multi-ethnic cross-sectional epidemiological study identified significant inverse associations of caffeinated coffee consumption with liver transaminases and the non-alcoholic fatty liver disease liver fat score (decaffeinated coffee intake showed no significant associations).¹⁵⁰ Similar



significant inverse associations with coffee intake were observed for serum AST and ALT in males and less strongly in females.¹⁵¹⁻¹⁵³

Bilirubin levels, while elevated compared to the control group in males of the high-dose group, were still within the historical reference range and were unaccompanied by direct histological changes or hematology findings; therefore, the change was not considered of toxicological concern. The decreased triglyceride and increased cholesterol levels noted are interpreted as caffeine related changes, and this pattern has been observed in other studies.^{127, 129, 136, 144, 154} Studies on rats receiving energy drinks revealed a similar pattern of increased cholesterol, although triglycerides were increased possibly due to the sugar content of the test articles.¹⁴⁴ Decreased triglycerides have also been attributed to the physiological effect of caffeine on increasing lipid droplet turnover, fat oxidation and oxidative phosphorylation in hepatic cells.¹²⁹ There have been mixed results with regard to the effects of caffeine and coffee on cholesterol/lipids in humans.^{127, 136, 154} Decreased triglyceride concentration found at all treatment levels is likely considered attributable to the pharmacological effects of caffeine on adipose tissue, which has historically correlated to reductions in fat pad weights.^{130, 155} Fat pad weight decreases were also noted in the current study in males and females at all dose levels and the caffeine control groups, and overall these results are thought to be related to caffeine and/or may be an indirect result of clinical reductions in body weight.

With regard to macroscopic findings at study termination, the small thymus and enlarged adrenal glands in individual animals of both the high dose GC and caffeine control females were considered by the study authors to be secondary to treatment-related stress.^{128, 156} With regard to organ weights, the significant differences in absolute and relative gonadal and retroperitoneal absolute and relative fat pad weights in males and females from all GC treatment and caffeine control groups were considered to be related to caffeine administration. This has been shown in other published studies.^{127, 130, 157, 158} In humans and rodents, caffeine ingestion elevates the metabolic rate and increases the oxidation of fat via lipolysis and release of catecholamines.¹⁵⁹⁻¹⁶¹ Wilcox et al. observed similar significantly reduced weights of fat pads as well as mobilized fatty acids after administration of caffeine and exercise to male rats.¹³⁰ Caffeine plus exercise resulted in greater fat pad loss than exercise alone. Sugiura et al. studied intraperitoneal adipose tissue (IPAT) in mice fed diets with caffeine, catechins or a combination of caffeine and catechins.¹⁵⁷ The caffeine group and the caffeine plus catechins group both showed statistically significant decreases in IPAT. Milanez et al. report that short term studies using caffeine resulted in decreased body fat in rats.¹²⁷ In humans, caffeine acts primarily as an antagonist of adenosine receptors; thus, the effects in humans include lipolysis, systematic catecholamine release and increased plasma free fatty acid concentrations.¹⁶²



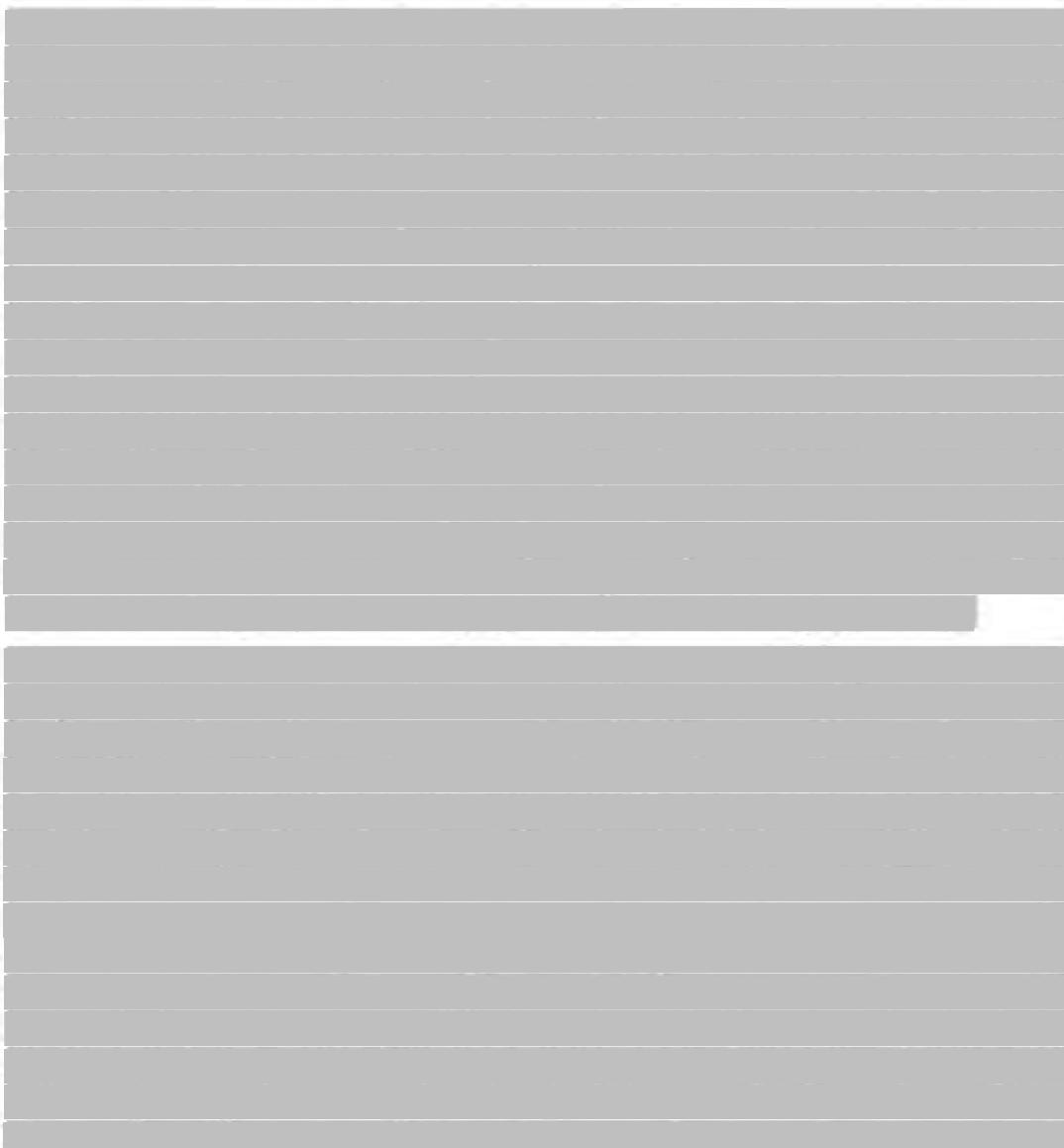
The absolute or relative reduction in the thymus, spleen or epididymitis weights, along with absolute and relative increases in adrenal gland weights are interpreted by the study authors to be secondary to treatment-related stress,¹⁵⁶ and/or reductions in body weight. As discussed in detail above, the effects on the salivary glands by GC were considered adaptive and not toxicologically relevant. The effects that were noted in all dose groups (decreases in fat pad weight, salivary gland hypertrophy, serum cholesterol, adrenal cortex hypertrophy and eosinophil count changes) also occurred in the caffeine group and/or have been attributed to caffeine in other studies.

Conclusions

Nearly all of the positive findings in the 90-day study that were related to GC (RUNA® Concentrate) treatment also occurred in the group treated with an equal amount of caffeine alone and are attributed to the pharmacologic effects of caffeine present in GC. Based strictly on body weight comparison, exposure to 150 mg/kg bw/day of caffeine (as was the case in the caffeine control group as well as the 5000 mg/kg bw/day GC group) would be equivalent to a 70 kg person consuming 10.5 g/day of caffeine (approximately 53 cups of coffee at 200 mg caffeine per cup) by gavage all at once. The low dose group represents exposure to approximately 2.5 g/day of caffeine for a 70 kg human, equivalent to consuming approximately 13 cups of coffee at once every day, which is still far higher than what is generally ingested by even the highest caffeine consumers. As detailed later in this report, safe caffeine consumption levels for humans have been agreed upon by numerous scientific and/or regulatory organizations.

In summary, there were no findings considered of toxicological concern that were otherwise attributable to GC (RUNA® Concentrate). Thus repeated administration by gavage of 1200, 2500 and 5000 mg/kg bw/day for 90 days was not considered to cause adverse effects or signs of toxicity in male or female rats other than those caused by caffeine, and the NOAEL, aside from caffeine exposure, (and thus for all components of the extract other than caffeine) was determined to be 5000 mg/kg bw/day; the highest dose tested.

The NOAEL reported by NTP for caffeine when it was administered *via drinking water* was 1500 ppm; equivalent to 151 and 174 mg/kg bw/day for male and female rats, respectively, and 167 and 179 mg/kg bw/day for male and female mice, respectively (which are similar to the 150 mg/kg bw/day level of caffeine given *via gavage* in the GC study high dose group).¹²³ Note that NOAELs are generally lower when a substance such as caffeine is administered via gavage as compared to administration in the food or water supply.¹⁶³ A summary of the NTP report follows:¹²³



Note that safe levels of caffeine consumption by humans have been established and are discussed in detail in subpart 6.2.

6.1.3 Human Studies on Guayusa

As previously described in the pharmacokinetic section (subpart 6.1.1), A guayusa leaf hydroethanolic extract (AmaTea®) containing 20% caffeine and 30% polyphenols by weight, and a green coffee extract (JAVA.g) containing 30% caffeine and 40% polyphenols by weight, were studied by Krieger et al. in a double-blind, randomized, crossover, clinical trial.¹¹⁷ In more detail, the subjects were 12 healthy male volunteers aged 21 to 34 years old. The men underwent physical



examination, medical history reporting and ECG analysis and were determined to be in good health. Those who regularly consumed more than 500 mg of caffeine per day were excluded. Subjects were instructed to abstain from caffeine consumption 24 hours prior to each study visit.

At each visit, subjects received one of three caffeine sources per the randomization schedule. The treatments were administered in bottled liquid form and subjects were required to drink the product in 5 min or less. Each caffeine source contained 200 mg of caffeine in 4 fluid ounces. The control was a synthetic source of caffeine. Baseline measurements of serum caffeine levels, urinary neurotransmitters (serotonin, GABA, dopamine, epinephrine, norepinephrine, and glutamate), blood pressure, and heart rate were obtained. Measurements of all neurotransmitters were taken 60 minutes post-dose; blood pressure and heart rate measurements were taken at 60 and 120 minutes; adverse events, subjective comments and incidences were taken over the entire 240 minutes of the visit.

All subjects completed the study per protocol, with the exception of one subject during his green coffee extract visit who had non-zero levels of caffeine at baseline. This subject was included in the per-protocol population.

The results showed no statistically significant changes in blood pressure or heart rate from baseline of each natural caffeine source compared with changes from baseline for the synthetic control. The AmaTea® stimulated a significantly lower increase in epinephrine compared with the synthetic control while the green coffee extract provoked an increase in epinephrine similar to the control. There were four adverse events, all of which were considered unrelated to the caffeine sources (a fractured clavicle and right toe abrasion at the green coffee visit, an upper respiratory tract infection at the AmaTea® visit, and right ankle pain at the synthetic control visit). None of the subjects made subjective comments regarding adverse effects related to the test substances.

6.1.4 Additional Scientific Studies

Swanson-Flatt et al. 1989 studied the effects of individual plant-derived preparations, including guayusa, and their effects on blood sugar regulation in mice.¹⁶⁴ A concentrated aqueous *I. guayusa* leaf extract was diluted in water (1 mL of the extract in 100 mL) and replaced drinking water in the mouse diet. Treatment lasted for 43 days. Guayusa did not adversely affect parameters of glucose homeostasis in non-diabetic or diabetic mice.

6.1.5 *Ilex guayusa* Regulatory Status

A thorough search for the current regulatory status of *I. guayusa* relevant to its use in food in the United States was conducted and no relevant information was located.



No mention of *I. guayusa* occurs in the Federal Register, Code of Federal Regulations Title 21, FDA's GRAS Notice Inventory or other federal databases that were searched.

On November 15, 2011, Health Canada added *I. guayusa* to the Natural Health Products Ingredients Database (NHPID), and on February 14, 2012, Health Canada added caffeine derived from *I. guayusa* leaves to the NHPID.

In February 2017, the Food Safety Authority of Ireland (FSAI) received an application from Runa, LLC for an opinion on the substantial equivalence of aqueous extracts of the dried leaves of *I. guayusa* with aqueous extracts of *I. paraguariensis* (which is not considered a novel food as it was in the EU market prior to 1997). They showed that the two extracts are similar in terms of macronutrients, caffeine and CA levels. FSAI was satisfied from the information that the two are substantially equivalent.¹⁶⁵ Aqueous extracts of dried leaves of *Ilex guayusa* are now an authorized novel food in the European Union, under the food categories "herbal infusions" and "food supplements". The maximum levels of use are stated as "in line with normal use in herbal infusions and food supplements of a similar aqueous extract of dried leaves of *Ilex paraguariensis*". The composition of the novel food is stated as 0.2–0.3 g/100 mL of carbohydrate, 19.8–57.7 mg/100 mL caffeine, 0.14–2.0 mg/100 mL theobromine, and 9.9–72.4 mg/100 mL chlorogenic acids.¹⁶⁶

6.1.6 Summary of Guayusa Safety

Overall, the toxicological studies on GC (RUNA® Concentrate) discussed in this subpart do not suggest any genotoxicity or other toxicological concerns with regard to ingestion of this ingredient, other than those caused by very high levels of caffeine. Of specific weight, repeated administration by gavage of RUNA® Concentrate for 90 days was not considered to cause adverse effects or signs of toxicity in male or female rats other than those caused by caffeine, and the NOAEL, aside from caffeine exposure, (and thus for all components of the extract other than caffeine) was determined to be 5000 mg/kg bw/day; the highest dose tested. There is also currently authorized European novel food status for aqueous extracts of dried leaves of *Ilex guayusa*.

6.2 Safety of Caffeine

The major safety conclusions of this subpart are comprised of:

1. Numerous toxicological and epidemiological safety reviews including those by authoritative bodies, suggesting that consumption of up to moderate levels of caffeine (400 mg/day for adults, 300 mg/day for pregnant women, and 2.5 mg/kg bw/day for children) is safe for humans.



2. The pharmacokinetic profile of caffeine suggests that it is rapidly absorbed, metabolized, and eliminated from the body.
3. The GRAS status of natural extractives of coffee (21 CFR §182.20), with the understanding that this regulation pertains to low levels used for flavorings.
4. The fact that caffeine consumption patterns have remained relatively consistent (or even declined) over the years despite the introduction of various new caffeinated beverages.^{13-15, 70-72}

Caffeine is a central nervous system (CNS) stimulant. It is structurally similar to adenosine, and its main action appears to be the antagonization of adenosine receptors (especially A₁ and A₂ receptors found in various tissues such as the heart and the CNS), along with possible inhibition of phosphodiesterase, likely at higher dose levels (with mild effects on energy metabolism).^{93, 167-171} It has flavoring capabilities due to its bitter taste.¹¹ Caffeine is thought to function as a natural herbicide and insect repellent in plants.^{172, 173} It is also found naturally at low levels in the nectar of *Coffea* and *Citrus* species where it appears to enhance pollinators' memory of reward via inhibition of adenosine receptors and long term potentiation of Kenyon cells (which function similarly to mammalian hippocampal neurons), resulting in the securing of pollinator fidelity.¹⁷⁴

Caffeine has been the subject of more scientific studies than likely any other food ingredient in history. Tens of thousands of studies have been published in the peer-reviewed literature on the physiological effects of caffeine and coffee consumption and its potential toxicological effects. Numerous comprehensive reviews and meta-analyses have been published on human and animal caffeine toxicological studies and general caffeine safety. To date, a number of governmental agencies and other scientific institutions that may be considered "authoritative bodies" have reviewed the safety of caffeine and reached conclusions and recommendations about the use of caffeine as a food/beverage ingredient. These opinions are freely available in the public domain and are described below, and they strongly support that there is expert consensus about the general recognition of safety of caffeine consumption within specified consumption limits that fall within the intended uses of RUNA® Concentrate.

As there is a plethora of human data available with regard to caffeine safety, and preclinical/animal study data was taken into account in various reviews that are summarized below and/or safety conclusions were made based on human data alone, specific animal data as relates to caffeine is not generally detailed or discussed in this dossier.



6.2.1 Toxicology and Safety Reviews of Caffeine by Authoritative Bodies

The organizations listed below that performed comprehensive reviews on the safety of caffeine use, consisting of governmental agencies or other highly respected scientific groups, may arguably be considered “authoritative bodies”. These groups evaluated a vast body of data in the primary and secondary published literature with regard to caffeine safety, and their conclusions are overall similar based on the research available at the time of each publication. They are considered consistent and representative of the totality of the body of safety evidence available in the public domain.

Below are summaries and findings of these reviews; they are listed in roughly chronological order. The conclusions are summarized and cited in Table 13 below, while additional detail, often using words taken directly from the reviews themselves, can be found in the subpart below the table. These reviews are hereby considered to be incorporated by reference into this dossier. Note that many of the studies and reviews described below were derived from research on the beneficial effects of coffee intake. The beneficial effects from coffee may also be attributed to the effects of the CAs found in coffee, and the coffee research is also relevant to safety of CAs.

Table 13. Major Conclusions on Caffeine Safety by Scientific and/or Regulatory Organizations

Publication and Citation	Year	Length of Report (# of Pages)	Major Conclusions Regarding Caffeine
Institute of Medicine Committee on Military Nutrition Research (IOM CMNR) ¹⁶⁷	2001	157	Doses of 100–600 mg caffeine may be used to maintain cognitive performance in the military. Based on the authors' review of the literature, such levels are not expected to pose any serious, irreversible acute or chronic health risks for military personnel.
Health Canada / Nawrot et al. ⁷⁴	2003	30	400 mg/day (~6 mg/kg for a 65-kg person) is not associated with adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (with consumption of adequate calcium), changes in adult behavior, increased incidence of cancer and effects on male fertility in the healthy adult population. Overall caffeine was considered not likely to be human carcinogen at doses \leq 500 mg/day. Reproductive-aged women should consume \leq 300 mg caffeine per day (equivalent to ~4.6 mg/kg bw/day for a 65-kg person) while children should consume \leq 2.5 mg/kg bw/day. Based upon the results from the Nawrot et al. publication, ⁷⁴ Health Canada developed the following maximum caffeine intake guidelines: Adults, 400 mg. Children aged 4–6, 45 mg/day. Children aged 7–9, 62.5 mg/day. Children aged 10–12 years, 85 mg/day. Women of childbearing age, 300 mg/day. ¹⁷⁵
European Food Safety Authority (EFSA) ¹³⁹	2015	120	Single doses of up to 200 mg (~3 mg/kg bw/day for 70 kg adult) are unlikely to induce clinically relevant changes in blood pressure, myocardial blood flow, hydration status or body temperature, to reduce perceived exertion/effort during exercise or to mask the subjective perception of alcohol intoxication. Single doses of 100 mg (about 1.4 mg/kg bw for a 70 kg adult) may increase sleep latency and reduce sleep duration in some adult individuals, particularly if consumed close to bedtime. 400 mg/day (~5.7 mg/kg bw/day) does not raise safety concerns for adults, including lactating women. Up to 200 mg/day is not of concern in pregnancy. Data was insufficient to determine a safe level for children and adolescents, but 3 mg/kg bw/day could potentially serve as a no concern level.



U.S. Dietary Guidelines Advisory Committee (DGAC) ^{55, 176}	2015	571*	Up to 400 mg/day in adults is not associated with increased long-term health risks such as cardiovascular disease, cancer or premature death, and moderate levels may confer certain health benefits. Data suggests that risk of miscarriage, stillbirth, low birth weight, and small for gestational age births is minimal given the average caffeine intake of pregnant women in the United States.
International Agency for Research on Cancer (IARC) / Loomis et al. ¹⁷⁷⁻¹⁷⁹	1991	523 (whole report)	Coffee is possibly carcinogenic to the human urinary bladder (Group 2B designation), no association with breast or colon cancer, inadequate evidence for other cancers. Caffeine is not classifiable as to its carcinogenicity to humans (Group 3 designation).
	2016/2018	2 (2016 conclusions published by Loomis et al.) / 501 (final monograph published in 2018)	Coffee is not classifiable as to its carcinogenicity to humans (Group 3 designation) with inadequate evidence in humans and animals. No consistent evidence for association with coffee and bladder cancer. Inverse associations with endometrial and liver cancer and coffee drinking. No association to a moderate inverse association with coffee consumption and breast cancer. Moderate evidence of an inverse relationship between coffee and colon adenomas, liver fibrosis and cirrhosis. No increased incidence in other tumors observed.
International Life Science Institute, North America (ILSI/NA) / Wikoff et al. ³⁶	2017	64	Systematic review of caffeine. Evidence generally in agreement with Health Canada (Nawrot, 2003) and supports that 400 mg caffeine/day in healthy adults is not associated with overt, adverse cardiovascular effects, behavioral effects, reproductive and developmental effects, acute effects or bone status. Consumption of up to 300 mg caffeine/day in healthy pregnant women is generally not associated with adverse reproductive and developmental effects. Limited data was identified for children and adolescent populations, although the available data suggests that 2.5 mg caffeine/kg bw/day remains an appropriate upper safe limit.

*This report covered many nutrients/substances aside from caffeine

6.2.1.1 Institute of Medicine Committee on Military Nutrition Research (2001)

An extensive review of the toxicity of coffee and caffeine was published by the Institute of Medicine Committee on Military Nutrition Research; (IOM CMNR) in 2001.¹⁶⁷ Part of the purpose of the report was to review the scientific data on the efficacy of caffeine in maintaining physical and cognitive performance in military operations, caffeine safety, appropriate formulations for administration during military operations, and to identify any ethical or other considerations. Another purpose was to review the effectiveness of caffeine compared to other compounds that have CNS-stimulating effects.

The publication included a comprehensive review of the myriad of clinical and pre-clinical studies on the safety of coffee and/or caffeine consumption in humans and rodents. Moderate caffeine consumption was defined in various clinical trials as up to 400 mg/day, although they state that some studies defined an upper moderate level to be 600 mg/day. A high caffeine exposure was defined as greater than 900–1,000 mg/day. The human fatal dose of caffeine was reported to be approximately 10–14 g (150–200 mg/kg bw); 10 g of caffeine can also lead to convulsions and vomiting.

With regard to potential health risks, the report summarized that caffeine-naïve individuals experience a small increase in blood pressure after acute dosing with caffeine. During chronic administration of caffeine, tolerance appears to develop, and chronic, long-lasting changes in blood pressure are usually not seen in individuals who consume caffeine routinely. While the acute pressor effects of



caffeine are well documented, there was no clear epidemiological evidence that caffeine consumption is causally related to hypertension. However, a number of studies have demonstrated that caffeine consumption produces a transient elevation in blood pressure that occurs regardless of whether or not the individual is a habitual user of caffeine. Thus, high caffeine intake may be an additional risk factor for hypertension at the individual level due to long-lasting stress or genetic susceptibility to hypertension (note that this has been disputed in more recent studies, systematic reviews and meta-analyses as discussed further below).

With regard to heart disease, the review summarized that in general, controlled clinical attempts to demonstrate effects of caffeine on increasing heart rate or inducing arrhythmia have been unsuccessful. The review found no increased risk of coronary heart disease associated with consumption of up to six cups of coffee per day. Thus, increased risk of cardiovascular problems resulting from the use of caffeine supplements by the military would not appear to be of major concern.

With regard to reproductive and developmental toxicity, the report summarized that caffeine consumption has been suggested as the cause of numerous negative reproductive outcomes, from shortened menstrual cycles to reduced conception, delayed implantation, spontaneous abortion, premature birth, low infant birthweight, and congenital malformations. As with most other aspects of caffeine consumption, there is a paucity of reliable data concerning the effects of caffeine on reproductive processes. The review stated that recent reviews of human studies suggest that some of the initial reported associations between caffeine and reduced fertility, teratogenicity, and other fetal and maternal effects in humans may be explained by confounding factors such as associated cigarette smoking, alcohol consumption, reporting inaccuracies, and other methodological errors. The authors concluded that moderate consumption of caffeine was not likely to increase the risk of spontaneous abortion.

With regard to osteoporosis, the review stated that in the large number of studies that have been conducted, there appears to be no consistent trend linking caffeine consumption to negative effects on bone mineral density or incidence of fracture. Early studies also indicated a significant effect on acute calcium diuresis; however, subsequent work indicated that this acute phase of excretion was accompanied by a later decrease in excretion of calcium in the urine. Later studies found either no significant effect of caffeine on calcium balance or negative balance only in subjects consuming less than half of the currently recommended intake of calcium.

With regard to fluid homeostasis, the report summarized that caffeine is a diuretic and has been found to increase urinary excretion within one hour of treatment. Significant increases have been observed in 3-hour urine output as well as in 24-hour urine output as a result of caffeine consumption in amounts of 250–642 mg. Data are inconsistent with respect to whether caffeine creates a total body water



deficit. The deficit may depend on the amount of caffeine consumed, the individual's history of caffeine use, and the total solute load of any accompanying food or beverage. However, the risk of water deficit may be increased when caffeine is used in situations already known to put military personnel at risk of dehydration, such as in hot or desert environments or in cold environments (note that more recent studies have found that caffeinated beverage consumption provides similar hydrating qualities as water; see subpart 6.2.3.9).

With regard to behavioral effects, the review stated that although a relatively low dose of caffeine (250 mg) produced favorable subjective effects (e.g., elation and pleasantness) and enhanced performance on cognitive tasks in healthy volunteers, higher doses (500 mg) led to less favorable subjective reports (e.g., tension, nervousness, anxiety, restlessness) and less improvement in cognitive performance than placebo. Negative effects may be more pronounced in nonusers than in regular users of caffeine.

The review found that use of caffeine by humans is generally not associated with abuse or addiction. Tolerance develops to some of the effects of caffeine when caffeine-containing beverages are consumed regularly. Withdrawal symptoms often occur with the abrupt removal of caffeine from the diet. The frequency of occurrence of withdrawal varies anywhere from 4 to 100 percent. The symptoms of cessation, when they do occur, are not long-lasting and are generally mild. These include headaches, drowsiness, irritability, fatigue, low vigor, and flu-like symptoms. This withdrawal phenomenon could conceivably lead to decrements in performance during military operations.

The report discussed that among the variables that may contribute to differences in caffeine sensitivity are baseline levels of stressor exposure and genetically mediated stress reactivity. Stress may include physical stressors (e.g., exercise), physiological stressors (e.g., heat stress, infection, sleep deprivation), or psychological stressors. After stressor exposures, stress-responsive neurohormonal and neurotransmitter systems are activated. Caffeine alters the degree of responsiveness of these stress-responsive systems to stressful stimuli. The degree to which responsiveness is altered varies according to previous caffeine consumption (habitual users versus nonusers).

The overall recommendations in the report were that caffeine in doses of 100–600 mg may be used to maintain cognitive performance, particularly in situations of sleep deprivation. Specifically, it can be used in maintaining speed of reactions and visual and auditory vigilance, which in military operations could be a life or death situation. A similar dose range (200–600 mg) of caffeine is also effective in enhancing physical endurance and may be especially useful in restoring some of the physical endurance lost at high altitude among military personnel.



The report further states that use of caffeine under conditions of sustained military operations would not appear to pose any serious, irreversible acute or chronic health risks for military personnel in situations where increased doses might be recommended. Caffeine use in sustained operations in hot or cold environments or at high altitudes may increase the risk of dehydration, so fluid and food intake of personnel should be closely monitored in these situations. Female military personnel should be advised of the potential for a small increased risk of spontaneous abortion in the first trimester of pregnancy.

Orally ingested caffeine is largely excreted as paraxanthine, the main metabolite of caffeine, and only small amounts of caffeine are excreted (in the urine) unchanged. The authors of the review stated, "The fact that the human body converts 70–80 percent of caffeine into paraxanthine with no apparent toxic effects following caffeine doses of 300–500 mg/day suggests that paraxanthine's toxicological potency is low." Excessive caffeine consumption may result in the biological accumulation of paraxanthine, which has a longer half-life than caffeine (exact $t_{1/2}$ value not given), and consequently result in "negative effects" by contributing to the potential pharmacologic effects associated with chronic caffeine consumption. Accumulated paraxanthine "may contribute to development of tolerance and withdrawal symptoms."

6.2.1.2 Health Canada/Nawrot et al. (2003)

In 2003, Health Canada authors published a comprehensive review of caffeine's general toxicity and its effects on the cardiovascular system, bone and calcium balance, and human behavior as well as its mutagenicity and genotoxicity, carcinogenicity, and reproductive and developmental effects.^{74, 175} As an aside, this has been considered one of the most extensive reviews on caffeine safety for many years, and is frequently cited.⁷⁴

The summary of the report per the abstract is as follows:

"Based on the data reviewed, it is concluded that for the healthy adult population, moderate daily caffeine intake at a dose level up to 400 mg/day (equivalent to 6 mg/kg body weight/day in a 65-kg person) is not associated with adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (with consumption of adequate calcium), changes in adult behaviour, increased incidence of cancer and effects on male fertility. The data also show that reproductive-aged women and children are 'at risk' subgroups who may require specific advice on moderating their caffeine intake. Based on available evidence, it is suggested that reproductive-aged women should consume \leq 300 mg caffeine per day (equivalent to 4.6 mg/kg bw/day for a 65-kg person) while children should consume \leq 2.5 mg/kg bw/day."



In more detail, the report states that the lethal dose for caffeine in humans is estimated at 10 g, although in specific cases death was reported after ingestion of only 6.5 g, while survival was also reported after ingestions of as much as 24 g. With regard to cardiovascular disease, clinical/epidemiological studies suggest that moderate caffeine intake (up to 400 mg/day) does not adversely affect cardiovascular health. With regard to bone metabolism and calcium balance, the authors stated that the significance of caffeine's potential to affect calcium balance and bone metabolism adversely is dependent on lifetime caffeine and calcium intakes and is biologically more relevant in women. Caffeine intakes of <400 mg/day did not have significant effects on bone status or calcium balance in individuals ingesting at least 800 mg calcium/day.

The report discussed that moderate consumption of caffeine in healthy adults has not been associated with major adverse effects on mood or performance, and most effects associated with higher consumption levels were considered to be self-limiting in nature. However, inconsistencies in the literature and individual differences in sensitivity to caffeine suggest that some people (e.g., those with anxiety disorders) need to be aware of possible adverse effects of caffeine and should limit their intake accordingly. Additionally, the literature supports the existence of caffeine withdrawal symptoms in some individuals, with variability in the severity of symptoms. Such symptoms were noted to be generally short-lived and relatively mild in the majority of those affected.

With regard to studies in children, the review states that results were sometimes conflicting and difficult to compare due to the use of different endpoints or assessment tools in different studies, and most studies used only a small number of subjects. The authors concluded that it is possible that the protracted development of the nervous system may render children more sensitive to any adverse events of caffeine, and they stated that in the absence of more robust data associated with low levels of administered caffeine, an upper intake of 2.5mg/kg bw/day is a reasonable amount on which to base risk assessments of caffeine consumption in children.

Although evidence for the mutagenic potential of caffeine is conflicting, it was considered unlikely by the authors of the review that at normal, physiologically relevant levels of consumption, caffeine would result in mutagenic effects in humans. With regard to carcinogenicity, evidence from several oral oncogenicity and chronic toxicity studies in mice and rats suggest that caffeine is not carcinogenic (up to dose levels of 291 and 230 mg/kg/bw/day, respectively). Observational studies on caffeine (as present in coffee) consistently showed that caffeine is not associated with cancer development at several tissue and organ sites (large bowel, stomach, prostate, liver, lung, vulva, breast). Caffeine was occasionally associated with cancer at several other sites in studies. With regard to the urinary bladder, the authors reported four cohort studies and 17 case-control studies showed no carcinogenic effect of consumption of five or more cups of coffee per day; however,



nine case-control studies did show a positive association, with three showing a dose-response (note: more recent reviews have found no consistent evidence of an association with bladder cancer as discussed below¹⁷⁸⁻¹⁸⁰). With regard to the pancreas, Nawrot et al. found eight cohort studies that showed no significant effect with doses of ≥ 500 mg/day while one study showed a positive effect. Similarly, 21 out of 24 case-control studies showed no effect on the pancreas; however, one showed a significant effect at doses over 400 mg per day, and two showed a dose-related response. When smoking was taken into consideration, the authors stated that positive responses were weakened. With regard to the ovaries, they found five case control studies showed no effect with doses ≥ 500 mg/day while two showed an effect. Lastly, in a case-control study, they found risk of basal cell carcinoma was associated with caffeine. Overall caffeine was considered not likely to be a human carcinogen at doses of ≤ 500 mg/day.

With regard to reproductive and developmental effects, the epidemiological studies that were reviewed by the authors suggested that consumption of caffeine at doses above 300 mg/day could reduce fecundability in fertile women. In men, consumption of dose levels above 400 mg/day were determined to have the possibility of decreasing sperm motility and/or increasing the percentage of dead spermatozoa (only in heavy smokers) but would be unlikely to adversely affect male fertility in general. Related to spontaneous abortions, there appeared to be reasonable grounds for limiting the consumption of caffeine to less than 300 mg/day in women who are, or who are planning to become, pregnant, although additional prospective studies to more carefully measure actual caffeine intake and to adjust for confounders such as the pregnancy signal were desired by the authors. Similarly, reducing consumption to below 300 mg/day in pregnancy (particularly in smokers or heavy alcohol drinkers) was considered prudent with regard to potential fetal growth interference effects. Caffeine consumption of less than 300 mg/day was considered unlikely to have an adverse effect on gestation length/preterm delivery. While caffeine was shown to be teratogenic at very high dose levels in animal studies, there was little evidence to support that moderate consumption of caffeine during pregnancy would cause morphological malformations, or adverse postnatal development.

Based on this review, Health Canada established the following guidelines with regard to maximum caffeine intake levels recommended for various populations¹⁷⁵:

- Adults: 400 mg/day
- Children aged 4–6: 45 mg/day
- Children aged 7–9: 62.5 mg/day
- Children aged 10–12 years: 85 mg/day
- Women of childbearing age: 300 mg/day.



6.2.1.3 European Food Safety Authority (2015)

In 2013, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies was asked by the European Commission to deliver a current scientific opinion on the safety of caffeine and on possible interactions between caffeine and other common constituents of energy drinks (such as taurine and D-glucurono- γ -15 lactone), alcohol, synephrine, and physical exercise. Bull et al. published a paper on the literature search that was the basis for this assessment.¹⁸¹ In 2015, EFSA released its scientific opinion on the safety of caffeine,¹³⁹ based on publications from 1997 onward.

The report assessed single and repeated doses of caffeine consumed alone and in combination with other products such as energy drinks and alcohol. The opinion addressed possible adverse health effects of caffeine consumption from all dietary sources, including food supplements, in the general healthy population and in relevant specific subgroups of the general population (e.g., children, adolescents, adults, the elderly, pregnant and lactating women, subjects performing physical exercise). The scientific assessment was based on human interventional and observational studies with adequate control for confounding variables that have been conducted in healthy subjects at recruitment. Whenever available, human interventional studies and prospective cohort studies were preferred over case-control and cross-sectional studies because of the lower risk of reverse causality and recall bias. Case reports of adverse events were not considered for the scientific assessment. Systematic reviews and meta-analysis were used whenever available.

EFSA concluded that for adults, single doses of caffeine up to 200 mg (corresponding to about 3 mg/kg bw for a 70-kg adult) are unlikely to induce clinically relevant changes in blood pressure, myocardial blood flow, hydration status or body temperature, to reduce perceived exertion/effort during exercise or to mask the subjective perception of alcohol intoxication. Single doses of 100 mg (about 1.4 mg/kg bw for a 70 kg adult) may increase sleep latency and reduce sleep duration in some adult individuals, particularly if consumed close to bedtime.

EFSA stated that daily caffeine intakes from all sources up to 400 mg per day (about 5.7 mg/kg bw) do not raise safety concerns for adults in the general population, including lactating women (although they excluded pregnant women). The EFSA Panel also stated that no health concerns in relation to acute toxicity, bone status, cardiovascular health, cancer risk or male fertility have been raised by other bodies in previous assessments for this level of habitual caffeine consumption, and no new data have become available on these or other clinical outcomes that could justify modifying these conclusions. Interestingly, they reported that in seven out of 13 countries examined, the 95th percentile of daily caffeine intake exceeded 400 mg. The proportion of all populations exceeding this level ranged from 5.2% to 32.9%.



In human pregnancy, EFSA found no studies on the health effects of single doses of caffeine. A daily intake of up to 200 mg was determined to not raise safety concerns for the fetus. This conclusion was based on prospective cohort studies showing a dose-dependent positive association between caffeine intakes during pregnancy and the risk of adverse birth weight-related outcomes (i.e., fetal growth retardation, small for gestational age) in the offspring. In those studies, the contribution of energy drinks to total caffeine intake was low (about 2%). With regard to lactating women, single doses of caffeine up to 200 mg and habitual caffeine consumption at doses of 200 mg per day consumed by lactating women in the general population were not found to give rise to safety concerns for the breastfed infant. At these doses of caffeine, daily caffeine intakes by the breastfed infant would not exceed 0.3 mg/kg bw, which is 10-fold below the lowest dose of 3 mg/kg bw tested in a dose-finding study and at which no adverse effects were observed in the majority of infants. There were no data found to characterize the risk of single doses of caffeine consumed by lactating women, and data on habitual caffeine consumption in this population subgroup was found to be scarce.

With regard to children and adolescents, EFSA found the information available was insufficient to base a safe level of caffeine intake, but the no concern level of 3 mg/kg bw/day derived for adults was considered to potentially serve as a basis to also derive no concern levels for children and adolescents. This is because caffeine clearance in children and adolescents is at least that of adults and because the limited studies available on the acute effects of caffeine on anxiety and behavior in children and adolescents support this level of no concern. Like for adults, caffeine doses of about 1.4 mg/kg bw may increase sleep latency and reduce sleep duration in some children and adolescents, particularly when consumed close to bedtime. They found that the estimated 95th percentile of caffeine intake from foods and beverages on a single day exceeded 3 mg/kg bw/day in adolescents (10–18 years) in 6 out of 16 countries examined. This level was also exceeded in children (3–10 years) in 9 out of 16 countries examined and in toddlers (12–36 months) in 3 out of 10 countries examined. The proportion of survey days in which the level was exceeded ranged from about 7–12% in adolescents, from 6–15% in children and from 7–37% in toddlers. Chocolate beverages were important contributors to total caffeine intakes in children and toddlers in most countries, and the use of a conservative caffeine value for this food category may have led to an overestimation of caffeine intakes in these age groups.

EFSA also concluded that other common constituents of energy drinks (taurine and D-glucurono- γ -15 lactone) or alcohol are unlikely to adversely interact with caffeine.

6.2.1.4 United States Dietary Guidelines Advisory Committee (2015)



The Dietary Guidelines for Americans (DGAC) is published every five years jointly by the Department of Health and Human Services and the USDA and provides a framework for US-based food and nutrition programs, health promotion and disease prevention initiatives, and research priorities.¹⁷⁶ Since 1985, DGAC, composed of nationally recognized experts in the field of nutrition and health, has been appointed to provide independent, science-based advice and recommendations for development of the guidelines.^{85, 176}

DGAC addressed the safety of coffee/caffeine for the first time in their 2015 report. They concluded that intake up to the equivalent of 3–5 cups of caffeinated coffee per day (or up to 400 mg/day) in adults was found not to be associated with increased long-term health risks, such as cardiovascular disease, cancer or premature death (DGAC evidence grade = strong) and, in moderate amounts, is actually associated with reduced risk of cardiovascular disease, type 2 diabetes, and Parkinson's disease in healthy adults (DGAC evidence grade = moderate).^{85, 176}

In addition, they found that consistent observational evidence indicates that regular consumption of coffee is associated with reduced risk of cancer of the liver and endometrium, and slightly inverse or null associations are observed for other cancer sites. The report also warns that coffee, as it is normally consumed, frequently contains added calories from cream, milk, and added sugars. Care should be taken to minimize these caloric additions. Limited evidence indicated that caffeine consumption is associated with a modestly lower risk of cognitive decline or impairment and lower risk of Alzheimer's disease. There was moderate confidence that moderate caffeine intake in pregnant women is not associated with risk of preterm delivery. Higher caffeine intake was associated with a small increased risk of miscarriage, stillbirth, low birth weight, and small for gestational age births. However, the report states that such data should be interpreted cautiously due to potential recall bias in the case-control studies and confounding by smoking and pregnancy signal symptoms. The DGAC recognized that there is limited data to identify a level of caffeine intake beyond which risk increases. Based on the existing data, the risk of miscarriage, stillbirth, low birth weight, and small for gestational age births was considered minimal given the average caffeine intake of pregnant women in the U.S. Lastly, DGAC stated that only limited evidence is available to ascertain the safety of high caffeine intake that might occur from large-sized energy drinks, and that concern is heightened when caffeine is combined with alcoholic beverages.^{85, 176}

6.2.1.5 International Agency for Research on Cancer/Loomis et al. (1991/2016)

WHO-IARC evaluates substances and then places them in one of four cancer-risk categories based on the combined weight of exposure data, biological data relevant to the evaluation of carcinogenicity to humans, evidence for carcinogenicity in



experimental animals, other relevant data in experimental systems and humans, and evidence for carcinogenicity in humans. Group 1 is for substances determined to be carcinogens in humans (meaning evidence of carcinogenicity is sufficient). Group 4 is for substances that are “probably not carcinogenic to humans” (meaning, at a minimum, there is “*evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of experimental data” but more commonly meaning there is “*evidence suggesting a lack of carcinogenicity*” in both humans and experimental animals). It should be noted that WHO-IARC has placed only one chemical into Group 4 out of over 1000 that have been evaluated.¹⁸²

1991 Conclusion

In 1991, WHO-IARC reviewed evidence related to both coffee and caffeine. It was concluded, due to limited evidence that “coffee is *possibly carcinogenic to the human urinary bladder (Group 2B)*”.¹⁷⁷ Note that coffee’s association with bladder cancer was later dismissed in the 2016 evaluation discussed below. The 1991 publication concluded that there is evidence suggesting a lack of carcinogenicity of coffee drinking associated with breast and colon cancer, and inadequate evidence for other cancers.

In the same 1991 WHO-IARC publication, it was concluded that “caffeine is *not classifiable as to its carcinogenicity to humans (Group 3)*”. There was “inadequate evidence for the carcinogenicity in humans or experimental animals of caffeine.” WHO-IARC found no evidence of carcinogenicity of caffeine in two rat studies deemed adequate for evaluation (no significant differences in incidence of tumors were found at any site), and, in general, human data showed no association between caffeine consumption and mortality from cancers at all sites (with the exception of a potential weak association with bladder cancer and caffeinated beverage consumption).

Additionally, administration of caffeine in combination with known carcinogens was found by WHO-IARC to result in decreased incidences of lung tumors in mice treated with urethane, of mammary tumors in rats treated with diethylstilbestrol and of skin tumors in mice treated with UV light or cigarette smoke condensate. Caffeine did not influence the incidence of bladder tumors induced in rats by *N*-nitroso-*N*-butyl(4-hydroxybutyl)amine or in pancreatic tumors induced in rats by 4-hydroxyaminoquinoline-1-oxide. In humans no association has been made in studies between caffeine and mortality from cancer at all sites. Four case control studies of breast cancer showed no association with methylxanthine intake. A slight increased risk was seen in premenopausal women in one study, but in general the relative risks suggested a protective effect.



2016 Conclusion

In May, 2016, a WHO-IARC Working Group of 23 scientists from ten countries re-evaluated the carcinogenicity of drinking coffee (as well as of maté and very hot beverages).^{178, 179} Note that caffeine was not evaluated in this working group. More than 1000 observational and experimental studies were available for the review. The greatest weight for the evaluation was given to well-conducted prospective cohort and population-based case-control studies that controlled adequately for important potential confounders, including tobacco and alcohol consumption.

The authors concluded that for bladder cancer, there was no consistent evidence of an association or an exposure-response gradient with drinking coffee based on ten cohort studies and several population-based case-control studies. The Group concluded that positive associations reported in some studies, and the reason for “limited evidence” reported for coffee in 1991 evaluation, could have been due to inadequate control for tobacco smoking, which can be strongly associated with heavy coffee drinking.

The Group found mainly inverse associations with regard to endometrial cancer and coffee drinking (based on the five largest cohort studies, several case-control studies and a meta-analysis). An inverse association was also found with regard to liver cancer and coffee drinking in cohort and case-control studies. They found that more than 40 cohort and case-control studies and a meta-analysis including nearly 1 million women consistently indicated either no association or a modest inverse association for breast cancer and coffee drinking. Similarly, cohort and case-control studies consistently showed no indication of pancreatic and prostate cancers associated with coffee drinking.

Data on more than 20 other cancers was available but judged by the authors to be inadequate for reasons including inconsistency of findings across studies, inadequate control for potential confounding, potential for measurement error, selection bias or recall bias, or insufficient numbers of studies. Moderate evidence of an association of coffee drinking with reduced risk of colorectal adenomas was noted by the Working Group and coffee drinking was also found to be associated with beneficial effects on liver fibrosis and cirrhosis.

The authors reviewed several long-term carcinogenicity studies (in rats and mice) and studies on tumor-promoting and cancer-preventing activity (in rats and hamsters). These studies were determined to have provided inadequate evidence in experimental animals for the carcinogenicity of coffee. Consumption of coffee was found to exhibit strong antioxidant effects in human studies, while genotoxicity results in humans were inconsistent. Coffee did not induce chromosomal damage *in vivo* in rodents. Coffee did show positive results in bacterial mutagenesis assays, but only without metabolic activation, and coffee promoted apoptosis in human cancer cell lines.



The overall conclusion of the 2016 evaluation was that coffee drinking was “unclassifiable as to its carcinogenicity to humans”. It was given a Group 3 designation.

6.2.1.6 International Life Science Institute, North America /Wikoff et al. (2017)

In 2017, the North American branch of the International Life Science Institute (ILSI/NA) published an updated review to the Nawrot et al., 2003⁷⁴ caffeine safety review to determine if the conclusions reached by Nawrot/Health Canada were still supported by the literature published since that time.⁸⁶ ILSI assembled an internationally recognized group of caffeine experts working with an independent consulting company for this endeavor. The publication is the first systematic review of the adverse effects of caffeine and investigated specific endpoints within five health outcome areas (acute toxicity, cardiovascular toxicity, bone and calcium effects, behavior, and development and reproduction) in four healthy populations (adults, pregnant women, adolescents (12–19 years) and children (3–12 years)). It spanned the primary literature from 2001 to 2015. The study was set up to use the dose levels that were considered to be safe by Health Canada in 2003 as comparators to data from more recent studies. In other words, the authors did not set out to identify a new safe value for caffeine but instead to ascertain whether or not the heavily cited values used in Nawrot, 2003 remain acceptable in light of new data. The “comparator” safe levels were 400 mg/day for adults (10 g for lethality), 300 mg/day for pregnant women, and 2.5 mg/kg/day for children and adolescents.

A total of 381 studies were found by the authors to have met the inclusion criteria for the entire systematic review, and 46 additional studies were reviewed that discussed the pharmacokinetics of caffeine contextually, aiming to capture all recent relevant papers for caffeine with specific focus on individual variation in metabolism and other pharmacogenomic variability. The majority of the literature reviewed involved adult populations (79%) whereas 14% involved pregnant women, 4% involved adolescents, and only 2% involved children.

Bone and Calcium Effects

The authors included 14 studies related to caffeine effects on bone and calcium. All of the studies involved adults (one study additionally evaluated adolescents). Most of the studies were observational, and caffeine exposures were typically self-reported. Endpoints characterizing the bone and calcium outcomes included metabolic impact on calcium homeostasis, bone mineral density and osteoporosis, and risk of fracture. The authors concluded with a moderate level of confidence that 400 mg caffeine/day was an acceptable intake that is not associated with adverse effects on bone or calcium endpoints, particularly under conditions of adequate calcium intake. The short-term nature of many of the studies made it difficult to determine long-term effects on calcium homeostasis. The key limitations in the



studies that precluded a higher level of confidence were the inability to fully accommodate for calcium intake, the high level of indirectness, as well as an uncertainty in exposure estimates.

Cardiovascular Effects

The authors found 202 studies related to cardiovascular disease that met their inclusion criteria. Of these, 11 studies involved children and/or adolescents, while the rest involved adults. The majority were randomized, double-blinded, crossover-controlled trials. Relevant measurements in the studies included blood pressure, heart rate, cardiovascular morbidity and/or mortality, arrhythmia, cholesterol, aortic stiffness/wave reflection, cerebral blood flow, plasma or urinary constituents (e.g., catecholamines, homocysteine), endothelial function, heart rate variability, heart rhythm, other hemodynamic measurements and ventricular function.

The authors concluded (with a moderate level of confidence) that 400 mg caffeine per day was an acceptable intake that is not associated with significant concern regarding adverse cardiovascular effects in healthy adults. For clinical endpoints, some findings suggested that intake higher than 400 mg/day may be safe; however, other data, particularly those for physiological endpoints, reported effects that occurred at doses lower than 400 mg/day. For such physiological endpoints (e.g., blood pressure), confidence in determining conclusions relative to the comparator was limited by the inability to ascertain the conditions and magnitude of change that would be considered adverse in a clinical or toxicological context. For these endpoints, the magnitudes of changes were relatively small and transient in nature. They may only be relevant in specific genetic subpopulations and may be subject to tolerance in habitual caffeine consumers. Also, because of the fact that the studies related to these parameters were generally short-term, the data does not provide evidence to characterize potential long-term effects. As the data for children and adolescents was limited to that from 11 studies, the evidence base was considered insufficient to render an absolute conclusion regarding the 2.5 mg/kg bw/day safety level. The available data for blood pressure and heart rate were inconsistent in these younger age groups; several studies reported physiological changes below the comparator (which may or may not be adverse) while other studies reported a lack of effect on these parameters following consumption of much higher levels (5 mg/kg/day or higher). When changes were observed, they were generally small in magnitude, and the lack of information demonstrating an association between chronic caffeine-mediated blood pressure increases relative to known cardiovascular risk factors shifted the evidence to support the comparator of 2.5 mg/kg bw/day.

Behavioral Effects

The authors included 81 studies in the review related to behavioral effects. The majority (approximately 77%) of the included papers were controlled trials using healthy adult populations, and only five of the included studies specifically



investigated children or adolescents. The endpoints in the studies included mood, sleep, withdrawal, headache and risk-taking behavior, as well as others that were considered to be less adverse such as hunger and bruxism.

Overall, the authors concluded that the more recent body of evidence generally supported the Health Canada comparator levels. While data showed that lower doses of caffeine may negatively affect some aspects of behavior (especially anxiety) and sleep, the changes were often low in magnitude and were more apparent in sensitive subpopulations (e.g., those with certain genotypes such as ADORA2A polymorphisms and/or those more prone to anxiety or sleep disruption, which highlights the inter-individual variability in sensitivity to caffeine's effects). Caffeine's ability to disrupt objective measures of sleep when administered later in the evening (i.e., close to bedtime) was not considered likely to reflect common consumer behavior due to self-regulating of caffeine intake (during certain times of day or altogether) to avoid negative effects on sleep. Additionally, effects of caffeine on sleep highlighted the difficulty of characterizing adversity versus desirable and/or anticipated effects (as caffeine is often ingested to avoid sleepiness). Otherwise, there was little to no evidence identified to suggest that <400 mg caffeine/day has any negative effects on mood states and in fact may provide some benefit in some cases (e.g., in fatigue and depression-related endpoints). The authors reported some inconsistency in data related to effects on headache, as they may have been linked to symptoms of caffeine withdrawal and consumer status. The evidence that caffeine is associated with increased risk-taking behavior in adults was considered sparse. The overall literature related to children and adolescents was scant, and even though the data was considered insufficient to render a final conclusion, the authors found no suggestion of adverse effects at doses near or less than 2.5 mg/kg bw/day. There was a moderate to high level of confidence in the body of evidence supporting the conclusions related to behavioral effects.

Reproduction and Developmental Effects

A total of 58 reproduction and developmental studies were considered by the authors to have met their inclusion criteria. The majority of studies involved caffeine exposure in pregnant women, for which the Health Canada/Nawrot comparator of <300 mg/day was applied. For the few studies evaluating non-pregnant women (e.g., studies evaluating fecundity or age at menopause) or men (e.g., sperm quality), the comparator for healthy adults of < 400 mg/day was applied. The majority of studies were observational (mainly cohort and case-control studies). Controlling for symptoms of the "pregnancy signal" such as nausea, aversion to smells or tastes and vomiting was considered critical, as they can influence caffeine intake. The authors explained that without specific analyses of caffeine aversion, it is difficult to ascertain whether an increased incidence of spontaneous abortion in a study is due to higher caffeine consumption or if reduced caffeine consumption is being



observed in healthier pregnancies due to the pregnancy signal (i.e., reverse causation).

Endpoints used by the authors for reproduction and development included fecundability and infertility, spontaneous abortion, recurrent miscarriage, stillbirth (including late spontaneous abortion), preterm birth, fetal growth (including small for gestational age/intrauterine growth restriction), birth defects, childhood behavior, childhood cancer, markers of maternal stress, pregnancy-induced hypertension and/or preeclampsia, and age at menopause. The authors concluded with moderate confidence that the body of evidence is generally consistent with the safe levels reported by Nawrot (<300 mg/day in pregnancy). Although some effects noted below this level could not be completely ruled out, such effects were primarily limited to isolated congenital malformations or childhood cancers and were of low magnitude. Effects on birth weight were also reported at intake levels below the comparator; however, when this endpoint was robustly studied in some papers, caffeine did not show effects below the comparator level.

Acute Toxicity

With regard to acute toxicity, 26 papers were considered by the authors to have met the inclusion criteria. All of the studies were case reports or case series, most of which were associated with emergency department visits or suicide-related events. Because the endpoints of interest in this outcome were considered rare (e.g., death or severe intoxication), the inclusion of case reports and case series were necessary to obtain any data.

The authors found that adverse events were generally associated with intake of very high doses of caffeine (up to 50 g) delivered over a relatively short time frame; approximately half of the studies involved caffeine in powder or tablet form and the remaining involved energy drinks or cola sources of caffeine. Confidence in the characterizations of exposures was low since they were almost always self-reported or reported by friends/family. Acute effects associated with caffeine consumption were described as having resulted in a wide spectrum of symptoms, the milder of which include headache, nausea, vomiting, fever, tremors, hyperventilation, dizziness, anxiety, tinnitus, and agitation. More severe effects have included abdominal pain, altered consciousness, rigidity, seizures, hypokalemia, rhabdomyolysis, increased blood lactate, supraventricular and ventricular arrhythmias, and myocardial ischemia. Such symptoms were considered expected at very high doses due to caffeine's ability to stimulate the CNS, decrease smooth muscle tone, increase peripheral vascular resistance, and increase cerebrovascular resistance. The authors concluded that the body of evidence related to acute toxicity was generally consistent with Nawrot's conclusion of potential death following acute exposures of 10 g of caffeine or higher although, due to the nature of the studies, the confidence in the evidence base was considered low to very low. For example, seven fatal case reports documented death following ingestion of



approximately 10 g of caffeine or higher, yet other reports documented survival after ingestion of levels significantly higher than 10 g, suggesting again that there is inter-individual variability in sensitivity to caffeine.

Conclusion

Overall, the ILSI, NA /Wikoff et al. (2017) systematic review concluded that the totality of evidence generally supports that consumption of up to 400 mg caffeine/day in healthy adults is not associated with overt, adverse cardiovascular effects, behavioral effects, acute effects or effects on bone status. They found the evidence also supports that consumption of up to 300 mg caffeine/day in healthy pregnant women is generally not associated with adverse reproductive and developmental effects. While limited data was identified for children and adolescent populations, the available evidence suggests that 2.5 mg caffeine/kg bw/day remains an appropriate recommendation overall.

6.2.2 Other Helpful Comprehensive Reviews on Caffeine/Coffee

As described above, many comprehensive reviews and opinions have been made by various “authoritative” governmental agencies and scientific institutions with regard to the safety of caffeine consumption. In addition to those investigations and opinions, a number of other comprehensive reviews on coffee/caffeine have been published in the literature that deserve mention, although they are considered more corroborative as they were not necessarily published as specific opinions of their organization or were more focused on coffee than caffeine specifically. Such reviews are described in more detail below.

6.2.2.1 Linus Pauling Institute (LPI)/Higdon and Frei (2006)

Scientists at the Linus Pauling Institute (LPI) published a review on coffee consumption and human health in 2006 and found that there is no evidence to indicate consumption of 3–4 cups of coffee per day—equivalent to about 300–400 mg of caffeine per day—is associated with health risks.⁹⁰ They stated that some groups, including people with hypertension and the elderly, may be more vulnerable to the adverse effects of caffeine and that it would be prudent for women who are pregnant, lactating, or planning to become pregnant to limit coffee consumption to 3 cups per day providing no more than 300 mg per day of caffeine. Limited data from short-term clinical trials suggested that caffeine intakes of 3 mg/kg bw/day or more may have adverse effects in children and adolescents. They stated that these findings are the basis for Health Canada’s recommendation that children should not consume more than 2.5 mg/kg bw/day of caffeine. Lastly, they concluded that more research is needed to determine whether long-term caffeine consumption has adverse effects on the health of children and adolescents.



In more detail, the review found that most prospective cohort studies have not found that coffee consumption is associated with significantly increased risk of heart disease or stroke. However, randomized controlled trials lasting up to 12 weeks have found that coffee consumption is associated with increases in several cardiovascular disease risk factors, including increased blood pressure and plasma homocysteine. They found little evidence that coffee consumption increases the risk of cancer. Although most studies did not find coffee or caffeine consumption to be inversely associated with bone mineral density in women who consume adequate calcium, positive associations between caffeine consumption and hip fracture risk in three prospective cohort studies suggest that limiting coffee consumption to 3 cups per day (300 mg of caffeine per day) may help prevent osteoporotic fractures in older adults. Although epidemiological data on the effects of caffeine during pregnancy are conflicting, the authors raised concern regarding the potential for high intakes of coffee or caffeine to increase the risk of spontaneous abortion and impair fetal growth (note that more recent studies and reviews^{86, 139} have concluded that caffeine consumption levels of <200–300 mg/day in pregnancy are safe with regard to endpoints for reproduction and development). Serious adverse effects from caffeine at the levels consumed from coffee are uncommon, but there is a potential for adverse interactions with a number of medications. Regular consumers of coffee and other caffeinated beverages may experience withdrawal symptoms, particularly if caffeine cessation is abrupt.

6.2.2.2 Facultad de Medicina, Valencia, Spain/Cano-Marquina et al. (2013)

Cano-Marquina et al. reviewed articles published between January 1990 and December 2012 with regard to coffee/caffeine and relevant health areas potentially affected by coffee intake.¹⁸³ The search yielded 10,625 references, which was reduced to 296 papers based on inclusion/exclusion criteria. The authors gave priority to meta-analyses and systematic reviews when available. They found that tolerance to caffeine often acts as a modulator of the biological actions of coffee and that the various forms of arterial cardiovascular disease, arrhythmia and heart insufficiency were unaffected by coffee intake. Coffee was found to be associated with a reduction in the incidence of diabetes and liver disease, and data on cancer seemed mainly inversely associated with coffee intake. Coffee consumption was found to potentially protect from Parkinson's disease while associations with osteoporosis risk factor were still considered under debate. Its effect on cancer risk was found to be dependent on the tissue concerned, although it appeared to favor overall risk reduction. Overall the authors concluded that coffee consumption appears to reduce mortality.



6.2.2.3 Northern Ireland Centre for Food and Health/Pourshahidi et al. (2016)

Pourshahidi et al. provided a comprehensive overview of the risks and benefits of coffee consumption on various health outcomes.¹⁸⁴ The authors performed a systematic search of the literature (from 1970 to June 30th 2015; in humans; in English) that returned 12,405 results. A total of 1,277 (many of which were observational) were determined to be eligible based on inclusion/exclusion criteria. Studies were grouped and discussed with regard to major diseases/conditions, at risk/vulnerable groups, and specific coffee bioactive constituents.

Cancer Effects

The reviewers found a total of 352 relevant studies related to cancer. The majority reported a beneficial or null effect of coffee consumption on cancer, with the exception of bladder/urinary tract cancers where the risks of coffee consumption were more commonly reported. An increased risk of bladder/urinary cancer was found to be typically associated with modifiers of risk (gender, age, smoking or alcohol status, genetic polymorphisms, type of coffee consumed (e.g., Turkish coffee), or degree of coffee consumption (e.g., 40+ cups per week)). The authors also found that some studies failed to demonstrate a dose-response, which suggests that such associations are non-causal. Similar risk modifiers were found in the observational evidence for other types of cancer as well (e.g., gastric, colorectal, pancreatic, breast, ovarian, and skin cancer). More consistently, the authors found a positive or beneficial association between coffee consumption and cancer risk, more often from intervention studies. They also found a protective or beneficial effect of coffee consumption on antioxidant status, oxidative DNA damage, urine mutagenicity, and DNA strand breaks/integrity. Overall, the authors found that data from intervention studies suggest that coffee can have a beneficial role with regard to reducing the risk of some cancers.

Cardiovascular Effects

The authors found a total of 273 relevant studies related to cardiovascular disease. They concluded that the majority of evidence reported adverse or null relationships between coffee consumption and hypercholesterolemia; however, this was mainly caused by the consumption of cafetière, French-press, Arabic, or boiled coffee, as compared to filtered coffee preparations. This negative effect of coffee on cholesterol was considered by the authors to be due to higher concentrations of diterpenes (especially in boiled coffee—note that diterpenes are not expected to be present in RUNA® Concentrate which is an aqueous extract, as diterpenes are lipid-soluble)¹⁸⁵ although, interestingly, diterpenes have also shown a lipoprotein(a)-reducing potential. The authors noted an inverse relationship between coffee consumption and triglyceride concentrations.

The literature on coffee and blood pressure/hypertension was reviewed by the authors. They stated that the pressor effect that has been noted in coffee consumers



may be caused by a coffee-induced increase in adrenaline concentrations. They found that a related effect was observed more often in coffee naïve individuals, with no blood pressure effect seen in habitual drinkers. While abstinence from coffee may decrease blood pressure in normotensive individuals, they found that some studies showed no effect on ambulatory blood pressure measurements or on the prospective risk of developing hypertension over time. On the other hand, they found coffee consumption may have benefits related to blood pressure (per human intervention studies conducted in both normotensive and mildly hypertensive adults) and effects may be more specifically related to an individual's genotype.

For some cardiovascular outcomes such as myocardial infarction, the authors found that increased risk in coffee drinkers is dependent on family history, CYP1A2 genotype and type of coffee preparation (boiled vs. filtered), highlighting the importance of adequately controlling for these and other confounders in such studies. They stated that although coffee polyphenols have been reported to have a beneficial effect on endothelial function, the opposite or at least a null effect is seen when coffee is consumed. For other outcomes, they stated that U- or J-shaped risks of coffee consumption have been reported, although differences in the definition of "moderate consumption" made it difficult to compare and draw adequate conclusions between the studies.

Metabolic Effects

With regard to metabolic health, the authors stated that coffee consumption consistently shows a beneficial (inverse) association with the risk of type 2 diabetes (per 126 studies). They stated that the associations are at least in part mediated by an improvement in insulin sensitivity and/or improved glucose tolerance. They found direct effects on glucose tolerance appeared to be caused by the antagonistic effect of CAs on glucose transport, shifting glucose absorption to more distal parts of the intestine. Other mechanisms of action were considered by the authors to include associations with low-grade systematic inflammation, oxidative stress, and sex-hormone binding globulin. They stated that important confounders might include the range of body mass index categories included within the study, as well as the use of hormone replacement therapy.

The authors found that coffee intake can also decrease energy intake (via effects on satiety hormones) and thus decrease body fat levels. Moreover, they stated that either the mannooligosaccharides or CAs in coffee may increase or stimulate postprandial fat utilization, thus, promoting excretion of fat in the feces. They found that although some studies have shown an adverse effect related to risk of metabolic syndrome, this was only relevant for higher coffee consumption (>3 cups/day), particularly of instant coffees with excess sugar and powdered creamer (i.e., the results must be interpreted with caution).



Neurological Effects

Coffee consumption was found by the authors to be positively linked to a decreased risk of a number of neurological disorders, with the most commonly reported being Parkinson's disease, cognitive decline/function, and mental health. They found 94 studies that reported links between coffee consumption and neurological outcomes. The beneficial associations were found to be potentially increased in one gender versus the other, depending on the disorder, and may also relate to genotype variations.

Gastrointestinal Effects

A total of 73 studies were found by the authors to have reported links between coffee consumption and gastrointestinal conditions (e.g., reflux, ulcers, heartburn, and dyspepsia). Although related negative findings were apparent in the literature from coffee consumption, the associations were found to be weak at best and either were only reported in univariate (not multivariate) analyses, were reported for (unusually) high coffee consumption, were perceived side effects by the consumer or patient rather than being tested/diagnosed, or were only reported in coffee-sensitive/susceptible individuals. They also found suggestions that variability in coffee-induced gastric responses may be caused by differences in bean processing (e.g., degree of roasting). The authors also found some beneficial effects of moderate coffee consumption on gut health (e.g., improved fecal microbiota and improved colonic fermentation) as reported by four different intervention studies.

Liver Effects

The authors found 72 studies that investigated the effect of coffee consumption on liver disorders, which showed a generally protective effect on the liver (with regard to liver enzyme levels, gall bladder disorders and alcohol-induced liver damage/inflammation/impairment). Confounders were considered to potentially include gender and smoking. Strong cafetière (vs. filtered) coffee, however, was found to possibly show the opposite effect. They found debate in the literature as to whether the compounds responsible for such effects are the diterpenes (e.g., kahweol within coffee oil; note, as previously explained, lipid-soluble diterpenes are not expected to be found in RUNA® Concentrate aqueous extract).¹⁸⁵

Mortality

The authors determined that coffee consumption is associated with a reduced risk of total/all-cause and cause-specific mortality, particularly for cardiovascular and coronary heart disease. They discussed that seemingly contrasting conclusions of some earlier studies (conducted 20+ years ago) found coronary or ischemic heart disease mortality risks were either related to sale of coffee rather than consumption, no/very low (0 to 1 cups/day) consumption, very high (6 to 9+ cups/day) consumption, or the associated risks were minimal. Similar to what was found for other conditions, the link between coffee consumption and mortality seemed to vary inconsistently by gender or hormone replacement and/or smoking status. Overall



consumption was found to be beneficial in the majority of evidence when populations are considered as a whole.

Other Effects

Although approximately half of the relevant studies reviewed by the authors showed a null effect on bone outcomes, a similar proportion also reported adverse effects (although only in lean versus overweight/obese individuals and in females, not males, and with high daily coffee consumption). The authors found evidence that the adverse effects on bone mineral density can be offset by the milk often consumed with coffee, are only evident in those with certain genotypes, and/or may not translate into an increase in fracture risk in the longer-term.

With regard to risks to pregnant women and relative to pregnancy complications, birth outcomes, or the health of infants, although risks were noted in 26 out of 50 studies, many were found to be linked with higher coffee consumption, and approximately the same number of studies (22 out of 50) also reported no related adverse effects. The authors found some studies that reported beneficial effects on certain pregnancy/infant health outcomes, such as the risk of pre-term delivery or childhood acute leukemia.

The authors found that beneficial effects of other “bioactive” components of coffee, such as CAs, phenolic acids, and melanoids added further support to the beneficial effect of this beverage. Overall, they concluded that the health benefits (or null effects) clearly outweigh the risks of moderate coffee consumption in adult consumers for the majority of the health outcomes considered.

6.2.2.4 Cambridge University, Harvard University, University of Cantania/Grosso et al. (2017)

Grosso and colleagues reviewed associations between coffee and caffeine and various health outcomes by performing an umbrella review of meta-analyses of observational studies and randomized controlled trials.¹⁸⁶ Coffee was found to be associated with a probable decreased risk of breast, colorectal, colon, endometrial, and prostate cancers; cardiovascular disease and mortality; Parkinson’s disease; and type-2 diabetes. Coffee was also associated with a rise in serum lipids but this result was affected by significant heterogeneity and was again associated with unfiltered coffee containing significant quantities of diterpenes (as previously explained, diterpenes are not expected to be found in RUNA® Concentrate). The authors stated that diterpenes may affect the LDL receptor, which is responsible for the endocytic processes of Apo B- and Apo E-containing lipoproteins and, consequently, may lead to extracellular accumulation of cholesterol. They found no evidence that long-term coffee consumption is associated with an increased risk of dyslipidemia or other outcomes related to a rise in serum lipids and concluded that coffee can be part of a healthful diet.



Caffeine was found by the authors to be associated with a probable decreased risk of Parkinson's disease and type-2 diabetes, as well as an increased risk of pregnancy loss, although the authors stated that the studies included in the meta-analyses did not stratify by smoking status, which is itself a known risk factor for pregnancy loss outcomes. The authors additionally stated that early caffeine therapy in newborns (administered intravenously) has been demonstrated to significantly decrease the risk of bronchopulmonary dysplasia (note also that while this review did not state a safe level of caffeine use in pregnancy, other reviews have determined that 200–300 mg/day is reasonable^{86, 139}). Acute caffeine doses were also associated with a rise in blood pressure although the authors found weaker effects demonstrated in long-term, habitual coffee drinkers, which may suggest tolerance and, thus, a lack of significant effects at the level of blood vessels.

6.2.3 Additional Recent Studies, Reviews and Information on Caffeine/Coffee

In addition to the more comprehensive reviews described above, additional scientific details about caffeine are described below, with a focus on pharmacokinetics publications, and studies that have been published since the most recent reviews discussed above in order to ensure that the most current scientific information and knowledge is covered in this GRAS report.

6.2.3.1 Pharmacokinetics

Caffeine extracted from plants (i.e., natural caffeine) can be distinguished from caffeine manufactured synthetically via carbon dating techniques,¹⁸⁷ but the two are otherwise identical molecules with the same chemical structure and are not expected to behave differently in the body.^{188, 189}

The pharmacokinetics of caffeine in healthy adults is well established. Once ingested, caffeine is rapidly absorbed, metabolized, and eliminated, and the short biological half-life of caffeine suggests negligible biological accumulation. The majority (99%) of ingested caffeine undergoes rapid gastrointestinal tract absorption in humans (within 45 minutes after oral consumption) and is rapidly distributed within the body; peak plasma time ranges from 15–120 minutes.^{74, 167} Due to being amphiphilic in nature, caffeine easily travels across biological membranes and the blood-brain barrier; after absorption it is rapidly and uniformly distributed throughout the body.^{74, 177}

The majority of ingested caffeine is metabolized in the liver (mainly by the CYP1A2 enzyme) into several metabolites including, via 3-demethylation, paraxanthine (major metabolite) and, via oxidation at various positions, 1,3,7-trimethyluric acid, theobromine and theophylline.^{74, 90, 190-193} Paraxanthine may be further metabolized to methylxanthines and methyluric acids.^{74, 192} For example, it may be hydroxylated



by CYP2A6 to form 1,7-dimethyluric acid or acetylated by *N*-acetyltransferase to form 5-acetylamino-6-formylamino-3-methyluracil, an unstable compound that may be deacetylated nonenzymatically to form 5-acetylamino-6-amino-3-methyluracil.⁹⁰ Only small amounts of caffeine are excreted in the urine unchanged.⁷⁴ The exception is in infants up to approximately 9 months old who have a greatly reduced ability to metabolize caffeine, excreting approximately 85% unchanged in the urine.⁷⁴

Orally ingested caffeine has an elimination half-life in humans ($t_{1/2}$) of 3–7 hours, which can be influenced by factors such as sex, age, oral contraceptives, pregnancy and smoking.⁷⁴ The most common agent that enhances caffeine metabolism is cigarette smoking via increasing the activity of CYP1A2, and caffeine also inhibits the metabolism and/or disposition of substances including several antibiotics and sedatives.⁷⁴ Pregnancy frequently alters the pharmacokinetics of compounds; for example, caffeine metabolism by CYP1A2 is known to decrease while renal clearance increases during the course of pregnancy, which is especially apparent during the third trimester.¹⁹⁴ The authors stated that only a small fraction of a caffeine dose is excreted unchanged into urine; the bulk is eliminated via *N*-demethylation in the liver. The effect of pregnancy on caffeine metabolism is bidirectional: renal clearance is enhanced while CYP1A2 activity reduces over the course of pregnancy. The decrease in CYP1A2 metabolism outcompetes the increase in renal function leading to increased caffeine concentrations and resulting in increased caffeine exposure throughout pregnancy. Changes in albumin levels (and hence caffeine binding) during pregnancy may also play a role in the increased caffeine serum concentrations noted. These increased serum levels of caffeine and other coffee constituents may play a role in the aversion to coffee noted by many pregnant women.¹⁹⁴

More recent pharmacokinetic studies on caffeine have focused on how individuals' genetic makeup lead to inter-individual differences in how caffeine is metabolized and excreted. Polymorphisms in the ADORA2A gene, which encodes the adenosine A2A receptor, can affect sensitivity to caffeine, especially with regard to anxiety responses, and can also affect caffeine consumption patterns.^{86, 195} Similarly, genomic variations in CYP1A2 alleles are associated with different patterns of caffeine metabolism.^{86, 193, 196-199}

Rybak et al. measured urine levels of caffeine and 14 of its known metabolites in samples from the cross-sectional NHANES 2009–2010 study using LC-tandem mass spectrometry.¹⁹² They found that caffeine and its metabolites were detectable in the urine of most individuals that were studied, and in general, dietary intake recordings significantly correlated with concentration and excretion rates. Median concentrations were 0.560–58.6 mmol/L and median excretion rates were 0.423–46.0 nmol/min. Urine concentrations and excretion rates for nine of the analytes (caffeine, theophylline, paraxanthine, 1-methylxanthine, 1-methyluric acid, 1,3-



dimethyluric acid, 1,7-dimethyluric acid, 1,3,7-trimethyluric acid, and 5-acetylamino-6-amino-3-methyluracil) had moderate correlations with recorded caffeine intake, making them potentially good biomarkers for caffeine consumption levels, while the remaining analytes had lower correlations. Urine concentrations and excretion rates for most compounds were significantly higher in men than in women and were highest in persons aged 40–59 years, which was consistent with the stated dietary caffeine intakes.

6.2.3.2 Overall Mortality

A beneficial association between daily coffee consumption and total all-cause mortality has been shown in a number of recent studies, such as the large NIH-AARP Health Study of 50–71 year olds,²⁰⁰ the large multi-ethnic, prospective, population-based Northern Manhattan Study,²⁰¹ a large Japanese cohort study,²⁰² large cohorts of diabetic men and women,²⁰³ as well as others.^{204, 205} An assessment of the association between filtered caffeinated coffee consumption and all-cause mortality in women with cardiovascular disease from the Nurses' Health Study found no association.²⁰⁶ A 2014 meta-analysis of 20 prospective cohort studies determined that the relative risk of total mortality for high versus low categories of coffee consumption was 0.86 (95% CI 0.80, 0.92).²⁰⁷ The pooled relative risk was similar whether $\geq 2\text{--}4$ cups/day or $\geq 5\text{--}9$ cups/day was used as the high group cut off. The authors concluded that coffee consumption is associated with a reduced risk of total mortality. Crippa et al. (2014) came to a similar conclusion in their 2014 meta-analysis of 21 prospective studies; they found that coffee consumption was inversely associated with all-cause mortality.²⁰⁸ Poole et al. (2017) found that coffee consumption is more likely to benefit than harm based on their umbrella review of meta-analyses on multiple health outcomes, although robust randomized controlled trials are necessary to determine if observational associations are causal.²⁰⁹ Interestingly, Liu et al. (2016) found that higher coffee consumption is associated with longer leukocyte telomeres among female nurses from the Nurses' Health Study, which are a biomarker of aging and whose shortening can be accelerated by oxidative stress.²¹⁰

6.2.3.3 Cancer

As described above, WHO-IARC (2018)/Loomis et al. (2016) reviewed the most current scientific data as relates to coffee's effects with regard to cancer.^{178, 179} They found no consistent evidence of an association between coffee drinking and bladder cancer, and mainly inverse associations with regard to endometrial and liver cancers. They found no association or a modest inverse association for breast cancer and no indication of pancreatic and prostate cancers associations. Data on more than 20 other cancers was available but were judged by the authors to be inadequate to make a conclusion for various reasons. Below is some additional literature on caffeine/coffee as relates to cancer, either on cancer types for which no conclusion



was made by WHO-IARC, important or key papers/reviews, or data that is more recent than May of 2016.

General Reviews

Comprehensive studies and reviews published on caffeine/coffee consumption and cancer have come to similar conclusions as those of the WHO-IARC/Loomis et al. paper. Lenore Arab authored a review of the epidemiologic evidence on coffee and cancer in 2010.²¹¹ It summarized meta-analyses and recent papers on site-specific human cancers among coffee consumers. The review found a strong and consistent protective association related to hepatocellular and endometrial cancers. There was also a borderline protective effect found for colorectal cancer. No association was found with breast, pancreatic, kidney, ovarian, prostate or gastric cancers. Bladder cancer appeared to the author to be associated with heavy coffee drinking consumption in some populations and among men (note that the more recent WHO-IARC conclusion described above was that there was no consistent association with bladder cancer and coffee consumption). Arab found that associations with childhood leukemia and mother's consumption of coffee were ambiguous.

Bohn and colleagues came to similar conclusions in 2014.²¹² After a review of the literature, they stated that epidemiological and experimental data generally indicate either neutral or beneficial effects of coffee consumption. They found evidence that consistently indicates coffee protects against liver cancer and also points toward protective effects for risk of colorectal cancer. They found no association between the overall risk of breast and prostate cancer and coffee intake, and for certain subgroups such as postmenopausal breast cancers, advanced prostate cancers, and breast and prostate cancer survivors, an inverse association with coffee intake was suggested. The authors also discussed the potential chemo-preventive mechanisms of coffee phytochemicals, which include inhibition of oxidative stress and oxidative damage and regulation of DNA repair genes and genes involved in detoxification processes as well as the processes of inflammation, apoptosis, angiogenesis, and metastasis.

Floegel et al. (2012) found no association between coffee consumption and total cancer risk after analyzing data from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which included 42,659 participants.²¹³ In 2011, Yu et al. published a meta-analysis of 59 studies consisting of 40 independent cohorts suggesting that overall, coffee consumption may reduce the total cancer incidence and has an inverse association with bladder, breast, buccal, pharyngeal, colorectal, endometrial, esophageal, hepatocellular, leukemic, pancreatic, and prostate cancers.²¹⁴

Ovarian Cancer

Studies have been mixed with regard to caffeine's effects on ovarian cancer risk, with the majority showing no correlation when consumption is moderate. For



example, a number of case-control studies have found no association or no dose-dependent associations between regular coffee consumption and ovarian cancer risk.²¹⁵⁻²¹⁹ A Danish case-control study suggested a modest decreased risk of ovarian cancer was associated with coffee and caffeine consumption.²²⁰

A 2008 prospective cohort study of 29,060 postmenopausal women in the Iowa Women's Health Study found a slight increased risk of ovarian cancer (using a multivariate model) in women who drank the highest levels of caffeinated coffee per day (defined as five or more cups per day). However, no statistically significant association was found between caffeine intake itself and ovarian cancer risk nor was there an association with total coffee intake or decaffeinated coffee intake. The authors stated that a component of coffee other than caffeine (or in combination with caffeine) could be causing the effect in drinkers of very high levels of caffeinated coffee.²²¹

A large Canadian prospective study (the National Breast Screening Study) found a borderline positive association with ovarian cancer risk in women who drank >4 cups of coffee per day.²²² The large prospective Netherlands Cohort Study on Diet and Cancer found no significant association with coffee consumption and epithelial ovarian cancer in postmenopausal women.²²³ Analysis of the Nurses' Health Study data found a significant inverse trend of ovarian cancer risk with caffeine and caffeinated coffee intake; however, the individual relative risks were not statistically significant. Caffeine was also inversely associated with ovarian cancer in postmenopausal women (RR range for all quintiles 0.71–0.75) and positively associated in premenopausal women (RR range 1.42–2.87 for all quintiles); however, neither was statistically significant.²²⁴ As the data are very inconsistent, no real conclusions can be derived.

Bladder Cancer

As mentioned a number of times previously, early studies on the risk of bladder cancer with regard to caffeine intake were mixed, with more studies showing no likely carcinogenic association with caffeine when consumed at moderate doses. WHO-IARC/Loomis et al.'s comprehensive review of cancer data in 2016 (final monograph published 2018) found no consistent evidence of an association or dose-response between coffee drinking and bladder cancer.^{178, 179}

Coffee consumption has been highly correlated with smoking habits (smoking is a known risk factor for bladder cancer).^{178, 179, 225} In a prospective study in Japanese men and women, no significant association was found between caffeine consumption and overall bladder cancer risk.²²⁶ When the data was stratified, the authors did find a possible positive association between the highest caffeine-level consumers and bladder cancer in non- or formerly smoking men; however, in men who smoked, the association was opposite (i.e., caffeine was protective). A 2007 review of the literature found no strong association between bladder cancer and



coffee consumption and that lack of dose-responses does not support causality in studies.¹⁸⁰ Similarly, Yu et al. found that coffee consumption may have an inverse association with bladder cancer in their 2011 meta-analysis.²¹⁴

Breast Cancer

A number of studies have been published in recent years with regard to breast cancer and coffee and/or caffeine consumption, and overall there appears to be no correlation other than a possible protective effect, as was concluded by WHO-IARC (2018)/Loomis et al., 2016^{178, 179} and a number of other reviews of the literature.^{211, 212, 214} For example, a large prospective study of African-American women found no associations, including when subgroups were considered, such as based on menopausal status and breast cancer hormone receptor status.²²⁷ Another large prospective analysis found no associations between coffee, tea, or caffeine and breast cancer risk in women living in France.²²⁸ Results of analysis of the large Nurses' Health Study data also found no relation between coffee and/or caffeine and breast cancer other than a weak inverse association in post-menopausal women.²²⁹ Evaluation of the large NIH-AARP Diet and Health cohort study data showed no evidence of an association between caffeinated coffee and either hormone receptor positive or negative breast cancer occurrence.²⁰⁰ Similarly, caffeine consumption before breast cancer diagnosis was not found to influence breast cancer specific survival or overall survival in the large Swedish Mammography Cohort.²³⁰

Analysis of data from the prospective Women's Health Study revealed no overall association between caffeine consumption and breast cancer risk,²³¹ which did not differ according to body mass index, menopausal status, or hormone usage. In this study, women who had a history of benign breast disease had a borderline significantly increased risk of breast cancer if they drank greater than 486.3 mg/day of caffeine (or 4 or more cups of coffee) per day. A potential increased risk of tumors greater than 2 cm diameter and/or hormone receptor negative cancers in caffeine consumers, which generally have worse outcomes, were also noted. However, the authors' stated that these findings may have been due to chance, and they differ from findings in several other large studies that found no association between caffeine consumption and risk of breast cancer according to receptor status.

Coffee was found to be associated with a decreased risk of breast cancer in women with a BRCA1 mutation who also had certain CYP1A2 alleles.²³² A smaller study found an association between increased coffee consumption and increased mortality in women treated for breast cancer; however, the authors hypothesized that coffee consumption may be a surrogate marker for fatigue and abnormal pro-inflammatory cytokine activity (often found in fatigued breast cancer survivors), as women with these symptoms may turn to coffee to help with energy such as that due to cytokine induced fatigue.²³³ Data from the Ontario Women's Diet and Health Study also found no association between caffeine intake and breast cancer other than a potential protective effect of large amounts (>5 cups/day) in postmenopausal women and



estrogen receptor negative breast cancers.²³⁴ Jiang and colleagues summarized findings in a 2013 meta-analysis that reviewed 59,018 breast cancer cases and a total of almost 1 million participants. They found no significant association between breast cancer risk and coffee or caffeine consumption other than a slight protective effect that was dose-dependent in postmenopausal women and in women with a BRCA1 mutation.²³⁵

Liver Cancer

Inverse associations have been consistently identified in the literature between coffee/caffeine drinking and liver cancer, as has been concluded by a number of comprehensive cancer reviews.^{178, 179, 211, 212} Hepatoprotective effects of coffee components, including caffeine, against liver fibrosis have been noted in a number of studies.²³⁶ A 2013 meta-analysis specific to coffee and hepatocellular carcinoma found that the risk of this cancer is reduced by 40% for any coffee consumption as compared to no coffee consumption. In newer research, according to Bamia et al. (2015), analysis of data from the large European Prospective Investigation into Cancer and Nutrition (EPIC) study found that increased coffee and tea intakes were consistently associated with lower hepatocellular cancer risk.²³⁷ The inverse associations in the study were substantial, monotonic and statistically significant. The findings were apparent for caffeinated, but not decaffeinated coffee. A 2017 meta-analysis of prospective cohort studies found that increased coffee consumption is associated with decreased risk of liver cancer and has no association with biliary tract cancer.²³⁸ Similarly, a 2016 meta-analysis also confirmed an inverse association between coffee consumption and hepatocellular carcinoma risk (as well as liver cirrhosis risk), which was detected among both the healthy population and those with chronic liver disease.²³⁹

Skin Cancer

Recent data indicates that caffeine consumption may also be protective against skin cancer, and mechanisms through which this may occur are beginning to be elucidated.²⁴⁰ A number of recent meta-analyses found that coffee intake may be inversely associated with incidence of malignant melanoma and basal cell cancer development.^{210, 241-243} A recent case-control study (the Yale Study of Skin Health in Young People) found that regular consumption of caffeinated coffee and hot tea was statistically significantly inversely associated with early onset basal cell carcinoma.²⁴⁴ Analysis of data from the large prospective Nurses' Health Study and the Health Professionals Follow-up Study found a significant inverse association between caffeine intake and basal cell carcinoma and no association with regard to squamous cell carcinoma or melanoma risk.²⁴⁵ Caini et al. (2017) found that consumption of caffeinated coffee was inversely associated with melanoma risk among men in the large multi-center prospective EPIC study.²⁴⁶



Endometrial Cancer

Recent large reviews of the literature have consistently reported inverse associations between endometrial cancer and coffee/caffeine consumption.^{178, 179, 186, 214} In 2013, the World Cancer Research Fund/American Institute for Cancer Research's panel related to its continuous update project on endometrial cancer concluded that coffee likely protects against endometrial cancer.²⁴⁷ They reviewed a total of eight studies in their meta-analysis, and overall analysis showed a 7% decrease in risk per one cup of coffee per day. A large prospective study found an inverse association between endometrial cancer and caffeinated coffee intake in women with a body mass index over 30 kg/sq.m.²⁴⁸ Using data from the Women's Health initiative study, Giri et al. (2011) concluded that caffeinated coffee consumption may be associated with lower endometrial cancer risk among obese postmenopausal women.²⁴⁹ In a prospective study that evaluated women in the NIH-AARP Diet and Health Study, Gunter et al. (2012) concluded that endometrial cancer incidence appears to be reduced among women that habitually drink coffee (which did not differ according to caffeine content).²⁵⁰ Analysis of data from the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial found a decreased risk of endometrial cancer with increased coffee intake.²⁵¹ A 2015 meta-analysis of 13 published studies concluded that coffee and caffeine intake might statistically significantly reduce the incidence of endometrial cancer, and these effects may be modified by BMI and history of hormone therapy.²⁵²

Colorectal Cancer

Large reviews of the cancer literature have found no negative effects, and a potential protective effect, of coffee on colorectal cancer.^{211, 212, 214} Analysis of the large European Investigation into Cancer and Nutrition study found no association between caffeine consumption patterns or genetic differences in caffeine metabolism and colorectal cancer risk.²⁵³ Similarly, analysis of the Nurses' Health Study and Health Professional's Follow-up Study data found no association between caffeine intake and the incidence of colorectal cancer.²⁵⁴ A 2014 study by Dik et al. assessed data from participants of the European Investigation into Cancer and Nutrition cohort study and found that neither coffee consumption patterns nor genetic differences in caffeine metabolism had a significant impact on colorectal cancer risk.²⁵³ A prospective analysis of subjects in the PLCO cancer screening trial by Dominianni et al. (2013) found that greater coffee intake was not associated with risk of colorectal cancer.²⁵⁵

Childhood Cancers

Several studies have been published in recent years exploring potential associations between infant/childhood leukemia and exposure to coffee and/or caffeine by pregnant mothers. Bonaventure et al. (2013)²⁵⁶ reviewed a total of 764 cases of childhood leukemia and 1,681 controls and found a positive association with increased maternal coffee consumption during pregnancy and acute leukemia; the



odds ratios increased with daily intake (p for the trend was <0.001 for > 2 cups per day versus less than 1 cup per week). Cola soda drinking was also slightly associated with lymphoblastic acute leukemia in the study. Tea consumption was not associated with any type of childhood leukemia. Other older studies have not shown an association between childhood leukemia and coffee/caffeine.

Milne et al., (2011)²⁵⁷ assessed 337 cases and 697 controls and found no evidence of an overall association between maternal coffee consumption and risk of acute lymphoblastic leukemia (ALL); in fact the odds ratio was less than 1 suggesting a potential (although not significant) protective role: (OR=0.89; 95% CI 0.61, 1.30). There was, however, some suggestion (although not significant) that consumption of > 2 cups per day by non-smokers during pregnancy could lead to a small increased risk of childhood leukemia (OR=1.44; 95% CI 0.85, 2.42). Tea consumption was inversely associated with childhood leukemia overall, although among ALL cases with balanced chromosomal translocations, the ORs for two cups or more of tea consumption tended to be elevated (OR=1.7; 95% CI 0.79, 3.68).

Several meta-analyses have been done using the data from the studies above. In the meta-analysis included in the Milne et al., 2011²⁵⁷ paper, the authors found no increased risk of leukemia with maternal low coffee consumption during pregnancy; however, 3 or more cups per day was associated with an increased risk, especially in the non-smoking subgroup (OR=2.32; 95% CI 1.51, 3.57) (of note, clearance of caffeine from the blood slows down during pregnancy while smoking is known to accelerate caffeine metabolism). Tea appeared to have an overall protective association.

Another meta-analysis was published in 2014 by Cheng et al.²⁵⁸ Compared with never/lowest drinkers, an adverse correlation between maternal coffee consumption during pregnancy and childhood leukemia was observed in ever drinkers (OR=1.22; 95% CI 1.04, 1.43), low to moderate-level drinkers (OR=1.16; 95% CI 1.00, 1.34), and high-level drinkers (OR=1.72; 95% CI 1.37, 2.16).

Thomopoulos et al. published a meta-analysis of case-control studies in 2015.²⁵⁹ They also found a positive association between high coffee consumption during pregnancy and childhood acute leukemia. Their analysis pointed to a threshold of 2 cups per day for overall leukemia while no threshold was noted for acute myeloid leukemia. Any (or low-to moderate) cola consumption was also associated with leukemia. On the contrary, they found an inverse association between low-to moderate maternal tea consumption and childhood leukemia.

The above results were based on case-control studies, and the associations cannot prove causation (or lack of causation) by coffee or caffeine. Some weaknesses in the studies include potential recall bias and/or recall error (this is a very important bias as many of the mothers were asked to recall their coffee intake during a pregnancy that occurred 10–15 years prior); the fact that data collection usually did



not distinguish between overall size of a “cup”, or whether it was instant, ground, regular or decaf coffee; and small numbers in the sub-group analyses. Additional research is needed in this area, and until more is known, the data supports maintaining current coffee recommendations during pregnancy of not more than 2–3 cups per day.^{86, 139} It should also be noted that the WHO-IARC Working Group’s 2016/2018 publications summarized that the evidence for an association between coffee and childhood leukemia (as well as a number of other cancers) was inadequate for one or more of various reasons including inconsistency of findings across studies, inadequate control for potential confounding, potential for measurement error, selection or recall bias, or insufficient numbers of studies. Similarly, one of the endpoints in the 2017 ISLI/Wikoff et al. systematic review related to reproduction and development was childhood cancers.⁸⁶ The authors stated that the very limited number of studies, combined with the significant impact of potential recall bias, precluded the development of a conclusion for this endpoint but highlights the need for additional research that accommodates this significant bias in the future. They concluded with moderate confidence that the body of evidence is generally consistent for the safe consumption levels during pregnancy that were previously reported by Nawrot et al. (<300 mg/day in pregnancy).

Other Cancers

Published reviews of the literature found no evidence linking coffee consumption with increased pancreatic cancer.^{178–180, 211, 260, 261} A slight inverse association between total coffee and tea consumption and risk of gliomas in individuals from various European countries was observed in a recent large cohort study while no associations were observed with consumption of coffee or tea and meningiomas.²⁶² A 2017 meta-analysis found no significant association between coffee consumption and renal cell carcinoma.²⁶³ A cross-sectional study of U.S. veterans did not find any association between coffee or tea consumption and risk of Barrett’s esophagus (a precursor to esophageal cancer).²⁶⁴

6.2.3.4 Cardiovascular Disease

Various large studies and reviews of the literature have found no effect of moderate levels of caffeine (e.g., 400 mg/day) on cardiovascular disease and there is some suggestion that it could even be protective in some circumstances.^{85, 86, 93, 176, 183, 184, 208, 213, 265–267} The ILSI comprehensive review concluded with a moderate level of confidence that 400 mg/day was not associated with significant concern regarding adverse cardiovascular effects in healthy adults.⁸⁶ Pourshahidi et al.’s review of the literature¹⁸⁴ found that adverse effects of coffee on blood pressure/hypertension were observed more often in coffee naïve individuals, with no effect seen in habitual drinkers. They found that some studies showed no effect on ambulatory blood pressure measurements or on the prospective risk of developing hypertension over time. Yet they also found that coffee consumption may have benefits related to blood pressure, and individual genotypes may play a role in caffeine’s effects.



In a 2016 review, Wilson and Bloom summarized that recently published studies (including prospective cohort studies, clinical investigations, and meta-analyses) generally show coffee consumption is safe for the heart.²⁶⁸ They did not find supportive evidence that chronic commonly consumed coffee levels raise blood pressure or cause atrial or ventricular arrhythmias. Effects on atherogenic lipid levels may be related to coffee brewing methods (levels may increase if the coffee is boiled versus filtered).

Turnball et al. published a comprehensive evaluation of the scientific literature in 2017 as pertains to cardiovascular diseases. They found that cardiovascular effects experienced by caffeine consumers at levels up to 600 mg/day are in most cases mild, transient, and reversible, with no lasting adverse effects. The point at which caffeine intake may cause harm to the cardiovascular system was not readily identifiable by the authors, in part because data on the effects of daily intakes greater than 600 mg is limited. They found that typical moderate caffeine intake is not associated with increased risk of total cardiovascular disease, arrhythmias, heart failure, blood pressure changes among regular coffee drinkers, or hypertension in baseline populations. Ding et al.'s meta-analysis in 2014 concluded that moderate coffee consumption was inversely associated with cardiovascular disease risk, with the lowest risk at 3–5 cups per day; heavy consumption was not associated with elevated risk.²⁶⁹

A 2007 review of in vitro, animal and human studies on coffee and cardiovascular disease concluded that only heavy consumption of coffee (>6 cups per day) is associated with increases in plasma cholesterol and LDL.²⁶⁵ They found that this effect appears to be due to the content of diterpene oils (which are removed in filtered coffee, and as previously explained, are also not expected to be found in RUNA® Concentrate) and not caffeine.

They summarized that moderate consumption of coffee may be protective against cardiovascular disease and that caffeine metabolites may have anti-inflammatory functions that could be beneficial to the heart. Studies looking at both caffeine and coffee showed no association with hypertension risk although an association was reported between diet and sugared colas and hypertension.²⁶⁵ The lack of association between caffeine and coffee (which is generally higher in caffeine content than cola) and hypertension suggest that the observed changes in risk could be due to something other than caffeine in either the coffee or the cola beverages. Those who metabolize caffeine at a slower rate may be at increased risk of nonfatal myocardial infarctions from intake of coffee.²⁷⁰

Coffee does not appear to adversely affect risk of atrial or ventricular premature contractions or fibrillation. Intake of caffeinated products (coffee, tea and chocolate) was not associated with ectopy (premature contractions) in a large dietary study in which subjects wore Holter monitors for cardiovascular tracking.²⁷¹ Analysis of data



from the Danish Diet, Cancer and Health study found no association between caffeine intake and risk of atrial fibrillation or flutter.²⁷² The Framingham Heart Study data found that even the highest quintile of caffeine ingestion (from coffee, tea, and other caffeinated beverages) was not associated with increased incident of atrial fibrillation risk.²⁷³ A 2014 meta-analysis by Cheng et al. of six prospective studies (including the two mentioned above) found that caffeine was weakly associated with a reduced risk of atrial fibrillation.²⁷⁴ Larsson et al. (2015) studied the association between coffee consumption and incidence of atrial fibrillation in two large prospective cohorts and then summarized the available evidence using a meta-analysis.²⁷⁵ They found no evidence that coffee consumption is associated with increased risk of atrial fibrillation. A 2011 review of the literature by Pelchovitz and Goldberger concluded that in most patients with known or suspected arrhythmia, caffeine in moderate doses is well tolerated and there is, therefore, no reason to restrict ingestion of caffeine (although the authors stated that care should be taken to avoid caffeine in situations in which catecholamines are thought to drive the arrhythmia, as well as in patients who note sensitivity to caffeine).²⁷⁶ A 2016 systematic review and meta-analysis of intervention studies on caffeine's effects on ventricular arrhythmias by Zuchinali et al. found no significant effect of caffeine consumption on the occurrence of ventricular premature beats.²⁷⁷ The authors stated that effects in this regard observed in animal studies are most probably the result of very high caffeine doses that are not regularly consumed on a daily basis by humans.

A 2008 review of clinical evidence of coffee consumption as specifically relates to blood pressure and hypertension found that while intake of caffeine can cause an acute short-term rise in blood pressure, intake of four or more cups of coffee per day could be protective against hypertension, especially in women, as shown in prospective observational studies. However, five cups of coffee per day or more has been shown to cause small elevations in blood pressure in randomized controlled trials. The authors of the review concluded that most evidence suggests that habitual coffee drinking is not related to risk of hypertension.²⁷⁸ A 2011 meta-analysis on coffee and blood pressure and cardiovascular disease concluded that in hypertensive individuals, caffeine intake (200–300 mg/day) produces acute increases in both systolic (8 mmHg) and diastolic (6 mmHg) blood pressure for up to three hours after consumption, similar to what has been shown in normotensive individuals. Overall evidence does not support an association between long-term coffee consumption and increased blood pressure or increased cardiovascular disease or cardiovascular event risk.²⁷⁹ The authors did suggest that additional studies be done with regard to caffeine intake in the hours prior to coronary and cardiovascular events to determine if there is a correlation with acute blood pressure increases from caffeine and such events. Genetic polymorphisms such as those related to caffeine metabolism also deserve further study with regard to potential risk of adverse events.^{280, 281}



A 2011 publication looked at data from the large Japan Collaborative Cohort Study for Evaluation of Cancer Risk, and found a lower risk of mortality from cardiovascular disease with moderate consumption of caffeine.²⁸² Analysis of data from the large Nurses' Health Study cohorts showed no linear association between caffeine consumption and hypertension risk although there was a statistically significant increased risk from cola beverages.²⁸³ However, the association was present across all soda types, and independent caffeine consumption was not associated with significant increased risk in the study; thus, the authors speculated that other compounds in soda beverages aside from caffeine are more likely responsible for the increased risk.

Consumption of four cups or more of coffee per day was associated with decreased levels of stroke in a 2012 meta-analysis that included nine studies.²⁸⁴ A large prospective study found no evidence that caffeine consumption increases the risk of coronary heart disease in men or women.²⁸⁵ Consumption of filtered caffeinated coffee was not associated with cardiovascular disease mortality after up to 24 years of follow-up of women with cardiovascular disease from another analysis of the Nurses' Health Study.²⁰⁶ Coffee consumption was not associated with developing cardiovascular disease between the 1980s and 2004 in large cohorts of diabetic men and women with no cardiovascular disease at baseline.²⁰³

6.2.3.5 Type 2 Diabetes

Coffee, CAs and caffeine consumption have been associated with a decrease in risk of developing type 2 diabetes over the long term, as has been determined in a number of recent comprehensive reviews of the literature.^{85, 176, 183, 184, 213, 266, 286, 287} Acutely, caffeine can impair insulin sensitivity and glucose metabolism,^{170–287} however these acute/short-term effects are in contrast to longer term beneficial associations that are well-described.^{287–289} Individual genetic polymorphisms (in, for example, CYP1A2) likely play a role in glycemic (and other) effects.¹⁹⁹

Decreased risk of developing type 2 diabetes has been associated with consumption of coffee/caffeine in large studies such as the Japan Collaborative Cohort Study for Evaluation of Cancer Risk, the European Prospective Investigation into Cancer and Nutrition (EPIC) study,²¹³ and a French women cohort study.²⁹⁰ Bhupathiraju and colleagues (2014) followed subjects in the Nurses' Health Studies and the Health Professionals Follow-up Study²⁹¹ and found that increasing coffee consumption over a four year period by more than one cup per day was associated with a lower risk of developing type 2 diabetes while decreasing coffee consumption by over one cup per day was associated with a subsequent higher risk.

A 2014 meta-analysis of prospective studies concluded that the pooled relative risk from 26 studies involving over a million subjects was 0.71 (95% CI, 0.67–0.76) for the highest level of coffee intake compared to the lowest level of intake.²⁹² A dose-response analysis found that incidence of diabetes decreased by 12% for every two



cups per day increment in coffee intake and 14% for every 200 mg/day increment in caffeine intake. Shi et al. performed a systematic review and meta-analysis in 2016 on randomized controlled trials that investigated the effect of acute caffeine intake on insulin sensitivity in healthy human populations (i.e., without diabetes).²⁹³ They found seven trials to examine and concluded that acute caffeine ingestion significantly reduces insulin sensitivity in healthy subjects, suggesting that the inverse association between coffee and diabetes might not be attributable to enhanced glucose control.

It should be noted that in individuals with diabetes, acute caffeine ingestion has been shown to have a short-term negative effect on blood glucose and insulin when consumed after consumption of sugar but not when consumed on its own.²⁹⁴ Under the former circumstances, caffeine appears to exaggerate post-prandial hyperglycemia and hyperinsulinemia, even in habitual caffeine consumers; the effect lasts up to three hours and is independent of exercise. Doses of caffeine used in these studies tended to be single large boluses of caffeine (≥ 250 mg), which may not reflect effects from more usual caffeine consumption patterns).²⁹⁴ Future studies will need to look at more long-term effects on blood sugar control and potential effects of reduction of caffeine intake in subjects with poorly controlled diabetes. Depending on outcomes, such studies could lead to changes in current dietary recommendations for this population with regard to caffeine consumption, similar to recommendations related to sugar consumption (another commonly consumed GRAS ingredient). Overweight but healthy (insulin-sensitive) males also showed a disruption of 2-hour glucose response after 100 mg dose of caffeine, without dose-dependence and with no further effect of increased other components of coffee such as CAs.²⁹⁵ The effect quickly resolved in these individuals and the physiological relevance is unknown at this time. In contrast to these findings in type 2 diabetics, there is early evidence that type 1 diabetics might benefit from caffeine intake due to its potential to decrease hypoglycemic episodes and allow for increased awareness of such episodes when they occur in this population.²⁹⁴ Genetic polymorphisms likely also play a role in individual glycemic responses in the presence of caffeine.¹⁹⁹

6.2.3.6 Reproduction

Not many randomized controlled trials have been performed in this area. A Cochrane review on restricted caffeine intake by mothers and effects on fetal, neonatal and pregnancy outcomes was published in 2013.²⁹⁶ Only two studies met the inclusion criteria, and only one contributed data to the outcomes of interest. In that study, 1200 pregnant women in Denmark were randomly assigned to drink caffeinated or decaffeinated instant coffee. No significant differences between groups with regard to birth weights, preterm births, or growth restrictions were uncovered.



Several 2014 reviews/meta-analyses reported slight positive associations between increased caffeine consumption by pregnant women, and low birth weights^{297, 298}, spontaneous abortions²⁹⁸, stillbirths²⁹⁸, and small for gestational age findings.²⁹⁸ However the sizes of the associations were modest within the range of usual intake and range of intake currently recommended for pregnancy and have been considered to be possibly explained by biases in the studies. For example, small but quantifiable increased associations between maternal caffeine intake and low birth weight per 100 mg/day increment were determined, but the authors stressed heterogeneity between studies and possible biases (such as reverse causation, residual confounding by smoking or pregnancy symptoms) making conclusions challenging to draw and that studies that adjusted for maternal education or socio-economic factors had significantly lower estimates than those that did not.^{297, 298} Greenwood et al., summarized that there is insufficient evidence to support changes in current caffeine consumption recommendations during pregnancy by scientific bodies (although they support maintaining the precautionary guidance information that is currently in place).²⁹⁷

Additional recent comprehensive analyses and reviews of current data regarding reproductive and developmental risks of caffeine consumption conclude that while there are some inconsistencies, the weight of evidence suggests that moderate caffeine exposure before or during pregnancy does not increase clinically relevant risks of subfecundity problems, congenital malformations, miscarriage, fetal death, preterm birth, or fetal growth retardation.^{163, 299} The American College of Obstetricians and Gynecologists released a committee opinion in 2010 that moderate caffeine consumption (less than 200 mg per day) does not appear to be a major contributing factor in miscarriage or preterm birth. They also stated that the relationship of caffeine to growth restriction remains undetermined.³⁰⁰

In a study investigating the effect of caffeine consumption during pregnancy and nursing on infant sleep, no association between maternal caffeine consumption and nighttime waking in infants at three months (the age at which infants are able to metabolize caffeine) was observed.³⁰¹

Various authors have expressed that there are a number of limitations in current studies, such as problems regarding accurate caffeine consumption estimates, lack of data on early miscarriages, potential reporting bias related to smoking (a known risk factor for low birth weight that often correlates with caffeine intake and may be under-reported by subjects due to negative connotations associated with smoking and pregnancy).^{85, 86, 297, 298} Possible confounding factors, such as lack of pregnancy signal symptoms, are also considered a major limitation; for example, studies that did not control for pregnancy signal symptoms have shown potential positive associations between caffeine consumption and spontaneous abortions.^{85, 86, 163, 299} Yet it is established that in general, women with viable pregnancies that go to term experience more frequent and severe nausea, vomiting, and aversions to various



smells and tastes first trimester compared to women whose pregnancies end in spontaneous abortion. The majority of women who decrease coffee consumption during first trimester do so because of a physical aversion to coffee that drives caffeine consumption in this group downward. Thus, it is possible that reduction in caffeine intake may be a marker of aversion and, thus, a healthier pregnancy.⁸⁶ This makes the pregnancy signal a crucial confounder that was not controlled for in most studies. Studies that attempted to control for nausea and vomiting during pregnancy have been less consistent in results.

As described previously, the endpoints in the 2017 ILSI/Wikoff et al. systematic review for reproduction and development included fecundability and infertility, spontaneous abortion, recurrent miscarriage, stillbirth, preterm birth, fetal growth, birth defects, childhood behavior, childhood cancer, markers of maternal stress, pregnancy-induced hypertension and/or preeclampsia, and age at menopause.⁸⁶ The authors concluded with moderate confidence that the body of evidence is generally consistent with the safe consumption levels for pregnancy that were previously reported by Nawrot et al. (<300 mg/day in pregnancy). The authors stated that although some effects noted below this level could not be completely ruled out, such effects were primarily limited to isolated congenital malformations or childhood cancers and were of low magnitude. They found the body of evidence for fetal growth showed inconsistent results making overall conclusions difficult. The biological significance of inverse effects on birth weight was more robustly evaluated in studies that included small for gestational age and intrauterine growth restriction measurements. On the whole, those studies were not found to provide support for effects below the comparator level.

6.2.3.7 Bone Health

Recent comprehensive reviews of the caffeine/coffee literature have revealed no health concerns related to bone or calcium endpoints with moderate consumption levels (i.e., 400 mg caffeine/day for adults), especially if calcium intake is adequate.^{86, 139} Recent data may even suggest a preventive effect of coffee on bone health. For example, in a 2016 paper by Choi et al. data from the 2008–2011 Korean National Health and Nutrition Examination Surveys was evaluated with regard to coffee consumption and dual-energy X-ray absorptiometry examination.³⁰² After adjusting for confounders they found that subjects in the highest quartile of coffee intake had a 36% lower chance of having osteoporosis and that coffee may have protective effects on bone health in postmenopausal women.

A 2013 study followed the Swedish Mammography Cohort from 1987–2008.³⁰³ The authors found no evidence of a higher rate of any fracture (including hip) with increasing coffee consumption. Higher coffee intake was associated with a slightly lower bone density, but it did not translate into an increased risk of fracture. In a



cross-sectional study of women in Brazil, no association was found between caffeine intake and bone mass.³⁰⁴

6.2.3.8 Neurological and Behavioral Health

In a review of the literature on caffeine's effects related to cognitive, mood and physical performance, McLellan et al. (2016) concluded that in doses up to approximately 300 mg (approximately 4 mg/kg bw), caffeine enhances a wide array of basic cognitive functions with minimal side effects by affecting alertness and attention.³⁰⁵ Caffeine's ability to enhance cognitive and physical function/performance was found to be dose-dependent, although response to a given dose shows large inter-individual variation. The authors concluded that caffeine is an effective strategy to counter both physical and cognitive degradation associated with sleep loss. Similar conclusions were described by Nehlig, 2016.³⁰⁶ Additionally, in reviewing more than a dozen studies related to caffeine's effects on aggression/risk-taking behavior, Turnbull et al. (2016) found no clear evidence for concern in this area, although stated that this should not preclude ongoing monitoring.³⁰⁷

Mood

While caffeine can disrupt sleep (especially if consumed closer to bed time) or raise anxiety at high doses (e.g., 400–800 mg in a sitting, or lower doses in individuals who are especially sensitive), experiencing such effects does not appear to have any significant lasting effects on health.^{306, 307} On the other hand, coffee and caffeine consumption have been associated with a decreased risk of depression, which was concluded by two different 2016 meta-analyses of the literature.^{308, 309} In the 11 observational studies that were analyzed in the Wang et al. meta-analysis, evidence of a dose-response relationship was found; the risk of depression decreased by 8% for each cup/day increment in coffee intake.³⁰⁸ Grosso et al. found a nonlinear J-shaped relation between coffee consumption and risk of depression in their 2016 meta-analysis, with a peak protective level of 400 mL coffee/day.³⁰⁹ In a review of three large cohort studies (the Health Professionals Follow-up Study and the Nurses' Health Studies I and II), an inverse association was found between caffeine consumption and risk of suicide.³¹⁰ Bioactive coffee constituents, including caffeine, may modulate parameters of neuro-inflammation, which may be a mechanism for effects on mood.³¹¹

Neurological Disorders

Moderate coffee/caffeine consumption has been found to be associated with reduced rates of age-related cognitive decline and reduced risk of developing neurological disorders such as Parkinson's or Alzheimer's diseases in some studies.^{85, 306, 312, 313} This may be particularly true for individuals who already have mild cognitive impairment, and various genetic factors may play roles in caffeine/coffee's



effects.^{314, 315} Caffeine doses of 3–5 mg/kg bw/day have been found to be neuroprotective in both epidemiological and preclinical studies.³¹⁶

Results of studies in animal models have suggested that coffee could play a preventative role in Alzheimer's disease, for example by lowering the concentration of associated neurotoxic peptides and protecting against oxidative stress. Human observational and prospective studies have also suggested a protective effect of coffee with regard to cognitive decline and Alzheimer's, although results have been mixed.^{312, 313, 315, 316} In two recent meta-analyses (2014 and 2015), the conclusions were that not enough research was available to suggest a specific association, and larger prospective studies are needed.^{317, 318} Regardless, moderate coffee/caffeine consumption appears safe in populations at risk for cognitive deficits.³¹³ In their meta-analysis of human studies relating caffeine to cognitive decline, Arab et al. (2013) found that for all studies of tea and most studies of coffee/caffeine, the estimates of cognitive decline were lower among consumers, although there was a lack of distinct dose-response.³¹⁹

Published reviews of the literature have found that coffee/caffeine may be associated with a decreased risk of Parkinson's disease.^{85, 93, 176, 183, 184, 315, 316, 320} This may occur via protection against underlying dopaminergic neuron degeneration and decreasing neuro-inflammation, as well as elevation of dopamine levels via caffeine's effects related to A₂ receptors.^{93, 316} A 2015 meta-analysis on Parkinson's disease risk found a linear dose-relationship for decreased disease risk with tea and caffeine consumption.³²¹ The study also found a protective effect of coffee, with a maximum strength of protection of approximately 3 cups per day. In 2015, Gaba et al. reviewed recent studies on nutrition and Parkinson's disease and found that coffee and black tea, but not green tea, seemed to be protective against the disease, most likely due to caffeine content.³²²

6.2.3.9 Diuresis and Hydration

Despite the lack of consistent evidence, a longstanding belief is that consumption of caffeine-containing beverages will have negative effects on fluid balance. Older studies of fluid balance tended to examine consumption of caffeine itself rather than caffeine in commonly consumed beverages. The use of experimental models such as fluid and dietary restriction accompanied by relatively prolonged periods of caffeine withdrawal do not necessarily reflect everyday consumption patterns, and numerous aspects of research design among human studies conducted in the 20th century also call into question the belief that caffeine disrupts fluid balance.³²³

While it was concluded in a 2003 review that large doses (at that time, ≥ 250 mg) of caffeine have an acute diuretic action,³²³ a 2015 meta-analysis cast doubt on this conclusion.³²⁴ Studies identifying urine volume following caffeine ingestion in healthy adults (as the primary outcome variable) were examined but only if sufficient information for calculating the effect sizes (ESs) was provided. Results



were determined from 28 investigations among 16 studies. The findings were threefold. Firstly, caffeine-induced diuresis was small in magnitude. Primary meta-analysis revealed a small but significant ES (ES = 0.29, 95% CI = 0.11–0.48, p = 0.001), although subgroup analysis showed an almost 6-fold greater ES in women (ES = 0.75) than in men (ES = 0.13). The difference between sexes may be attributed to the metabolism of caffeine, which is mediated by the activity level of CYP1A2.

Secondly, the diuretic effect was not observed with physical activity (PA) (this was also concluded in an International Society of Sports Nutrition position paper³²⁵ from 2010), likely due to the increased sympathoadrenal activation that accompanies exercise, which stimulates the release of catecholamines. This constricts the renal arterioles, lowering glomerular filtration rate. Following this logic, increased PA intensity and longer PA duration both mediate a greater release of catecholamines, lessening the likelihood of caffeine-induced diuresis. Thirdly, significant heterogeneity was observed, yet neither the dosages of caffeine nor the duration of investigations explained the heterogeneity. The findings of the meta-analysis help to discredit the belief that caffeine ingestion leads to excessive fluid loss via diuresis in healthy, active adults.

In a 2014 cross-over study, male habitual coffee drinkers were controlled for physical activity, food and fluid intake over three day periods and were given either coffee (4 mg/kg caffeine) or water.³²⁶ No differences were observed across hematological markers or 24-hour urine volume, osmolality, creatinine levels, or body mass between the trials, and the authors concluded that moderate coffee consumption provides similar hydrating qualities to water. Similarly, in a 2013 trial of healthy young men who did not regularly ingest caffeine, investigators discovered that a moderate dose of caffeine did not affect fluid distribution or total body water, even after adjusting for body composition, daily water intake, and habitual physical activity.³²⁷ The caffeine dose was 5 mg/kg bw/d (350 mg in a 70 kg individual), approximating five shots of espresso (30 mL each) or seven servings of tea. These results are in agreement with others reporting no changes in hydration with caffeine intake.^{328–330}

6.2.3.10 Self-regulation of Caffeine Intake

Individuals tend to be aware of their personal tolerance to the objective and subjective cognitive/energizing/physiological effects of caffeine through experience over time and use this awareness to moderate their intake accordingly.¹⁹³ For example, the caffeine safety reviews by Health Canada and ILSI suggest that self-regulation of caffeine intake reduces caffeine's potential to produce anxiety and/or sleep disturbances in adults.^{74, 86} This is also demonstrated by the fact that caffeine consumption levels have remained stable despite new caffeinated beverage additions to the market.^{13–15, 70, 72, 73, 331}



It is known that subsets of individuals intentionally consume high levels of caffeine for its perceived positive effects on alertness, as a countermeasure for sleep deprivation, for improved energy, and/or for other physiological responses associated with it. Individuals with a larger body mass, faster metabolism, or certain genetic variations likely are able to consume higher amounts of caffeine (as compared to other individuals) safely. In contrast, individuals that metabolize caffeine more slowly are more likely to self-limit consumption. Genetic polymorphisms in metabolizing enzymes, such as on the loci 15q24 (between CYP1A1 and 1A2, the latter of which metabolizes caffeine), and 7p21 (near AHR, known to regulate CYP1A2), as well as certain ADORA2A genetic polymorphisms related to adenosine receptors have been linked to caffeine consumption patterns.⁸⁶, 193, 196-198

6.2.4 Current Regulatory Status of Caffeine

The following is a summary of U.S. regulations related to coffee and caffeine:

- In accordance with 21 CFR §182.20, essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *Coffea* spp. are GRAS in the United States for their intended use. It is understood that this regulation is intended to refer to use in relatively small amounts for flavoring.
- Cola-type beverages are allowed to contain 0.02% caffeine, or approximately 0.2 mg/mL (~ 47 mg per 8 oz.), according to 21 CFR §182.1180.
- Caffeine is allowed as a stimulant Over-the Counter drug pursuant to 21 CFR §340.50 and §340.10. The directions must be 100–200 mg per dose, and a dose may be taken every 3–4 hours. Product warnings must include that “too much caffeine may cause nervousness, irritability, sleeplessness, and, occasionally, rapid heart beat.”

6.2.5 Energy Drinks, and Caffeine Interaction Concerns

6.2.5.1 FDA Opinions

In the past, there has been some concern voiced regarding potential interactions between caffeine and other ingredients in energy drinks that might potentiate toxicity in ways not obviously apparent in safety studies conducted on the individual ingredients.³³² FDA has stated that they have not found in their review of the literature information that calls into question the safety of specifically taurine and guarana as currently used in beverages, that their research has shown that caffeine consumption has remained relatively stable despite the entry of energy drinks into the marketplace, and that energy drinks contribute only a small portion of caffeine consumed, even for teens.³³² FDA has cited 400 mg of caffeine per day (equivalent



to 4–5 cups of coffee) “as an amount not generally associated with dangerous, negative effects” for healthy adults in a May 3, 2013 statement.³³³

Several federal workshops occurred in 2013 with the aims of gathering information about caffeine and energy drinks and identifying critical data gaps.^{17, 334-336} The workshops were intended to be a sharing of information, and no conclusions regarding safety were made. After these workshops, Michael Taylor (FDA’s Deputy Commissioner for Foods and Veterinary Medicine) blogged on August 26, 2013 about caffeine, and stated that valuable scientific input was received, and FDA is committed to incorporating what they learned into their ongoing scientific assessment, and will consider future regulatory options on that basis.³³⁷

6.2.5.2 European Union Opinions

The European Union’s Scientific Committee on Food (SCF) evaluated the safety of caffeine for use in energy drinks in 1999 and concluded that the contribution of energy drinks to overall caffeine intake does not appear to be a matter of concern for non-pregnant adults.³³⁸ With respect to pregnant women, SCF concluded that most of the available data suggest there is no problem if total intake is below 300 mg caffeine/day. With respect to children, SCF concluded that consumption of energy drinks could represent an increase in daily caffeine exposure compared with their previous intake, which could result in transient behavioral changes, such as increased arousal, irritability, nervousness or anxiety. They also found no apparent reason for concern about carcinogenic or mutagenic effects of caffeine at normal levels of intake. SCF’s 1999 opinion was upheld without changes in its 2003 updated opinion on energy drinks.³³⁹

The EU released subsequent reports related to the safety of energy drinks in 2003 and 2009.^{339, 340} Note that the European Food Safety Authority (EFSA) was established by 2009; thus, the latter report was produced by EFSA’s Scientific Panel on Food Additives and Nutrition Sources added to Food (ANS), rather than by SCF. Based on data reviewed concerning the individual mechanisms of action of taurine and caffeine affecting the cardiovascular system, CNS, and kidneys, the Committee made the following conclusions in these reports:

1. In 2003 SCF concluded, “if there are any cardiovascular interactions between caffeine and taurine, taurine might reduce the cardiovascular effects of caffeine.”
2. In 2003, regarding the CNS, SCF stated, “if there were any interaction, taurine might reduce caffeine-mediated excitation [of the CNS]” but “noted that caffeine and taurine act on different [CNS] receptors” and concluded, “the potential for interactions between caffeine and taurine has not ruled out the possibility of stimulatory effects from both substances at the level of the central nervous system.” Of note, at the time of the 2003 report, concerns



over an apparent taurine related stimulatory action on locomotor activity in rats in an unpublished 13-week oral toxicity study had not yet been laid to rest. In the 2009 report, ANS evaluated a 2007 pharmacokinetic study in rats that found oral administration of taurine does not increase brain taurine levels,³⁴¹ as well as an unpublished new 13-week oral neurotoxicity study in rats. Based on the results, the committee concluded, “[this] largely rules out the possibility of stimulatory effects from taurine at the level of the central nervous system,” implying that additive or synergistic CNS interactions (i.e., potentially toxic interactions) between caffeine and taurine are unlikely.

3. The 2009 report concluded, “additive interactions between taurine and caffeine on diuretic effects are unlikely.”³⁴²
4. The 2003 and 2009 reports concluded the unlikelihood of any interactions between caffeine and D-glucurono-γ-lactone.

In 2015, EFSA released its scientific opinion on the safety of caffeine, in which it also considered the safety of caffeine interactions with common constituents of energy drinks.¹³⁹ The panel reviewed the literature on effects of single and repeated doses of caffeine consumed either alone or in combination with other constituents of energy drinks. The conclusions in the abstract were as follows: “Single doses of caffeine up to 200 mg (about 3 mg/kg bw for a 70-kg adult) do not give rise to safety concerns. The same amount does not give rise to safety concerns when consumed < 2 hours prior to intense physical exercise under normal environmental conditions. Other constituents of “energy drinks” at typical concentrations in such beverages (about 300–320, 4000 and 2400 mg/L of caffeine, taurine and D-glucurono-γ-lactone, respectively), as well as alcohol at doses up to about 0.65 g/kg bw, would not affect the safety of single doses of caffeine up to 200 mg. Habitual caffeine consumption up to 400 mg per day does not give rise to safety concerns for non-pregnant adults. Habitual caffeine consumption up to 200 mg per day by pregnant women does not give rise to safety concerns for the fetus. Single doses of caffeine and habitual caffeine intakes up to 200 mg consumed by lactating women do not give rise to safety concerns for breastfed infants. For children and adolescents, the information available is insufficient to derive a safe caffeine intake. The Panel considers that caffeine intakes of no concern derived for acute caffeine consumption by adults (3 mg/kg bw per day) may serve as a basis to derive single doses of caffeine and daily caffeine intakes of no concern for these population subgroups.”

6.2.5.3 Health Canada Opinions

In 2010, an independent expert advisory panel on caffeinated energy drinks was convened to review the scientific literature and adverse reaction reports associated with energy beverages. Health Canada (2012) then analyzed recommendations provided by the panel and, along with its own risk assessment and data collection, decided upon a proposed approach to manage energy drinks. Some aspects of the



approach included classifying the beverages as foods and setting certain safety requirements for the products. In order to be eligible for marketing authorization, an energy drink must contain 200–400 ppm caffeine but shall not exceed 180 mg per single serving container or per serving in multiple serving containers. Caffeine content (from all sources) must be declared on product labels along with the statement: "High caffeine content." Certain cautionary statements are also required, including warnings not to mix with alcohol; not recommended for children, pregnant or breastfeeding women, or individuals sensitive to caffeine; and not to consume more than a specified number of servings per day.³⁴³

Rotstein et al. (2013), authors from Health Canada, published a paper entitled "Energy Drinks: An Assessment of the Potential Health Risks in the Canadian Context."³⁴⁴ In the document, a typical energy drink was considered to contain 80 mg of caffeine per 250 mL serving. With respect to caffeine, the authors utilized previously concluded safe levels of caffeine consumption and applied them to energy drink consumption (up to 400 mg for a healthy adult, up to 300 mg for reproductive-aged women, and up to 2.5 mg/kg bw/day for children and adolescents).⁷⁴ Caffeine intake concerns related to energy drink consumption by children were considered limited given that children are less likely to obtain these products on their own and that parents are expected to keep energy drinks out of children's diets. Adults and pregnant women were considered capable of monitoring their own caffeine intake and would be more likely to recognize acute adverse effects from excess intake and moderate their consumption accordingly. Adolescents were identified as a potential higher risk group that could exceed recommended caffeine intake levels via energy drink consumption, and it was suggested that attention to the levels of caffeine present in large volume energy drink containers may be warranted. Health Canada's recent guidelines were considered likely to mitigate some of the risks related to possible overconsumption of energy drink products in these areas.

6.2.6 U.S. Food and Drug Administration on Caffeine and Alcohol, Pure Powdered Forms

In 2010, FDA issued warning letters to a number of manufacturers of caffeinated alcoholic beverages stating that such use of caffeine was not approved by FDA and is considered unsafe.¹⁷ These manufacturers have since removed their caffeinated alcoholic beverages from the market. One of the manufacturers had submitted a GRAS notification to FDA (designated GRN #347) on the use of caffeine as a flavoring ingredient in alcoholic beverages at a level of up to 200 ppm. However, the notification was later withdrawn.

In 2014 the FDA issued an alert to consumers regarding the dangers of pure powdered caffeine,³⁴⁵ and issued warning letters to various distributors in 2015



because such products were considered to be dangerous and to present a significant or unreasonable risk of illness or injury to consumers.³⁴⁵⁻³⁴⁹ In April 2018 FDA released a guidance for industry on highly concentrated caffeine in dietary supplements.^{350, 351} In this guidance, FDA made clear that highly concentrated powdered or liquid caffeine products, in which consumers are expected to be able to precisely measure out safe portions, will most likely be considered adulterated by FDA. This is because toxic or lethal doses of caffeine could inadvertently be consumed if measurements are not done correctly.

It should be underscored that RUNA® Concentrate is not intended for use in beverages containing alcohol and is not intended to be sold in pure powdered form to consumers.

6.2.7 Summary of Recent Scientific Studies on Caffeine Safety

As described above, caffeine (naturally occurring and added) has been the subject of enormous numbers of scientific studies for many decades, likely more than any other food ingredient. Much of the caffeine safety evidence has been gleaned from studies that evaluated coffee consumption. Coffee contains more than two thousand chemical constituents, especially small molecular weight flavor and aroma chemicals and high molecular weight bio-polymers.³¹ Thus, it is possible that effects seen could be from constituents other than caffeine, and effects specifically from caffeine cannot be explicitly discerned. However, coffee can be considered a surrogate of caffeine consumption, and if the vast majority of studies on coffee show no increases in disease risk, but actually beneficial effects, then the caffeine in that coffee may also be assumed not to increase risk. The lack of association with disease risk shown in the overwhelming majority of studies summarized above supports the conclusion that consumption of up to moderate levels (400 mg/day for adults, 300 mg/day for pregnant women, and 2.5 mg/kg bw/day for children) of caffeine is safe. Importantly, as detailed in Part 3, caffeine consumption patterns have remained relatively consistent (or even declined) over the years despite the introduction of various new caffeinated products into the marketplace. Further, the caffeine consumption estimates from current proposed uses of RUNA® Concentrate are below these established safety thresholds.

While attention has been given to the issue of caffeine overexposure in energy beverages or co-exposure with alcohol, these exposure scenarios are not considered relevant to the intended uses of RUNA® Concentrate evaluated in the current safety assessment. In their evaluation of caffeine-containing energy drinks, scientific and regulatory authorities have generally concluded that common energy drink constituents are unlikely to adversely interact with caffeine, and the previously established safety thresholds for caffeine (400 mg/day for adults, 300 mg/day for



pregnant women, 2.5 mg/kg bw/day for children and adolescents) remain protective of consumer health and safety.

6.3 Safety of Chlorogenic Acids

CAs are components of guayusa leaves and comprise approximately 5.2% of RUNA® Concentrate. As estimated in Part 3, exposure to CAs from the addition of RUNA® Concentrate to energy beverages is expected to be approximately 380 mg/day (4.7 mg/kg bw/day) at the lifetime 90th percentile.

This subpart provides a safety narrative for CAs, much of which is derived from research on coffee, which contains the same major CA compounds as guayusa (e.g. 3- and 5-CQA, 3- and 5-FQA, and 3,4- and 3,5-diCQA).^{7, 88, 352}

As previously stated, confusion in the literature arises in CA nomenclature in part from the use of trivial names and in part from the availability of two numbering systems for the cyclohexane ring, and the failure of some authors to define which system is being used in a particular publication. It is possible in most cases to determine which system of numbering has been used, and herein any notable non-IUPAC numbering has been changed to IUPAC (1976) numbering and the change noted explicitly. Where it is impossible to define which system has been used, no change is made, and this also is noted explicitly.

The major safety conclusions of this subpart are:

1. The pharmacokinetic profile of CAs suggests that they are rapidly absorbed, metabolized, and eliminated from the body.
2. CAs from CoffeeBerry® ethanol extract are substantially similar to CAs in guayusa; a NOAEL for CAs from a 90-day feeding study of CoffeeBerry® ethanol extract provides a margin of safety of greater than 100 for exposure to CAs from RUNA® Concentrate.
3. Corroborative animal studies showing no abnormal or toxicological effects in Sprague-Dawley rats when pure 5-CQA (presumably IUPAC) was consumed at 1% of the diet for 3 weeks, equivalent to approximately 1000 mg/kg bw/day; no side effects from a green coffee bean extract containing 28% total CAs related to general health, body and organ weights and clinical and physical chemistry parameters; and an acute study of CAs from Crofton weed showing no toxicity up to 1.5 g/kg bw.
4. Clinical studies on green coffee extracts and CAs (one of which reported safe consumption of 750–900 mg/day of CAs from green coffee (as Svetol™) for 12 weeks, and others showing safe consumption of lower levels of CAs for up to 16 weeks) do not suggest adverse effects of consumption of CAs by humans.



6.3.1 Pharmacokinetics of Chlorogenic Acids

Upon ingestion, some absorption of CAs occurs in the stomach/small intestine (with mechanisms of absorption varying depending upon the compound), while small amounts are hydrolyzed by cytosolic esterases in the mucosa. CAs that are not absorbed in the small intestines (approximately 70%) move into the large intestine where the colonic microflora metabolize the compounds into highly absorbable derivatives (e.g., caffeic acid, ferulic acid, quinic acid and their glucuronate/sulfate/methylated conjugates).^{40, 43, 93, 353-364} An ex vivo absorption experiment using a pig jejunal mucosal model using 0.2–3.5 mM concentrations of various CA compounds found that absorption rate and mechanism was dependent on the physiological properties of the compound.³⁶² The diCQAs were the least absorbed (trace levels) followed by CQAs (1%) and FQAs (2%). Absorption occurred mainly through passive diffusion with active efflux playing a significant role, with the exception of 4-CQA and 4-FQA for which there appears to be saturable facilitated transport.^{358, 362-364} Using liquid chromatography-electrospray ionization-tandem mass spectrometry, Matsui et al. (2007) were able to identify eleven compounds (3x CQAs, 3x FQAs, 3x diCQAs, and the metabolites caffeic acid and ferulic acid) in human plasma after consumption of a beverage containing 300 mg CAs.³⁶⁵ A significant portion of CAs and other phenolic acids are metabolized by inducible phase II xenobiotic systems into, for example, glucuronidated, sulfated and methylated metabolites.^{40, 43}

Major CA-related compounds absorbed in the small intestine (short T_{max} of approximately one hour) are unmetabolized CQAs, FQAs, sulfated CQALs, and, at higher concentrations, caffeic acid-3'-*O*-sulfate and ferulic acid-4'-*O*-sulfate. Metabolites originating from the colon (longer T_{max} of approximately 4.3–5.2 hours) include compounds such as dihydrocaffeic acid, dihydrocaffeic acid-3'-*O*-sulfate, dihydroferulic acid and dihydroferulic acid-4'-*O*-sulfate.^{93, 359, 366} Absorption of CA parent compounds and their metabolites in humans suggest that their bioavailability could be greater than that of other dietary flavonoids and phenolic compounds.^{359, 360, 361} Median apparent half-lives from oral dosing of the various parent CA compounds and small intestinal absorption metabolites range from 0.3–1.2 hours, and large intestinal absorption metabolites ranged from 0.7 to 3.9 hours.³⁶⁶ Metabolic pathways for the CAs (after ingestion of coffee) are shown in Figure 5 below.

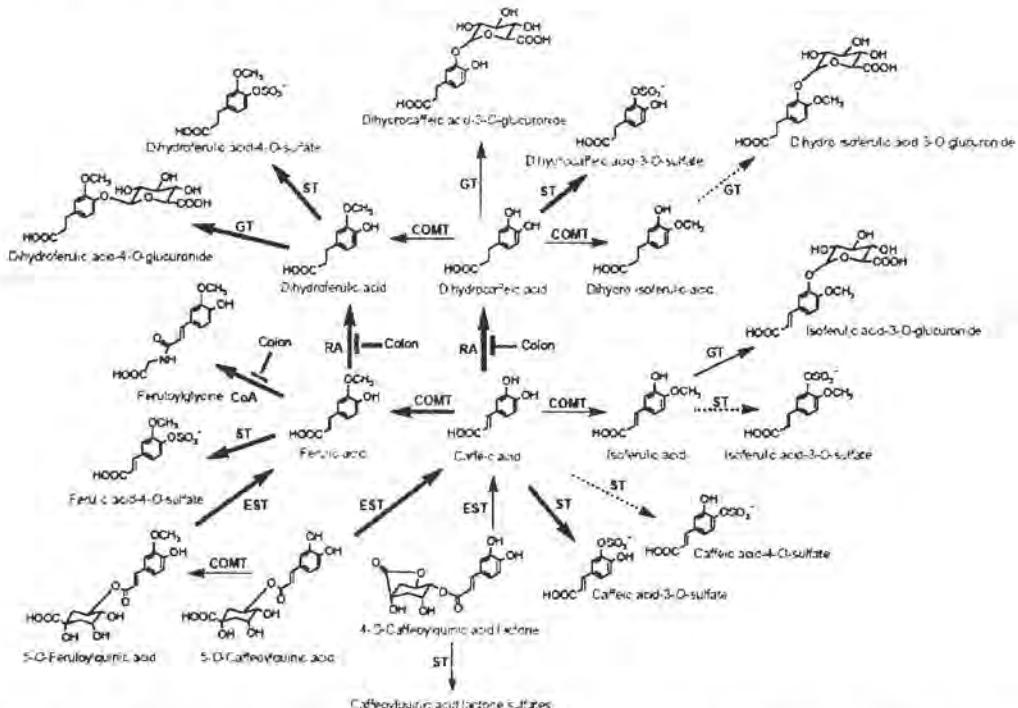


Figure 5. Metabolism of Chlorogenic Acids Following Ingestion of Coffee by Human Volunteers (borrowed with permission from del Rio et al., 2010).⁴⁰

*Note that while only 5-O-CQA and 5-O-FQA are illustrated, their respective 3- and 4-isomers would be metabolized in a similar manner. COMT=catechol-O-methyltransferase, ET=esterase, RA=reductase, GT=UDP-glycuronyltransferase, ST=sulfuryltransferase. Bold arrows indicate major routes, dotted arrows are minor pathways. Steps blocked in subjects with an ileostomy and hence occurring in the colon are indicated.

A double-blind crossover study was conducted in part, to determine the absorption of CAs from green coffee extract.¹⁸⁸ The study also evaluated the pharmacokinetics of caffeine from the green coffee extract compared to synthetic caffeine, and the study is, thus, also described in the caffeine pharmacokinetic subpart. Sixteen healthy male subjects, aged 18 to 45, were randomly assigned to take a single dose of product 1 (approximately 60 mg and 238 mg of botanically sourced caffeine and CAs derived from 480 mg green coffee extract) or product 2 (60 mg of synthetic US Pharmacopeia caffeine), in an 8 oz. beverage, with 5 days between test visits. Fifteen subjects completed all of the study visits, tests and procedures. A serving of Product 1 contained 103 mg 3-CQA (5-CQA IUPAC), 46.4 mg 4-CQA and 43.7 mg 5-CQA (3-CQA IUPAC). Blood samples were collected for analysis 1 hour prior to dosing and approximately 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 hours post dosing.



Levels of the CQA compounds (and their conjugates) were analyzed, and the pharmacokinetic data are shown in the table below:

Parameters	3-CQA (5-CQA IUPAC)	4-CQA	5-CQA (3-CQA IUPAC)
C _{max} (ng/mL)	11.4	6.84	7.20
Median T _{max} (hours)	1.0	1.0	1.5
AUC _{0-4h} (h·ng/mL)	27.3	16.1	15.7

The order of magnitude for the AUC and the rate of appearance after oral consumption appeared to be directly related to the dosing or concentration of each individual compound. No treatment related adverse events occurred in the study.

Farah et al. (2008) also found the “apparent bioavailability” of CAs after consumption of green coffee bean extracts to be relatively high in humans, although it is noted that the data from this study differs compared to the vast majority of other studies.³⁶¹ Ten subjects ingested 400 mg of a hydroalcoholic decaffeinated green coffee bean extract (Svetol™) containing a total of 170 mg CAs, including 45.2 mg 5-CQA (IUPAC), 36.7 mg 4-CQA, and 39.1 mg 3-CQA (IUPAC). Additional CA compounds included diCQA isomers (3,4-, 3,5-, and 4,5-diCQA at 16.3 mg), FQA isomers (3-, 4-, and 5-FQA at 22.4 mg), and other minor constituents. After ingesting the extract capsules, serum was collected hourly up to 8 hours to determine the pharmacokinetic profiles of the CA compounds and their metabolites. Considerable inter-individual variation in concentrations of the serum and urine CA compounds/metabolites was observed between the 10 subjects; the pharmacokinetic data are shown in the table below:

Parameters	3-CQA	4-CQA	5-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	Total CAs
C _{max} (μmol/L)	0.9 ± 1.4	1.4 ± 1.1	5.9 ± 4.2	1.5 ± 1.6	2.7 ± 2.7	2.5 ± 3.0	14.8 ± 11.7
Median T _{max} (hours)	4.0 ± 2.6	3.6 ± 2.2	3.3 ± 2.4	2.6 ± 1.8	3.2 ± 2.5	3.3 ± 2.5	3.1 ± 2.6
AUC _{0-4h} (h·μmol/L)	3.0 ± 4.5	4.3 ± 5.4	17.9 ± 15.3	5.0 ± 4.9	8.7 ± 8.3	6.8 ± 5.7	45.6 ± 37.1

The FQAs were not detected in the plasma of any of the subjects (which is a difference compared to other studies). Small amounts of caffeic, ferulic, isoferulic and *p*-coumaric acids were found in the plasma and were considered to have been formed from metabolism of the CAs in the lumen, mucosa and/or liver. The four major urinary phenolic compounds excreted after green coffee consumption were sinapic acid (formed from ferulic acid), gallic acid (formed from quinic acid), *p*-hydroxybenzoic (formed from gallic acid) and dihydrocafeic acids; together they represented approximately 85% of the phenolic compounds identified in the urine. The apparent bioavailability for CA compounds/metabolites (evaluated as the plasma levels of CAs and cinnamic acids divided by the CA levels consumed) varied considerably among subjects, ranging from 7.8–72.2% with an average of 33.1 ±



23.3%. Due to the variability in pharmacokinetic data between participants, the half-life of CA compounds could not be established.

A human study by Olthof (2001) in which ileostomy effluent from seven healthy patients without colons was collected and analyzed (eliminating colonic and bacterial degradation of tested compounds), found that ingested 5-CQA (IUPAC) exhibited $33 \pm 17\%$ absorption while the absorption of ingested pure caffeic acid was nearly complete at $95 \pm 4\%.$ ³⁶⁰ Only small amounts of ingested 5-CQA (trace amounts) and ingested caffeic acid (up to 11%) were excreted intact in subjects' urine. The authors concluded that while part of the CA from food will enter into the blood circulation, the majority will reach the colon and be further metabolized there.

Intestinal absorption and metabolism of 385 μmol CAs consumed in 200 mL coffee in another group of ileostomy volunteers was analyzed using HPLC-MS.³⁶⁷ Approximately 71% of CAs and their metabolites were found in the ileal effluent within 24 hours. Of the compounds recovered, 78% were the original compounds found in the coffee while 22% were metabolites (including free and sulfated caffeic and ferulic acids). Excretion of metabolites in the urine accounted for approximately 8% of the initial intake in those with an ileostomy. In contrast, excretion in the urine of volunteers with an intact colon accounted for approximately 29% of initial intake, highlighting again the importance of colonic metabolism.

Studies in rats have reported low absorption of intact CQAs from the small intestine but high absorption of CA gastrointestinal (gut flora) metabolites.^{368, 369} The results of an absorption study in which rats ingested a 5-CQA (IUPAC)-supplemented diet (0.25% by weight) indicated that 15–32% of ingested 5-CQA is hydrolyzed in the cecum, while small amounts (< 1%) were hydrolyzed in the stomach and small intestine.³⁷ The same study reported some “intact” gastric absorption of 5-CQA (IUPAC). The elimination half-life of caffeic acid, a major metabolite of CAs, after oral administration to female Sprague Dawley rats, was reported as 3.1 hours (true half-life after i.v. dosing was 1.75 hours).³⁷⁰

In summary approximately 30% of CAs and/or their metabolites are absorbed in the small intestines, while the remaining 70% are metabolized by gut microflora in the large intestines and further absorbed or eliminated in the feces. In studies on green coffee extract, CQA compounds were found to be absorbed, although inter-individual variation in pharmacokinetic values may be considerable. The CA compounds and their metabolites generally have T_{\max} values of less than 5.5. hours and apparent $t_{1/2}$ levels (following oral administration) of under 4 hours. CQAs are excreted as sulfate or glucuronide conjugates.



6.3.2 Studies on CoffeeBerry®, an Extract of Whole Coffee Fruit

A set of toxicological studies was published in 2010 by Heimbach et al., on the whole coffee fruit of *C. arabica*, including the pulp and the green coffee bean, under the trade name CoffeeBerry® (FutureCeuticals, Momence, IL).³⁷¹ Three forms were evaluated in the publication: (1) a whole powder produced by quick-drying and grinding the berries into a fine powder, (2) a water extract produced by freeze-drying an aqueous extract of the whole powder, and (3) an ethanol extract produced by freeze-drying a water-ethanol extract of the quick-dried whole powder. The studies are included here because of the content of CAs in the extracts.

6.1.2.1 Composition of CoffeeBerry®

The composition of CoffeeBerry® is shown in Table 14 below based on available data in the Heimbach et al. publication.

Table 14. Composition of CoffeeBerry® Extracts³⁷¹

Parameter	CoffeeBerry® Whole Powder	CoffeeBerry® Water Extract	CoffeeBerry® Ethanol Extract
Appearance	Tan/Brown Powder	Brown Powder	Brown Powder
Extraction Solvent	—	Water	Water: Ethanol
Solids	≥ 90%	96%	90%
Solubility in water	Partially	100%	100%
*Total Phenolic Acids (described as chlorogenic acid (CA), and caffeic, quinic and ferulic acids)	≥ 2%	5%	35–40%
Total Phenolic Acids (All CA Isomers)	n/a	n/a	45–65%**
Caffeine	0.7–1.0%	≤ 1.0%	0.6–9.08%

*Additional characterization in a subsequent paper of the CoffeeBerry® ethanol extract from FutureCeuticals was found to contain approximately 42 % CAs, with the majority as 5-CQA (IUPAC), followed by 4- and 3-CQA and other compounds.³⁷²

**Data not reported in the Heimbach et al. publication; data was provided by AFS based on composite CoffeeBerry® Forte N580 Lot #06964459, supplier FutureCeuticals® Corp, Momence, IL using ultra-high performance liquid chromatography.

n/a = data not available

Detailed compositional analysis of the CoffeeBerry® product is also presented in Mullen (2011); the authors report total CA content (expressed as 5-CQA (IUPAC) equivalents) as 42% by weight for the single-step ethanol CoffeeBerry® extract.³⁷² As the major CAs are substantially similar in both coffee and guayusa, the safety studies on CoffeeBerry® extracts (discussed below) are considered relevant to the current safety evaluation and GRAS conclusion for RUNA® Concentrate, especially with regard to the content of CAs.

6.1.2.2 CoffeeBerry® ethanol extract studies

CoffeeBerry® ethanol extract was tested for potential toxicity by the gavage route in Sprague-Dawley (Hsd:SD) rats for a period of 14 days based on OECD Guideline



407 and US FDA Redbook 2000, IV.C.3a. The overall conclusion was that the test article was well-tolerated by both male and female rats up to the highest dose tested, 4000 mg/kg bw/day; as such it was considered appropriate to use this dose in the longer 90-day repeated dose animal feeding study (described below).³⁷¹

A 90-day feeding study with the CoffeeBerry® ethanol extract was performed (note that this was the only CoffeeBerry® extract utilized in a 90-day study). The study was compliant with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM(98)17 OECD, Paris, 1998) and U.S. FDA Good Laboratory Practices (21 CFR §58, 1987). The study protocol generally followed OECD Guideline 408, EPA Guideline OPPTS 870.3100 and US FDA Redbook 2000, IV.C.4.a. Rats were housed in individual stainless-steel cages in a room set to maintain a temperature of 18–23 °C, a relative humidity of 49–57%, and a 12-h light/dark cycle. Animals were divided into one of four groups (n=10/sex) in which the test article was mixed into the feed at 0, 12,500, 25,000, or 50,000 ppm. Based on food intake values during the study, males ingested approximately 0, 846, 1723, and 3446 mg/kg bw/day of the extract, respectively, while females ingested 0, 965, 2030, and 4087 mg/kg bw/day of the extract, respectively.

Ophthalmological evaluations occurred at onset and on day 88 of the study. A functional observational battery (FOB) was performed at the end of the study. Measurements of grip strength and foot splay were taken prior to termination and means calculated. At the same time motor activity was monitored and evaluated for one hour. Blood samples were collected at termination of the study for hematology and clinical chemistry analyses. All animals were sacrificed at the end of the study and subjected to full necropsy and microscopic examination of selected tissues/organs.

Abnormal clinical signs included black ocular discharge (noted in a couple of rats from controls and treated groups of both sexes) and hyperactivity (noted in a couple of mid- and low-dose rats). The signs were considered either transient or minimal and non-adverse. No toxicological or treatment-related ophthalmological or FOB findings or effects on motor activity were observed in any of the treatment groups. Overall and weekly mean body weight and mean daily body weight gain of all treated rats were comparable with controls with the following exceptions: females showed a significant increase in body weight during weeks 4, 7, 11, and 12 (low-dose group), weeks 5 and 8 (mid-dose group), and weeks 10–12 (high-dose group); and females showed a significant change in daily body weight gain during week 1 (increased in low-dose group), overall (increased in low-dose group) and week 6 (decreased in mid-dose group). Overall and weekly feed consumption and mean daily feed efficiency of all treated rats were generally comparable to controls with the following exceptions: females showed a significant increase in feed consumption during weeks 5, 8, and 10 and overall (mid-dose group), and during weeks 4, 8, 10, 12, and 13 and overall (high-dose group) suggesting an overall dose-



response from days 0 to 91 although the effect was not considered by the authors to be adverse or toxicologically significant, with which we concur as there were no overall or dose-related effects on body weight or body weight gain. Females also showed a significant change in feed efficiency during week 1 (increased in low-dose group) and week 6 (decreased in mid-dose group).

Hematology, including coagulation, and clinical chemistry parameters showed no adverse changes. The only statistically significant changes reported were increased mean platelet concentration (mid- and high-dose males), decreased eosinophil concentration (low-dose males), decreased sorbitol dehydrogenase activity (mid-dose males), decreased alkaline phosphatase activity (high-dose males), decreased triglyceride concentration (high-dose males), increased glucose concentration (low-dose males and females), increased cholesterol concentration (high-dose females), increased sodium concentration (mid-dose females), and increased chloride concentration (mid-dose females). The findings were considered non-adverse and not related to exposure because the magnitudes of the changes were not considered clinically significant and/or the changes were not accompanied by any other correlating pathological findings.

There were no test substance-related changes in blood cell morphology, and serology showed no detectable titers against the tested pathogens and antigens. The only statistically significant change reported in urinalysis was increased urine volume in high-dose males (8.3 ± 4.8 ml) compared to controls (3.5 ± 1.5 ml), but this was not considered adverse since there were no supporting clinical chemistry or histopathology findings. Macroscopic examination revealed no gross abnormalities related to treatment with the test substance. Some incidental findings such as fluid-filled bladders (mostly males of all groups) and fluid-filled uteri (females of all groups) were reported. There were some statistically significant differences in absolute and relative (to body or brain weight) organ weights compared to controls, but none were accompanied by histopathological changes that would suggest toxicological relevance to treatment with the test substance (the authors did not report historical control values and did not comment on whether or not the weights fell within historical control ranges). The organ weight differences were a decreased relative brain weight to body weight in females (all dose groups); increased liver weight (absolute and relative to body and brain weight in high-dose females and increased relative to brain weight also in mid-dose females); increased absolute kidney weight in mid- and high-dose male and females; increased relative kidney weight (compared to body weight in high-dose males and females and compared to brain weight in mid- and high-dose males and females); increased absolute heart weight in high-dose females; and increased heart relative to brain weight in mid- and high-dose females (data tabulated in Heimbach et al., 2010; in their Table 3)³⁷¹. Again, there was no correlating histopathology noted with regard to these findings. The authors stated that "The organ weight changes in the kidneys



(dose-dependent increases in both sexes from 10–17%) were reviewed in detail by three board-certified veterinary pathologists who state that weight variations are often the most difficult anatomical changes to find microscopic correlates to since a 10–15% increase in weight/volume will translate into a 5–6% increase in a given plane, which cannot be detected by the human eye if it is evenly distributed or spread over a wide tissue area. Overall the increased absolute and relative kidney weights were considered to be of no safety concern given the lack of corresponding blood work and notable histopathology.”

Reported histopathological changes were considered incidental and related to the orbital sinus bleeds (the method by which blood samples were obtained) or related to the age and strain of the rats used in the study. These were episcleral inflammation, periocular muscle inflammation, microgranuloma involving the conjunctiva, inflammation, necrosis, hemorrhage, and fibroplasia of the Harderian gland, nephropathy, pulmonary alveolar histiocytosis, pituitary gland cyst, and ectopic thymus in thyroid gland.

In summary the highest concentration of the CoffeeBerry® ethanol extract tested of 50,000 ppm, equivalent to 3446 and 4087 mg/kg bw/day for males and females, respectively, was considered by the authors to be the NOAEL for the 90-day feeding study.³⁷¹ This is equivalent to approximately 1206 mg/kg bw/day of CAs based on the minimum concentration stated in the study for the test article.

The mutagenic potential of all three CoffeeBerry® products was evaluated in a bacterial reverse mutation assay based on OECD Guideline 471, EEC Directive 2000/32, L 136, Annex 4D, B 13/14, and EPA Health Effects Test Guidelines, OPPTS 870.5100. None exhibited mutagenic potential in the assay at concentrations ranging from 31.6–5000 µg/plate using strains TA98, TA100, TA1535, TA1537 and WP2 uvrA in the presence and absence of S9 liver microsomal fraction.³⁷¹

6.1.2.3 Summary of CoffeeBerry® studies

In summary, the CoffeeBerry® studies are considered relevant to the safety evaluation of RUNA® Concentrate due to the content of CAs. The CoffeeBerry® ethanol extract was not mutagenic in a bacterial reverse mutation assay and did not show toxicity in a 90-day feeding study up to the highest dose tested.

A margin of safety related to the exposure of CAs in RUNA® Concentrate based on the CoffeeBerry® ethanol extract 90-day study NOAEL can be calculated. The margin of safety result for the CAs is shown in Table 15 below and is greater than the usual expected margin of safety for a food ingredient of 100 (21 CFR §170.22). It should be noted that while the NOAEL of 1206 mg/kg bw/day was used for this calculation based on the minimum level of CAs stated in the publication of 35%,³⁷¹ a more detailed publication on the composition of CoffeeBerry® ethanol extract by Mullen et al. (2011) determined a content of 42% CAs.³⁷² If these higher



percentages were used to calculate the NOAEL for the CAs, they would provide even higher margins of safety.

Table 15. Margin of Safety Calculations for Chlorogenic Acids from RUNA® Concentrate based on the CoffeeBerry® Ethanol Extract 90-day Feeding Study

90 th Percentile Lifetime Exposure to CAs from RUNA® Concentrate	Exposure Margin of Safety (NOAEL/EDI) for CAs from RUNA® Concentrate (NOAEL of CAs from CoffeeBerry® = 1206 mg/kg bw/day CAs)
4.7 mg/kg bw/day (see Part 3.4.1)	257

6.3.3 Review of Toxicological Literature Chlorogenic Acids (1998)

In 1998 a review of the toxicological-related literature on CA was prepared by Tice et al., of Integrated Laboratory Systems for the National Institute of Environmental Health Sciences, and the National Toxicology Program.³⁷³ In the review CA was defined as 3-CQA (5-CQA IUPAC), although it is also mentioned that CAs can also refer to other related compounds including CQAs, FQAs and diCQA; thus the term “CA” is used in this subpart as it is in the review. Relevant literature on caffeic acid was also reviewed.

The two substances were nominated for review based on their occurrence in high concentrations in food and the apparent lack of carcinogenicity data on them. The executive summary of the review included the following pieces of information about CA/s: Hydrolyzation occurs in the stomach and intestines of rats, forming caffeic and quinic acids. Few toxic effects resulting from acute exposure have been noted. In rats, CA dosed intraperitoneally (i.p.) at 4000 mg/kg induced death in 4 out of 6 animals. I.p. doses lower than 2437 mg/kg were non-lethal. In rats, CA feeding was associated with reduced kidney and adrenal weights (1% CA in the diet for 3 weeks, with no associated histopathology findings³⁷⁴) and hyperplasia of the forestomach of 17% of animals (2% CA in the diet for 4 weeks) (it should be noted that there is no human counterpart for the rodent forestomach; hyperplasia may be due to tissue irritation and may not be relevant to humans³⁷⁵).

A developmental toxicity study in rats (5–500 mg CA/kg/day given i.p. on days 5 through 12 of gestation—CA is defined in the original study by a 2D diagram that could be either 5-CQA or 3-CQA) found that treatment did not induce maternal or fetal mortality. No CNS defects were observed. A total of 30/289 fetuses had rib defects and one failed to develop the mandible while the control group did not show such an effect (0/356). Note: the CA effect was not dose dependent in the treated



groups; the dose groups of 5–40, 60, 100 and 500 mg/kg bw/day had irregular or fused rib findings in 18, 2, 6 and 4 rats, respectively.³⁷⁶ It should also be noted that the dosing in the study was i.p. as opposed to oral; thus, the relevance of the results to oral administration are unknown as metabolism of CA by the two administration routes would be different. For this reason, the results are not considered especially relevant to dietary intake.

CA was noted to induce strand breaks in DNA in acellular test systems that favored formation of hydrogen peroxide and oxygen radicals, particularly in the presence of transition metals. However it was not mutagenic in standard bacterial mutagenicity assays (also discussed in a publication by Fung et al., 1988 on behalf of the National Cancer Institute (and reviewed by Seifried et al., 2006) and by Stich et al. of the British Columbia Cancer Research Center.³⁷⁷⁻³⁷⁹) It induced mitotic gene conversion in *Saccharomyces cerevisiae* strain D7 under conditions of alkaline pH in the absence of S9, but not in the presence of S9. CA also induced forward mutation at the tk locus in mouse lymphoma L5178Y cells in the presence of S9 (the induced mutant frequency was 8-fold higher than that of the solvent control in the assay with metabolic activation^{377, 378}). CA did not induce 8-azaguanine resistance in Chinese hamster V79 cells but was clastogenic in mammalian cells in vitro. Induction of chromosomal aberrations was seen in Chinese hamster ovary cells treated with CA in the absence of S9; addition of S9 eliminated the clastogenicity (original research by Stich et al., 1981³⁷⁹). However, importantly, CA did not induce chromosomal damage in rats in the in vivo micronucleus assay—male Sprague-Dawley rats administered two oral CA doses of 150 mg/kg 24 hours apart showed no increases in the frequencies of bone marrow micronucleated polychromatic erythrocytes. While the authors gave no overall genotoxicity conclusions, it appears that while CA has been shown to have genotoxic effects in certain in vitro assays (more often, although not always, in the absence of metabolic activation-only suggesting the effect might not be real after normal metabolism occurs), more standard bacterial mutagenicity assays and importantly an in vivo rat micronucleus assay have shown negative results.

Intravenous injection of CA did not induce allergic reactions in monkeys that were first sensitized by topical applications of sera from humans who were allergic to green coffee. In mice, topical application of CA inhibited TPA-induced edema of the ear.

A search of the National Toxicology Program website (<https://ntp.niehs.nih.gov/>, accessed June 10, 2018) provided no indication that further testing was performed on CA after this initial review of the literature, other than a *Salmonella* genotoxicity test that was reported as “negative” (no additional data was available regarding the *Salmonella* test). While reasoning for the lack of additional testing was not uncovered, it is presumed that CA was not considered a compound of any significant toxicological concern.



6.3.4 Other Studies on Chlorogenic Acids

Three-Week Feeding Study using Crystalline CA isolated from Green Coffee

In a 1975 study by Eklund et al., male Sprague-Dawley rats (3 weeks old) received casein diets supplemented with 1% (by weight) pure crystalline CA (from Sigma, presumed 5-CQA (IUPAC)) prepared from green coffee for 3 weeks (n=5) or casein diets only as a control (n=5).³⁷⁴ The average daily food intake for the treated and control groups was not reported. However, using the Lehman method to calculate mg/kg bw from percent in the diet, the estimated exposure to CA from 1% in the diet is approximately 1000 mg/kg bw/day (1 mg/kg feed = 0.1 mg/kg bw for young rats).¹³²

The animals were housed in individual metabolic cages with free access to food and water, and daily food intake and body weights were recorded. Urine was collected daily for volume and pH measurements, and feces were collected daily to measure nitrogen content. Animals were sacrificed after the treatment period. Blood was collected from the abdominal aorta at this time for analysis of serum levels of hemoglobin, hematocrit, white blood cells (WBC), and thrombocytes. The following organs were weighed: liver, spleen, kidneys, adrenals, testes, and heart. Microscopic examination of selected tissues (liver, pancreas, small and large intestines, adrenals, gonads, spleen, heart, lungs, and bone marrow) was performed.

The CA supplementation did not result in any significant differences in growth, protein intake, protein efficiency ratio, biological values, digestibility and nitrogen balance compared to control. There were no significant differences in hematology or urine volume or pH. Slightly lower ($p=0.016$) kidney and adrenal weights were reported for the treated animals with all other organ weights being comparable to that of the control. No correlating histopathology was observed in either the kidney or adrenals nor were any abnormalities seen in other tissues/organs. CA did not alter the digestive and nutritive value of the casein diet as similar fecal and urine test parameters were observed between groups. In summary, CA supplementation resulted in no abnormal or toxicological effects in Sprague-Dawley rats when consumed at 1% of the diet for 3 weeks, equivalent to approximately 1000 mg/kg bw/day.

Six-Week Feeding Study of Green Coffee Bean Extract

A 6-week feeding study by Suzuki et al. in 2002 investigated the hypotensive effect of a hot water green coffee bean extract that was subjected to ion-exchange chromatography in males of two rat strains: spontaneous hypertensive rats (SHR) and Wistar Kyoto (WKY) rats aged 7 weeks; the study also contained various toxicological endpoints.³⁸⁰ The extract was 28% CAs (no further description of CAs in the extract were given, but it is assumed that the CA profiles would be similar to that of other green coffee extracts), 6% caffeine and 50% water by weight.



SHR animals were fed moderate fat (MF) diets supplemented with 0, 0.25, 0.5, or 1% of the extract ($n=8$), and WKY rats received MF diets with 0 or 1% extract ($n=8$). Test article consumption values were not provided; however, using the Lehman method,¹³² the amount of extract consumed by animals ingesting diets supplemented at 1.0% can be estimated as approximately 500 mg/kg bw/day, equivalent to approximately 140 mg/kg bw/day green coffee CAs.

Food intake was measured daily and body weights weekly; urine and serum were collected at the end of the test period for analysis. Ingestion of the extract did not alter food intake, final body weights, urinary volume, or heart rate values for any of the treated rats compared to their respective controls. Systolic blood pressure (SBP) values were reduced in the treated SHR rats compared to the SHR control rats; SBP values for the WKY rats receiving green coffee bean extract were comparable to those of WKY control rats. The general health of the animals was not altered, and the treatments did not alter the weights of the liver, kidneys, spleen, or testes compared to controls. The extract treatment did not alter fasting cholesterol, triglyceride, sodium, potassium, or insulin levels in the SHR strain at any test concentration (additional serum parameters were tested and also were not altered by the test article); these plasma parameters were not assessed/addressed in WKY rats.

The authors additionally studied the effects of gavage dosing of pure 5-CQA (IUPAC) (0, 50, 100 or 200 mg/kg bw) in male rats. SBP was measured at 3, 6, 9, 12 and 24 hours after oral administration. The test article produced a dose-dependent hypotensive effect in the SHR strain of rats (returning to pretreatment levels 24 hours after administration), and no effect on heart rate.

In conclusion, no adverse effects related to consumption of an aqueous/ion-exchange extract of green coffee beans containing 28% CAs and 6% caffeine were noted up to the maximum dose of approximately 140 mg/kg bw/day of CAs with regard to general health, body weights, organ weights, and chemistry parameters. The extract and pure 5-CQA (IUPAC) were shown to have a hypotensive effect in the SHR (hypertensive), but not in the normotensive rat strain. This study functions as corroborative evidence of safety as relates to constituents such as CAs and caffeine.

Acute Study of Three CA Extracts from *Eupatorium adenophorum*

CAs extracted from *Eupatorium adenophorum* (Crofton weed) was tested in an acute toxicological study in mice and reported in a 2016 publication.³⁸¹ As in green coffee beans, the three main CAs in the plant are 5-CQA (IUPAC), followed by 3-CQA, and 4-CQA. Sixty ICR mice were randomly divided into three treatment groups (10/sex/group).

Three extracts with 5-CQA (IUPAC) contents of 6.11%, 22.17%, and 96.03% were given to the mice at a single dose of up to 1.5 g/kg bw (note that in the abstract of the publication, it states that the high dose was 1.5 g/kg bw; however, in several



other sections of the paper it states the high dose was 15 g/kg bw). The powdered products were dissolved in distilled water and administered to the mice via gavage. Animals were monitored for signs of toxic effects and mortality for 14 days. The mice were weighed initially and then every 7 days throughout the study. No deaths or toxic effects such as abnormal behavior were observed at any dose. Weights of mice continued to increase. No treatment-related gross pathological changes were observed in any of the organs examined (kidney, liver, lung, spleen, heart, colon and thymus; histopathology was not performed). The three different products were determined to have no toxicity up to the high dose tested.

Reproduction Studies

No oral dose reproduction studies were identified related to CAs. As described above, a developmental toxicity study in rats using i.p. dosing (5–500 mg of CA (from Sigma, presumed 5-CQA IUPAC) per kg/day on days 5 through 12 of gestation) did not result in maternal or fetal mortality, or CNS effects (non-dose dependent rib defects that were noted are described above and results are not repeated here).³⁷⁶ Reproduction/teratogenicity potential for CAs may be inferred to some degree from studies on coffee. For example, Nolen (1982) described a study in which rats were given full strength or 50% or 25% dilutions of decaffeinated brewed or instant coffees as a replacement for their drinking water for about five months from weaning, considered equivalent to human consumption of 50, 25 or 12 cups of coffee per day, respectively.³⁸² The concentration of CAs was not disclosed; thus, no specific comparisons can be made other than to assume that CAs were present at some level. None of the coffee treatments had a significant effect on reproductive parameters compared to controls, such as conception rate, number born, or number weaned. Body weights of 4-day old pups and pups at weaning were statistically significantly decreased in the full-strength coffee group. Ten days after weaning their first litters, the rats were mated a second time to the same male as before. During this second pregnancy in the study, no significant effects were noted related to early embryotoxicity measured in dams sacrificed on day 13 of pregnancy or fetal toxicity in dams sacrificed on day 21. No significant fetal abnormalities associated with coffee treatments were observed in either soft-tissue or skeletal examinations, although there was a significant increase in unossified sternebrae in the fetuses from the dams given the full-strength regular coffee. Fetal body weights were also decreased in this group, but not statistically. This result was considered by the authors to be a common finding in teratogenic studies related to a transient delay in development or a result of nonspecific stress that when seen as an isolated event is not considered to be a teratogenic response.^{383, 384} In addition, these are similar to the effects seen in both drinking water and gavage developmental toxicity studies conducted with caffeine. The unossified sternebrae were shown to be completely reversed by the time fetuses allowed to deliver were 6 days old.³⁸⁵



Therefore, since they did not occur in the decaffeinated groups, they are not likely due to the presence of CAs in these coffees.

In a population study of 7,855 live births in California, maternal decaffeinated coffee consumption showed no increased odds of small-for-gestational age birth, low birth weight, or preterm delivery compared to women who drank neither decaffeinated nor caffeinated coffee.³⁸⁶ However, while it can be assumed that there were CAs present in the decaffeinated coffee beverages consumed, there was no analysis of actual CA levels, so no specific conclusions can be made and the study merely corroborates the safety of CAs in a general sense.

Many of the reviews described in the caffeine subpart on reproductive effects (subpart 6.2.3.6) were based on coffee consumption studies and can also be generally inferred to support the safety of CAs as well. As described in that subpart, there are a number of limitations in current studies, such as problems regarding accurate caffeine consumption estimates, lack of data on early miscarriages, potential reporting bias related to smoking and importantly the lack of controlling for pregnancy signal symptoms as a major confounding factor.^{85, 86, 163, 297-299} The majority of women who decrease coffee consumption during first trimester do so because of a physical aversion to coffee that drives caffeine consumption (and thus also consumption of CAs) in this group downward (i.e., the pregnancy signal). Thus it is possible that reduction in coffee intake may be a marker of aversion and thus a healthier pregnancy; many studies have not controlled for this effect.⁸⁶

6.3.5 Human Studies

Many single dose/acute human studies of up to 500 or 1000 mg CA in various population groups have been published and have not been associated with adverse effects.³⁸⁷⁻³⁹⁴ Additionally, a number of longer clinical studies using various preparations of CAs or green coffee extracts are listed and described in more detail below.

Nine subjects completed a placebo-controlled, double-blinded cross-over intervention study using a beverage containing 0 or 600 mg CA for five days, to determine effects on energy metabolism and sleep quality.³⁹⁵ CA was associated with a shortened sleep latency, and an increase in fat oxidation and parasympathetic activity during sleep, but there was no effect on sleep architecture, sleeping energy expenditure, or overall sleep quality. No side effects or adverse events were reported.

Svetol™ is a green coffee bean alcohol-extract standardized to 45–50% CA (containing equal amounts of 3-, 4- and 5-CQA),^{87, 396} which was given to subjects in several different clinical trial designs. One was a 12-week randomized placebo-controlled study, in which 30 volunteers consumed either 11 g/day (5 cups) of an



instant coffee blend containing 200 mg Svetol™ per 2200 mg of the coffee blend, calculated to be equivalent to consumption 330–440 mg/day CAs for the placebo group versus 750–900 mg/day CAs for the test group.⁸⁷ The group consuming Svetol™ had a slight but significant decrease in weight and body fat compared to controls at the end of the study ($p < 0.05$). The test article was well tolerated (all participants completed the study according to the protocol) and none of the participants reported any treatment-related side effects. This study supports the safety of green coffee CAs at a dose of approximately 750–900 mg/day for 12 weeks.

Another Svetol™ study was a randomized placebo controlled double-blind trial with 50 participants with a body mass index of over 25 (described in two separate papers).^{397,398} In this study, subjects were given either Svetol™ (n=30, 400 mg taken in divided doses for a total of 180–200 mg CAs/day) or placebo (n=20) capsules for 60 days. After the two months of treatment, a reduction of weight and body mass index was observed in the treated group compared to controls ($p < 0.001$), while muscle mass to fat mass ratio was increased slightly. Tolerability was comparable between the groups, and no participant left the study due to side effects.

A special coffee beverage containing 435 mg CQAs per 750 mL daily serving from green coffee beans, was given in an open study to 33 individuals for four weeks, with additional four week washout periods before and after.³⁹⁹ Blood samples were taken at the beginning and end of each study phase, as were body weight/composition measurements, and intake of energy and nutrients were recorded. During the treatment phase, DNA damage as measured with a Comet assay was reduced, while glutathione and glutathione activity were increased. Body weight and energy intake were also reduced during the treatment phase. No adverse events were reported in the study.

Eighteen healthy male subjects were given a test beverage with or without 329 mg CAs (containing CQAs, FQAs and diCQAs, although specific ratios or source not presented) daily for 4 weeks in a placebo-controlled, double-blind, crossover study.⁴⁰⁰ The authors did not observe any differences in body weight, body mass index or body fat ratios between the two groups before and after intervention, although serum glucose was decreased and energy expenditure was marginally increased in the treatment group. They did not report any adverse events in the study.

Similarly, 20 healthy males with decreased vasodilation responses consumed a test drink containing a green coffee extract (28% total CAs and 6% caffeine) for four months (140 mg CAs/day).⁴⁰¹ During the study period, none of the subjects exhibited poor health or rapid weight gain/loss, or dropped out. Improved vaso-reactivity was noted in the test group. The test group also had a statistically significantly decreased homocysteine level at the end of the study (not considered an adverse effect) and there were no significant changes in insulin, blood sugar,



triacylglycerol, phospholipids, free fatty acid, total cholesterol, HDL cholesterol, and LDL cholesterol, or mineral components such as Ca, Mg, serum iron, and serum zinc. According to health care records and administered questionnaires in the study, the extract did not cause any side effects.

An investigation of a green coffee extract containing ~31% CAs given to Japanese individuals with mild hypertension found the test article to be safe at the dose level of 140 mg/day CAs.⁴⁰² Information about the type and additional composition of the extract was not provided. Participants (n=28) in this double-blind, placebo-controlled randomized clinical trial ingested either the placebo (n=14) or the test article (n=14, extract containing 140 mg CAs/day) for 14-weeks. The clinical safety with respect to side effects was judged by a physician based on a questionnaire survey of subjective symptoms (no information about the survey was provided) administered to the subjects during the run-in, treatment, and post treatment periods. There were no apparent, including serious, side effects in either group, and all subjects completed the study. With regard to clinical chemistry/hematological parameters, the ingestion of CAs did not result in any changes in the white and red blood cell counts, hemoglobin, enzyme levels, lipid profiles and sugars. The exposure also did not result in any “significant change in serum iron, magnesium, copper, zinc, or vitamin B1.”

A Japanese double-blind, randomized, controlled study evaluated 183 subjects with mild hypertension who drank one cup of coffee per day containing zero, 82 mg, 172 mg, or 299 mg of CAs (not otherwise specified) in a hydroxyhydroquinone (HHQ)-free coffee background (HHQ is generally formed via the roasting process of coffee manufacturing and is thought to potentially mitigate some of CAs’ beneficial effects) or a regular coffee control containing both HHQ (1.7 mg) and CAs (299 mg).⁴⁰³ The intervention period was four weeks, and no subjective or objective symptoms related to the treatment were reported, although a dose-related benefit was seen related to blood pressure. There were also no treatment related changes in clinical chemistry parameters measured. These results were in agreement with another study performed by the same authors using a low HHQ coffee with just one CAs group (299 mg/day in a beverage) for 12 weeks, which also showed no adverse effects.⁴⁰⁴ A similar randomized double-blind placebo controlled study of 21 Japanese individuals with mild hypertension and vascular failure found that consumption of 300 mg of CAs in a low HHQ beverage for 8 weeks was beneficial to blood pressure and oxidative stress but had no other effects on parameters such as pulse, body weight, cardiac output, or urine volume.⁴⁰⁵ No adverse events related to the test article were noted in the interviews taken by physicians, and there were no clinically relevant changes in blood chemistry or urinalysis test values.

An open study in Japan tracked almost 17,000 individuals who were given 30 free cans of a beverage containing 270 mg CAs (including CQAs and FQAs not otherwise specified) and reduced HHQ.⁴⁰⁶ The subjects checked in via a website



and reported beverage consumption as well as health parameters for 4 weeks and up to 12 weeks for some individuals. Out of the original 25,441 participants, approximately 65% completed the ad libitum consumption period, which was considered to suggest good acceptability of the beverage for everyday use.

A multicenter, randomized double-blind, placebo-controlled study assessed the effect of green coffee bean hot water-extract containing 54% CAs (not otherwise characterized) and 12% caffeine in 117 male participants with mild hypertension.⁴⁰⁷ Participants ingested 46 mg, 93 mg or 185 mg of the extract (up to ~100 mg CAs for the high dose group) daily for 28 days. No adverse effects related to treatment were observed in clinical exams (hematology and blood chemistry), physical exams, or history taking.

A 2011 systematic review and meta-analysis of green coffee extracts and weight loss in humans reported that none of the randomized controlled trials included in their analysis reported any adverse effects.⁴⁰⁸ A pilot study that was not included had two participants drop out due to adverse events associated with the intake of a green coffee extract, which included a headache and urinary tract infection. However, without a control group, it is impossible to determine if the events were random or related to treatment.

In summary, multiple green coffee bean extract human clinical studies found levels of 100–600 mg CAs taken daily for 5 days to 16 weeks (presumably in addition to background consumption of CAs from other food sources) to be well tolerated and did not cause known adverse events. One study reported safe consumption of 750–900 mg/day of CAs from green coffee (as SvetolTM) for 12 weeks. None of the studies reported any signs of abnormal or toxicologically concerning outcomes.^{87, 398, 400-402, 407, 409}

6.3.6 Chlorogenic Acids Possible Modes of Action

This dossier is related to the safety, as opposed to the efficacy, of RUNA[®] Concentrate, and neither the ingredient nor its constituents are intended to treat or prevent disease; however, exposure to CAs has been associated with beneficial effects on blood sugar and blood lipid regulation, as well as endothelial health and blood pressure, the proposed mechanisms of which could be of interest as relates to possible insights into the ingredients' safety.⁴¹⁰⁻⁴¹² A wide variety of mechanisms have been proposed and investigated to explain the various biological effects of CAs. While the mechanisms summarized below have been demonstrated to some degree, their biological significance or importance is less clear. For example in EFSA's 2011 opinion on the substantiation of health claims related to coffee and/or CAs from coffee, they concluded that cause and effect relationships have not been established between consumption of CAs in coffee maintenance of normal blood glucose concentrations, protection of DNA, lipids or proteins from oxidative



damage, or maintenance or achievement of a normal body weight in humans.⁴¹³ Loader et al. 2017 also concluded in their review that effects of CAs on blood pressure are not convincing enough to merit a Health Canada health claim.⁴¹⁴ The overall lack of clinically relevant evidence in support of important *in vivo* biological outcomes and to the nature of the mechanisms described below, suggest that these mechanisms are not expected to present significant effects, or more importantly, safety concerns. Importantly, CAs are ubiquitous in foods (especially coffee), and the intended use of RUNA® Concentrate is not expected to significantly impact exposure to CAs, as demonstrated in Part 3 of this report.

Several reviews in 2013 and 2014 address the various mechanisms by which coffee components and CAs may function with regard to an inverse relationship with type 2 diabetes mellitus.^{287, 415} The authors state that various studies show long-term and habitual use of coffee (including decaffeinated) may help maintain normal glucose tolerance and improve insulin sensitivity, although more work is required to firmly establish benefits and determine if there are any side effects. CAs' antioxidant effects appear to have a beneficial role on the inflammatory aspects of diabetes; for example, the authors explain that CAs dose-dependently inhibit activation of NF- κ B and reduce oxidative stress. The authors also noted that CAs inhibit glucose-6-phosphatase. CAs also may have some function in insulin sensitization and may increase glucose in muscle cells and have shown antidiabetic potential *in vitro* and in diabetic and obese rat models, as well as in healthy models. CAs can inhibit the activity of α -glucosidase, which also can affect post-prandial blood glucose concentrations.^{287, 415} The antioxidant and glucose modulation actions of CAs may also be hepatoprotective, by suppressing liver fibrogenesis and counteracting steatogenesis.²³⁶

CAs appear to have some degree of ability to inhibit glucose absorption in the small intestine. In one double-blinded randomized crossover study (1 week washout between experimental phases), 12 healthy adult subjects with normal weight received sugar sweetened instant coffee beverages with or without enrichment with CAs or an equal volume of sweetened water.⁸⁷ The CA-enriched beverage contained CA-rich (equal amounts of 5-, 4- and 3-CQA) green coffee bean extract Svetol™. The non-enriched instant coffee beverages were made with Nescafé® Gold Norwegian blend, both regular and decaf, both of which contain typical amounts of CAs. All beverages were sweetened with 25 g of sucrose per 400 mL water, and 10 g of each instant coffee were added for the treatment groups, resulting in the enriched beverage containing approximately 682–818 mg CAs and the non-enriched regular and decaffeinated beverages containing approximately 300–400 mg CAs (note, CA content of beverages was calculated; it was not directly reported in the study). In an oral glucose tolerance test with the study beverages serving as the glucose source, plasma glucose AUC was statistically significantly reduced (~6.9%) over 2 hours following ingestion of the CA-enriched beverage compared



to the sugar water control while there were no significant differences in AUC compared to control following ingestion of the non-enriched regular or decaffeinated beverage.

In a rat study, similar results were observed when fasted animals were pretreated with 3.5 mg/kg CA (as 5-CQA from Sigma, presumed IUPAC) 10 minutes prior to a 200 mg/kg glucose bolus.⁴¹⁶ Peak glucose levels were statistically significantly lower (21.8% at 10 minutes and 17.8% at 15 minutes) in the CA pre-treated animals compared to controls. The authors demonstrated that CA statistically significantly inhibits hepatic glucose-6-phosphatase (which is mainly located in the liver and regulates blood glucose levels by hydrolyzing glucose-6-phosphate into glucose and phosphate as the terminal step in gluconeogenesis and glycogenolysis) *in vitro* in a dose-dependent fashion. However, in an *in situ* liver perfusion experiment CA failed to inhibit glucose production by glycogenolysis or gluconeogenesis. Concentrations of CA perfused into the liver (along with Krebs-Henseleit buffer) did not differ from those flowing out via the hepatic vein suggesting that CA uptake by rat hepatocytes did not occur to any significant degree. Finally, intravenous infusion of 70 mg/kg CA had no effect on glycemic response. Thus, the authors concluded that the effects of CA pretreatment on plasma glucose were likely due to reduced intestinal absorption.

Johnston et al. performed a human study that also suggested an antagonistic effect of CA on glucose transport.³⁸⁷ Nine healthy fasted volunteers took part in a 3-way randomized, crossover study in which they consumed 25 g of glucose in 400 mL of caffeinated coffee, decaffeinated coffee, or water. The coffees contained 2.5 mmol CA. Glucose and insulin concentrations tended to be higher in the first 30 minutes after caffeinated coffee consumption than after consumption of decaffeinated coffee or the control ($P < 0.05$ for total and incremental AUC for glucose and insulin). Glucose-dependent insulinotropic polypeptide (GIP) secretion decreased with both caffeinated and decaffeinated coffee drinks (the rate of absorption of glucose determines the magnitude of the GIP response), suggesting a decreased rate of intestinal absorption of glucose. Glucagon-like peptide 1 secretion increased 0–120 minutes ($P < 0.01$) after decaffeinated coffee consumption compared with the control. While glucose and insulin profiles were consistent with the known metabolic effects of caffeine, gastrointestinal hormone profiles suggested delayed intestinal glucose absorption.

Ong et al., investigated the effect of CA in glucose transport in skeletal muscle.⁴¹⁷ CA was found to stimulate glucose transport in skeletal muscle via the activation of AMP-activated protein kinase (AMPK).

In their 1997 paper, Hemmerle et al. described that CA was a novel inhibitor of microsomal glucose-6-phosphatase and that detailed kinetic studies suggested that glucose-6-phosphate translocase was the site of inhibition.⁴¹⁸ CA and various



derivatives of CA were able to inhibit glucose-6-phosphate hydrolysis in intact rat liver microsomes. That same year, Arion et al. expanded on the mechanism by showing that while CA inhibits glucose-6-phosphate hydrolysis in intact microsomes, it is without effect in fully disrupted microsomes and it binds to T1 on the glucose-6-phosphate transporter.⁴¹⁹ The T1 binding was found to be freely reversible. In 2010, Henry-Vitrac et al. looked at effects of CAs in an in vitro structure-activity relationship study.³⁹⁶ Glucose-6-phosphate hydrolysis was measured in the presence of Svetol™ (which, as previously described above, is a green coffee bean alcohol-extract standardized to 45–50% CA (equal amounts of 3-, 4- and 5-CQA^{87, 396}) or CAs in intact human liver microsomes. Svetol™ significantly inhibited hydrolysis of glucose-6-phosphate and it was determined that CAs (CQAs and diCQAs) were the chief compounds mediating this activity. The structure-activity analysis showed that variation in the position of the caffeoyl residue is an important determinant of inhibition of glucose-6-phosphate hydrolysis.

CA may also have an inhibiting effect on complex carbohydrate-hydrolyzing enzymes, which in turn can decrease absorption of carbohydrates after food intake.⁴²⁰ For example, CA was found to inhibit α -amylase and α -glucosidase in vitro in a dose-dependent manner (2–8 μ g/mL), although the effect was less than that of caffeic acid.⁴²¹

CA also seems to have a beneficial effect on blood lipids. Mechanisms may include reducing LDL oxidation susceptibility and decreasing LDL-cholesterol and malondialdehyde levels, inhibition of fat absorption and activation of fat metabolism in the liver, reduction of hepatic triglyceride accumulation, and possibly inhibition of HMG-CoA reductase.^{415, 422}

Zheng et al., (2014) found that CA, especially in combination with caffeine, suppressed fat accumulation and body weight gain in a study of 40 mice by regulating the activities and mRNA and protein expression levels of hepatic lipid metabolism-related enzymes.⁴²³ The mice were randomly assigned to four groups and fed diets containing no CA or caffeine, CA, caffeine, or CA plus caffeine for 24 weeks. The rats fed CA plus caffeine showed a decrease in body weight and intraperitoneal adipose tissue weight, a significant decrease in serum and hepatic concentrations of total cholesterol, triacylglycerol and leptin, increased activities of carnitine acyltransferase and acyl-CoA oxidase, and decreased activity of fatty acid synthase. The mRNA and protein expression levels of AMPK, carnitine acyltransferase and acyl-CoA oxidase were up-regulated in this group as well. These authors concluded CA plus caffeine suppresses fat accumulation and body weight gain by regulating the activities and mRNA and protein expression levels of hepatic lipid metabolism-related enzymes and that these effects are stronger than those exerted by CA and caffeine individually.



Svetol™ (again, this is a green coffee extract that is rich in CA) was found to have lipolytic activity in vitro, in that it was able to liberate free fatty acids from freshly isolated human adipocytes after exposure of approximately 192 hours of incubation at mM concentrations of CA.⁴²² The results were not correlated with the caffeine content of the substance.

CAs' antioxidant effects appear to lead to a reduction in oxidative stress and improved endothelial function and nitric oxide bioavailability in the arterial vasculature, and may lead to the beneficial effects on blood pressure that have been observed.^{412, 414, 424-431} While endothelial benefits from acute consumption of CAs have been shown (within a few hours), effects from more chronic consumption in humans are less clear.^{430, 432} Additionally, when CAs are consumed with caffeine such as in a cup of coffee, the acute short term beneficial effects on endothelial function, such as those measured via flow-mediated dilation, may be modified, with some confusion in the literature as to whether caffeine has an acute short-term beneficial or detrimental effect.⁴³³⁻⁴³⁷ Acute effects on left ventricular polarization do not appear to occur with either caffeine or CAs.⁴³⁸ Hydroxyhydroquinones, a byproduct of coffee roasting, may also mitigate beneficial endothelial effects from ingestion of CAs.⁴³⁹ A metabolite of CAs (and also a metabolite from foods rich in ferulic acid such as wholegrain cereals), ferulic acid-4-O-sulfate, has been shown to have specific vasorelaxant activity.⁴⁴⁰

Fuentes et al. (2014) also reviewed the effects of CA (presumably 5-CQA IUPAC) on endothelial function and stated that CA attenuates oxidative stress that leads to the beneficial reduction of blood pressure through improved endothelial function and nitric oxide bioavailability in the arterial vasculature.⁴²⁴ They stated that mechanistically, in endothelial cells CA can inhibit of monocyte-like adhesion, adhesion molecule expression (VCAM-1, ICAM-1 and E-selectin), NF-κB translocation and reactive oxygen species production. They also suggested that CA may inhibit hydrogen peroxide-induced dysfunction and apoptosis in endothelial cells, which may be related to its effects on suppressing oxidative stress and upregulating the endothelial nitric oxide synthase pathway. Lastly, they reviewed that CA significantly reduced apoptosis by up-regulation of expression of the Bcl-2 gene and down-regulation of Bax gene expression.

Several reviews on the effects of CAs on blood pressure have been published. The most recent is by Loader et al (2017).⁴¹⁴ The authors located four animal studies that all found CAs to significantly reduce systolic blood pressure in spontaneously hypertensive rats when given at single or longer-term doses (8 weeks). The acute effect appeared to be dose-dependent (for 5-CQA IUPAC) with maximal effects observed at 300 mg/kg bw. The authors suggested that CA or its metabolites might act to scavenge reactive oxygen species, which improves nitric oxide availability and endothelial function, attenuating blood pressure. Eight human studies related to CAs and blood pressure met the authors' inclusion criteria, and compared with



control groups, CA supplementation showed significant reductions in systolic blood pressure in three studies and in diastolic blood pressure in two studies. No reductions were seen in the remaining studies. The authors summarized that the effects of CAs on blood pressure reduction were not likely to be large enough to infer long-term benefits, and no clear dose-response effects were observed nor was an effective dose established.

Onakpoya et al. (2015) performed a systematic review and meta-analysis of randomized clinical trials on the effects of CAs on blood pressure.⁴²⁵ They identified five studies (including 364 participants) and also found that supplementation with CAs results in a statistically significant reduction in systolic blood pressure and small reductions in diastolic blood pressure. The effect sizes were moderate, and the clinical relevance was stated as “modest at best”. They also stated that results should be interpreted with caution because of moderate-to-large statistical heterogeneity in the analysis, small sample sizes, and variations in study designs.

Zhao et al. also reviewed the scientific evidence related to CAs’ impact on blood pressure in 2012.⁴²⁶ They similarly summarized that basic and clinical investigations imply that the consumption of CAs can have an anti-hypertensive effect. They stated that the metabolites of CAs attenuate oxidative stress, leading to blood-pressure reduction through improved endothelial function and nitric oxide bioavailability in the arterial vasculature.

It should be noted that the studies discussed on specific health effects or general lack of adverse effects in the caffeine safety subpart above were often based on associations with coffee intake. The results of such studies are hence often also relevant to intake of CAs, and they generally show a lack of adverse effects.

In summary, while various mechanisms have been investigated with regard to the effects of CAs on various health parameters, the relevance of their overall effects do not appear to be clinically significant or suggestive of safety concerns. Importantly, CAs are ubiquitous in the diet, and again, as shown in Part 3, exposure estimates suggest that RUNA® Concentrate’s use in energy drinks will not significantly increase consumption of CAs as compared to background consumption of coffee in the population.

6.3.7 CA Studies in Combination with Toxins/Toxicants

While not necessarily directly relevant to safety of CAs, a number of publications have shown protective effects of CAs in the context of various toxins/toxicants, with the mechanism mainly attributed to its antioxidant properties. Recent examples include reduced toxicity-induced injuries in animal models and/or cell cultures related to the liver,⁴⁴¹⁻⁴⁴⁵ ischemia and reperfusion,⁴⁴⁶ neuronal toxicity and cell death,⁴⁴⁷ endothelial dysfunction,⁴⁴⁸ and myocardial infarctions.⁴⁴⁹



Additionally, CAs significantly reduced the frequencies of micronucleated polychromatic erythrocytes induced by whole body exposure to λ -radiation,⁴⁵⁰ inhibited duck hepatitis B virus when given to ducklings orally at a dose of 100 mg/kg bw/day, twice per day,⁴⁵¹ and has shown chemoprotective potential in rats and hamsters.⁴⁵²⁻⁴⁵⁵ Of note, no adverse effects were observed in the various studies at doses equivalent to up to approximately 6.5 g for a human adult.⁴⁴²

6.3.8 Effects of CA on Mineral and Thiamine Absorption

It has been proposed that dietary phenolic compounds in general possess the ability to hinder the absorption of non-heme dietary iron due to luminal complex formation within the gastrointestinal tract. In an early study, iron absorption was determined in 125 healthy adults following ingestion of a control meal to which 3.8 mg of double radio-labeled (⁵⁵Fe and ⁵⁹Fe) iron sulfate was added or the same meal to which known equimolar quantities of pure phenolic compounds (30.5 mg CA presumed to be 5-CQA (IUPAC)) was added.⁴⁵⁶ The effects of oregano, spinach, coffee, and tea (foodstuffs containing phenolic compounds) were also investigated. A 10 mL 0.01 M HCl solution containing 3 mg iron sulfate and 20 mg ascorbic acid was used as a reference standard and administered orally on two consecutive days. Blood samples were tested for erythrocyte ⁵⁵Fe and ⁵⁹Fe content and relative absorption was calculated. A statistically significant, decrease (33% relative to reference standard; p<0.001) in iron absorption was observed in the CA experiment.

Broadly speaking, there was equal inhibition of iron absorption by tannic acid, gallic acid, and oregano when considered in terms of galloyl groups per unit weight suggesting direct complex formation between iron and galloyl groups. CA and catechin do not contain galloyl groups, but instead contain catechol groups, and the degree of inhibition of iron absorption by CA was statistically significantly lower compared to gallic acid (p=0.005). The study concluded that galloyl groups strongly interfere with iron absorption and are the major contributor to this observation with respect to phenolic compounds, while the influence of catechol groups was smaller and of only minor importance. In fact, inhibition by the CA (33%) was not only less than pure gallic acid (52%) but was also generally less than the phenolic containing foodstuffs tested (relative decrease 38, 61, 68, and 69% for spinach, coffee, tea, and oregano, respectively). Of note, approximately 75% of the inhibition due to coffee was attributed to galloyl groups with the remaining 25% attributed to its CA and phytate content.

In a study in anemic rats using a closed cavity intestinal loop administration procedure, CA statistically significantly, dose-dependently (up to a plateau at 1.7 mM) inhibited non-heme iron (⁵⁹Fe radio-labeled iron citrate) absorption and subsequent tissue distribution.⁴⁵⁷ At the low dose of 0.28 mM, inhibition of iron absorption was delayed, not being observed until 120 minutes post-treatment. The



authors hypothesized that inhibition was due to an inhibitory effect of CA in brush border iron transporters.

Hurrell et al., investigated the effects of various polyphenol containing beverages, including instant coffee (the only high CA beverage tested), on iron absorption from an iron fortified bread roll in healthy human adults.⁴⁵⁸ The authors reported that all tested beverages statistically significantly inhibited iron absorption and that the inhibition was dose-related based on total polyphenol content, regardless of the specific compounds present. Addition of milk to coffee had no effect on iron absorption. The authors also reported that coffee and tea consumption in the U.S. does not contribute to the prevalence of anemia according NHANES II data, suggesting that their results could not easily be extrapolated to a population consuming a varied diet of complex composite meals.

The effect of polyphenols from potatoes on iron absorption (assessed as ferritin synthesis) was investigated.⁴⁵⁹ Potatoes were subjected to simulated in vitro digestion, and 5-CQA (IUPAC) was the major phenolic compound released in the digestive filtrate, followed by 3-CQA (IUPAC) and 4-CQA. Caco-2 cells (a commonly used human colon carcinoma cell line that undergoes differentiation and polarization, and acquires characteristics of mature enterocytes) were then incubated with the various filtrates and CA was the main polyphenol taken up by the cells, although at low levels. Next Caco-2 cells were incubated with ferric chloride and ascorbate, to induce ferritin synthesis, with or without treatment with the potato filtrates (diluted to 10, 20 and 25% of the final concentration); untreated ferritin synthesis-induced cells served as controls, and experiments were also set up using digestive enzymes in order to discriminate between the effects of polyphenols and enzymes present. A concentration dependent statistically significant reduction in ferritin levels was observed for all treatment conditions (enzymes alone and all potatoes) compared to the controls at the mid- and high-concentrations; no significant differences were observed with any treatment at the low concentration. However, the effect of enzymes alone was moderate and treatment with each of the three potato infiltrates was statistically significant compared to digestive enzymes alone.

The effect of dietary CA (as CQA (most likely 5-CQA, IUPAC), at a dose equivalent to 4 g in a 65 kg human) on absorption of dietary zinc and copper has also been investigated in rats.⁴⁶⁰ The absorption of zinc (⁶⁷Zn) was statistically significantly reduced compared to controls in rats fed CA (5.4% and 25% absolute and relative reductions, respectively, compared to controls) or caffeic acid (5.9% and 27% absolute and relative reductions, respectively, compared to controls); however, no differences in copper (⁶⁵Cu) absorption were observed.

The 1999 World Health Organization (WHO) report on thiamin deficiency stated that polyphenols, such as caffeic acid, CA, and tannic acid, are thiamin antagonists



that interfere with thiamin absorption by forming non-absorbable thiamin disulfide.⁴⁶¹ Symogyi and Bönicke investigated the anti-thiamin activity of phenolic compounds in general and concluded that it was related to the number and positions of hydroxyl groups on phenol derivatives.⁴⁶² Simple phenol derivatives with varying numbers and positions of hydroxyl groups were investigated. Phenol, which has a single hydroxyl group, did not inactivate thiamin nor did resorcinol with two hydroxyl groups in meta-position. In contrast, catechol with two hydroxyl groups in the ortho-position exhibited high anti-thiamin activity (similar to that of caffeic acid) while hydroquinone with two hydroxyl groups in para-position exhibited medium anti-thiamin activity. The presence of a third hydroxyl group in meta- or para-position when the other two hydroxyl groups were in ortho-position significantly attenuated anti-thiamin activity. Cinnamic acid (no hydroxyl groups and an aliphatic side chain) and cinnamic acid derivatives with zero or one hydroxyl group did not possess anti-thiamin activity. 5-CQA (IUPAC) consistent with its caffeic acid moiety's ortho-hydroxyl groups, also exhibited high anti-thiamin activity. Thus, to the extent the number and positions of hydroxyl groups on the hydroxycinnamic acid moiety of CA can be relied on to predict potential for anti-thiamin activity, the caffeoylquinic and dicaffeoylquinic acids can be predicted to exhibit anti-thiamin potential while the feruloylquinic and *p*-coumaroylquinic acids can be predicted to be devoid of anti-thiamin activity.

The mechanism of thiamin inactivation by caffeic acid has been investigated and determined to be a two-phase reaction characterized by a very rapid, reversible ring opening to yield a thiamin sulphydryl derivative followed by a slower, oxygen, temperature, and pH dependent, irreversible oxidation resulting in thiamin disulfide, an inactive form, and reactivation of caffeic acid resulting in a cyclic thiamin inactivation reaction.^{463, 464} Phase two of the above reaction depends on redox cycling of the phenolic derived benzoquinone, which explains the observations of Symogyi and Bönicke given that meta-substituted diphenols are poor oxidizing agents due to the inability to form a meta-benzoquinone.

In order to understand whether these in vitro results are important in vivo, Somogyi and Nägeli investigated the anti-thiamin effects of roasted coffee (12–14% CA (dry weight) and 0.2% CA (as consumed in the coffee)) in human adults.⁴⁶⁵ Following a standardized breakfast, one liter of the prepared coffee was consumed in seven portions over three hours and each subject served as their own control in a crossover design employing water as the control following an eight-day washout. Urinary thiamin excretion over 8 to 10 hours was measured in serial collections at predetermined time intervals (as well as blood thiamin in some subjects). There was a small decrease in blood thiamin six hours following coffee consumption compared to no change following water ingestion. Urinary thiamin excretion was decreased by an average of 45.5% following coffee consumption compared to water (although in two of 15 subjects, the inverse effect was observed). While the authors were



unable to explain the inverse results, they noted that analytical error could not be excluded. Similar results (average decrease in urinary thiamin excretion of 35.8%) were obtained in second experiment using a simplified procedure with a single urine collection, two hours following the last dose of coffee or water. In this experiment, no inverse effects were observed. In another study, the authors repeated the experiment using coffee and decaffeinated coffee with water as the control, as well as including a 10 mg dose of thiamin 1 hour before beverage consumption.⁴⁶⁶ Both decaffeinated and regular coffee decreased thiamin excretion compared to water, and in most subjects thiamin excretion was lower following decaffeinated coffee compared to regular coffee suggesting that caffeine does not contribute to the anti-thiamin activity of coffee and may attenuate it to some degree.

In summary, there is some evidence that CA is able to decrease absorption of iron and zinc and may possess some anti-thiamin activity; conversely it can also prevent iron induced hydroxyl radical formation.^{456-460, 467} While iron deficiency is reasonably common in the population, it is well accepted that iron is best absorbed as "heme iron" (e.g., that found in meat) due to the fact that non-heme iron absorption can be reduced by phytates, tannates from tea, polyphenols, and bran (and as shown here, CA) for example.⁴⁶⁸ Similarly, while diets high in fiber and phytates are known to reduce zinc absorption, zinc deficiencies are uncommon in healthy individuals.⁴⁶⁹ Thiamin deficiency is also most commonly found in individuals who are alcoholics or those who subsist on highly refined carbohydrates, and we were unable to find associations in the literature with coffee consumption or CA intake.

CAs are ubiquitous in foods in the U.S. diet, including fruits, vegetables, grains and more. The intended uses of RUNA® Concentrate are not expected to substantially increase consumption of CAs at the 90th percentile compared to consumption by coffee drinkers as shown in Part 3, which, as stated by Hurrell et al.,⁴⁵⁸ is not associated with prevalence of anemia according to NHANES II data. Thus, consumption of RUNA® Concentrate under the conditions of its intended use is not expected to negatively affect absorption values of iron and zinc or produce a clinically relevant anti-thiamin effect in the general population.

6.3.9 Summary and Conclusions Regarding Safety of Chlorogenic Acids

CAs, found in numerous foods, especially coffee beans with the same major CA compounds as are found in guayusa, are rapidly absorbed, metabolized and eliminated from the body. A 90-day oral toxicity study on CoffeeBerry® ethanol extract suggests that CAs from green coffee beans are safe with a NOAEL of 1206 mg CAs/kg bw/day (the highest dose tested in the study) that allows for a margin of safety of greater than 100 with regard to exposure to CAs from RUNA® Concentrate. Corroborative animal studies support the safety of the major classes of



green coffee CAs, and human studies show a lack of adverse outcomes from consuming CAs from green coffee extracts—one human study showed a lack of adverse events after consumption of a beverage containing 750–900 mg/day of CAs from green coffee extracts for 12 weeks.

6.4 Catechin Safety

Catechins are a form of flavan-3-ols (also called flavanols, a subcategory of flavonoids) and include compounds such as ECG, EGCG, EGC and more,³⁰ and are present in foods such as tea, chocolate, grapes and red wine.⁴⁷⁰ RUNA® Concentrate is approximately 0.36% catechins, and the estimated 90th percentile lifetime exposure based on use in energy drinks is 26.3 mg/day (0.32 mg/kg bw/day), as discussed in Part 3.

6.4.1 Absorption and Metabolism of Catechins

C_{max} values for flavan-3-ols range from 25 to 126 nM and t_{max} values from 1.6 to 2.3 hours, which reflect absorption in the small intestine.^{30, 40, 471} Appearance of unmetabolized flavonoids in the blood is unusual and studies show that catechins and epicatechins are absorbed and excreted to a greater extent compared to other flavonoids,³⁰ although absorbed levels are relatively low compared to intake.⁵⁶

Del Rio et al. (2010) determined that catechins are indeed absorbed in the small intestine and appear in the circulatory system predominantly as glucuronide, sulfate and methylated metabolites via liver metabolism.⁴⁷² The colonic microflora also metabolize catechins, which makes up the majority of the urinary catabolites found after consumption of green tea flavan-3-ols. Bioavailability of catechins was approximately 39%, and a great variability in urinary excretion of colonic metabolites among participants was considered likely related to differences in colonic microflora profiles.

Of the individual catechin species, absorption of non-gallated catechins (EC and EGC) appears to be more efficient than the gallated catechins (EGCG and ECG).^{43, 56} Data suggests that catechins are rapidly excreted and tissue sequestration is minimal.^{30, 470} Peak plasma levels of catechins and their intestinal and hepatic metabolites typically return to baseline levels within 24 hours of initial consumption.⁴³

6.4.2 Toxicity Studies and Reviews on Catechins

While catechins are generally considered antioxidant and protective for various health states, there have been some toxicological concerns related to liver toxicity when taken at higher doses, especially with regard to EGCG. The concentration of



catechins in RUNA® Concentrate is fairly low, at 0.36%, and high level (90th percentile) consumers are only expected to consume 26.3 mg/day (0.32 mg/kg bw/day) catechins from its intended use. While RUNA® Concentrate does contain EGCG, it is the lowest of the catechins present that were measured, and the total catechin levels per serving are low compared to that found in green/black tea.

In 2017, Dekant et al. published a review of the safety of catechins to determine a safe level for use as a dietary supplement,⁴⁷³ and in 2018 EFSA published a scientific opinion on the safety of green tea catechins.⁴⁷⁴ Both concluded that consumption of EGCG from tea infusions or tea extract-based beverages are generally not considered a safety concern. EGCG has been suspected of being responsible for liver toxicity reported in humans who consumed it as a dietary supplement. EFSA estimated mean daily intake of EGCG from green tea infusions to be 90–300 mg/day in the EU adult population, while high level exposure was up to 866 mg/day EGCG.⁴⁷⁴ While green tea infusions have sometimes been associated with liver damage in predisposed humans, such events have not been observed in controlled studies and may be due to the presence of confounders.⁴⁷³ In animal studies, EGCG's potency for liver effects is highly dependent on conditions of administration (NOAELs are significantly lower in fasted animals or after bolus dose administered via gavage), thus feeding or divided dose studies are considered better NOAELs for human risk characterization.

EFSA concluded that from clinical studies reviewed, there was no evidence of hepatotoxicity below 800 mg EGCG/day up to 12 months, although there was an association with a product containing 80% ethanolic extract of EGCG at a dose of 375 mg/day.⁴⁷⁴ Dekant et al. concluded a tolerable upper intake level of 300 mg EGCG/day for food supplements, which gives a two-fold safety margin to clinical studies that did not report liver effects, and a margin of safety of greater than 100 related to the NOAELs in animal studies that used dietary administration of green tea catechins.⁴⁷³ The two study NOAELs specifically used were from a 90-day study in “fed” dogs, which resulted in a NOAEL of 460 mg EGCG/kg bw/day, and a two-year rat feeding study using a green tea extract, which resulted in a NOAEL of 551.9 mg EGCG/kg bw/day. Importantly, again the authors noted that with regard to green tea infusions and green tea extract-based beverages, adverse events have not been reported and such products are considered safe to consume in the range of historical safe use.

Chengelis et al. evaluated the potential adverse effects, if any, of two standardized green tea catechin preparations: one that underwent heat sterilization (GTC-H; 33% total catechins w/w) and one that was not heat-sterilized (GTC-UH; 63.7% total catechins w/w).⁴⁷⁵ A decaffeinated preparation of the GTC-H (GTC-HDC; 31.4% total catechins w/w) was also evaluated to ascertain if any effects were due to caffeine. The preparations were administered to rats once daily at levels up to 2000 mg/kg/day for 28 days. There were no deaths attributable to the GTC preparations.



The clinical condition of the animals, functional observational battery, motor activity, clinical pathology evaluations, organ weights, and gross necropsy findings were unaffected by any of the preparations. GTC-HDC or GTC-UH dosing had no effects on body weights or microscopic findings, whereas lower body weights and food consumption were observed in the 1000 and 2000 mg/kg/day GTC-H group males. The NOAEL for localized gastric effects for GTC-H was 1000 mg/kg/day. No other target organs were identified. Thus, the NOAEL for systemic toxicity following oral administration was 2000 mg/kg/day for GTC-H, GTC-HDC, and GTC-UH under the conditions of this study.

Isbrucker et al. published three safety studies on Teavigo, which is a high (~90%) concentration EGCG extract of *C. sinensis* leaves. With regard to genotoxicity, the extract was tested in *Salmonella* and L5178Y tk mouse lymphoma cell assays. No mutagenic activity was detected in the bacterial system. A statistically significant increase in mutation frequency was noted in the mouse lymphoma assay (as has been described previously)⁴⁷⁶ in the presence of S9; the findings were considered an extension of peroxide formation under the reducing conditions of the media which was also evaluated in the present study. Oral administration of 500, 1000, or 2000 mg EGCG/kg to mice did not induce micronuclei formation in bone marrow cells, and administration of 400, 800, or 1200 mg EGCG/kg/day in their diet for 10 days did not induce bone marrow cell micronuclei and produced plasma EGCG concentrations comparable to those reported in human studies. Intravenous injection of 10, 25 and 50 mg EGCG/kg/day to rats resulted in much higher plasma concentrations and demonstrated an absence of genotoxic effects. It was concluded that Teavigo (EGCG) was not genotoxic.⁴⁷⁷

An oral acute dose of Teavigo delivering 2000 mg EGCG/kg was lethal to rats; whereas, a dose of 200 mg EGCG/kg induced no observed toxicity. The dietary administration of EGCG preparation to rats for 13 weeks was not toxic at doses up to 500 mg/kg/day. Similarly, no adverse effects were noted when 500 mg EGCG preparation/kg/day was administered to pre-fed dogs in divided doses. This dose caused morbidity when administered to fasted dogs as a single bolus dose, although this model was considered an unrealistic comparison to the human condition. From these studies a NOAEL of 500 mg Teavigo/kg/day was established.⁴⁷⁸

Teavigo preparations were administered to pregnant rats during organogenesis and development. In an initial preliminary study using subcutaneous and gavage routes, there was no evidence of any direct embryo-fetal toxicity, although some maternal toxicity was seen. In the main teratogenicity study, feeding pregnant rats diets supplemented at 1400, 4200 or 14,000 ppm during organogenesis was non-toxic to dams or fetuses. A two-generation study in rats fed 1200, 3600 or 12,000 ppm EGCG preparation showed no adverse effects on reproduction or fertility. The highest dose reduced the growth rate of offspring, and there was a slight increase in pup loss. A growth effect among pups was also seen at 3600 ppm in the second



generation. The lowest dose (1200 ppm) was considered the overall NOAEL. As dams consumed twice the amount of feed during the crucial lactation period, the NOAEL was equivalent to 200 mg/kg/day Teavino.⁴⁷⁹ Again, exposure to *total catechins* at the 90th percentile from RUNA® Concentrate is estimated to be low at 0.32 mg/kg bw/day.

6.5 Safety of Theobromine

The concentration of theobromine in RUNA® Concentrate is very small compared to that found in other foods in the U.S. diet (such as chocolate), as previously discussed. There is no specification for theobromine in the extract; however, analysis suggests it contains approximately 0.03%, and in Part 3, 90th percentile lifetime exposure from RUNA® Concentrate was calculated to be approximately 2.2 mg per day (0.03 mg/kg bw/day). A bar of dark chocolate can contain 450–1394 mg theobromine.⁵⁷ Because of this low level, only a brief safety review of this compound is provided.

Theobromine is an alkaloid that is structurally related to caffeine, and like caffeine, it can block adenosine receptors and inhibit phosphodiesterase.⁴⁸⁰ Its serum half-life in humans ranges from 6.1 to 10 hours, and up to 18% is excreted unchanged in the urine (major metabolites are 7-methylxanthine and 3-methylxanthine).^{115, 480}

IARC published a monograph on theobromine in its 51st volume in 1991.¹¹⁵ The authors discussed that theobromine is readily absorbed in humans from food and evenly distributed in body fluids. In “large doses” (e.g., 0.8–1.5 g from cocoa) they stated theobromine can cause sweating, trembling and headaches. However, ingestion of theobromine from chocolate at a dose of 6 mg/kg bw/day, for example, has shown no effect on clinical parameters in humans.

With regard to animal studies, IARC found that very high doses of theobromine (250–300 mg/kg bw) cause thymic atrophy in male and female rats. The effect was seen only at much higher levels in mice (850 mg/kg bw/day) and hamsters (1840–1880 mg/kg bw/day). A study in male dogs found that 100–150 mg/kg bw/day for 21–28 days and various doses over one year reported a degenerative and fibrotic lesion in the right atrial appendage of the heart, however the finding was considered unique to the dog since no such appendage exists in humans.

The IARC authors reviewed that while testicular atrophy, aspermatogenesis and related findings have been shown in rats given theobromine, the effects were not seen in dogs given 25, 50, 100 or 150 mg/kg bw/day for over a year. Additionally, rats fed cocoa powder containing theobromine (~2.5%) and caffeine (~0.19%) at concentrations of 0, 1.5, 3.5 and 5.0% for three generations (males and females were given the powder for 12 and 2 weeks, respectively, prior to mating, with a total methylxanthine dose of up to 126 mg/kg bw/day) showed no consistent dose-related



effect in any reproductive index. While teratogenic effects were observed in rabbits after given theobromine by gavage, they were not seen after dietary administration. No teratogenic effects from theobromine were seen in rats.

Overall IARC found no relevant carcinogenicity data available in the literature. After reviewing relevant genotoxicity and other data, the panel found that there is inadequate evidence for carcinogenicity in humans from theobromine, and it is not classifiable as to its carcinogenicity to humans. They designated it a Group 3 compound (the same designation as was given to coffee and caffeine as discussed above). The low level of exposure that is expected from RUNA® Concentrate (90th percentile lifetime exposure calculated to be approximately 2.2 mg per day or 0.03 mg/kg bw/day), is not expected to be of any safety concern and will likely not affect dietary background exposure levels.

6.6 Safety of Isoflavones

RUNA® Concentrate does not have a specification for isoflavones, although analysis showed that the liquid extract consists of approximately 0.08% of these compounds. In Part 3, 90th percentile lifetime exposure from RUNA® Concentrate was calculated to be approximately 5.8 mg per day (0.07 mg/kg bw/day).

Consumption of isoflavones has been associated with a number of health benefits that are hypothesized to result from the phytoestrogenic and antioxidant nature of these compounds.⁴⁸¹ Isoflavones have been consumed by humans as part of soy-based diets for many years (the 75th percentile of intake has been reported to be as high as 65 mg/day in some Asian populations) without evidence of adverse effects.¹¹⁶

EFSA's 2015 risk assessment on isoflavone intake in dietary supplement form by peri- and post-menopausal women with regard to harmful effects on the mammary glands, uterus and thyroid, stated that supplements generally provide 35–150 mg/day of isoflavones.⁵³ While isoflavones can possess estrogenic properties based on their structure, EFSA found that human data did not support the hypothesis of an increased risk of breast cancer from observational studies, nor of an effect on mammographic density nor on the proliferation marker Ki-67 expression in interventional studies. No effect was found on endometrial thickness or histopathological changes in the uterus after up to 30 months of supplementation with 150 mg/day of soy isoflavones. After 60 months some non-malignant histopathological changes were reported. Thyroid hormones levels were also not changed following intake of isoflavones from food supplements.

Beaton et al. (2010) found that a high soy protein isolate containing 61.7 ± 7.35 mg isoflavones/day, expressed as aglycone equivalents) for 57 days did not affect various semen parameters or quality in healthy adult men.⁴⁸² A meta-analysis of



human studies in 2009 also showed that soy protein and isoflavone intake had no significant effects on serum testosterone, sex hormone-binding globulin or free androgen index in men.⁴⁸³ Isoflavone intake appears to significantly inhibit bone resorption and stimulates bone formation, especially in post-menopausal women, showing a beneficial effect on bone health.^{116, 484}

Soy intake has been associated with a reduced risk of breast, prostate, and endometrial cancers, as well as cardiovascular disease in some studies.¹¹⁶ Some concern regarding isoflavone consumption has been hypothesized in breast cancer survivors (especially those with estrogen receptor-positive cancers); however, there is not enough evidence to discourage breast cancer survivors from consuming soy foods in moderation, according to a review by the Linus Pauling Institute.¹¹⁶ Soy protein based infant formulas have been available since the 1960s, and contain up to 9.4 mg total isoflavones per 8 fl. oz. serving; no clinical concerns regarding nutritional adequacy, sexual development, thyroid disease, immune function, and neurodevelopment have been found in infants that consume these products according to reviews.¹¹⁶

The low level of exposure that is expected from RUNA® Concentrate (90th percentile lifetime exposure was calculated to be approximately 5.8 mg per day or 0.07 mg/kg bw/day), is not expected to be of any safety concern and will likely not significantly affect dietary background exposure levels.

6.7 Safety of Other Components of Guayusa Leaf Aqueous Extract

As mentioned previously and shown in Table 5, other constituents of RUNA® Concentrate include moisture, minerals, protein, sugars, fat, and fiber. These substances are so ubiquitous in foods, and consumption of them from this ingredient is not expected to impact the levels already consumed from the background diet. The body is expected to act upon these common constituents through physiological processes of digestion and absorption, distribution, metabolism and excretion that are utilized for these food constituents when obtained ubiquitously from a wide variety of other foods in the human diet.

6.8 Safety of *Ilex paraguariensis* (Yerba Maté)

6.8.1 Comparison of *Ilex paraguariensis* and *Ilex guayusa* constituents

I. guayusa is closely related to *I. paraguariensis*—both are evergreen shrubs/trees belonging to the *Aquifoliaceae* family; the leaves of the latter are used for the drink yerba maté, or maté. While *I. guayusa* is found in the upper Amazon basin of



Colombia, Ecuador, Peru and Venezuela,² *I. paraguariensis* is more often found in the Southern region of South America, in Argentina, Brazil, Paraguay and Uruguay.^{485, 486} Like *I. guayusa*, infusions or decoctions of the aerial parts of *I. paraguariensis* have long been regularly consumed as caffeinated beverages as yerba maté, which is appreciated for its unique bitter taste and stimulant (caffeine) properties.^{47, 286, 485, 486} South American indigenous people have consumed yerba maté for centuries, and the plant has made its way into beers, candy and other non-traditional products in the recent past.⁴⁸⁶ It has been estimated that the per capita consumption of maté in Uruguay is 6–8 kg/year (roughly 200–300 mg/kg bw/day),⁴⁸⁶ and the plant and maté beverage have penetrated the U.S. and European markets as well.^{47, 54, 486}

Heck et al. conducted a scientific review of yerba maté (*I. paraguariensis*) in 2007.⁴⁷ Similar to guayusa leaves, maté leaves contain CAs and caffeine, along with small amounts of theobromine and theophylline. The average concentration of caffeine in maté beverage has been estimated at approximately 78 mg per cup.⁴⁷

While some observational studies have linked maté consumption with an increased risk of developing cancer (especially esophageal, oral, lung, bladder, renal, and other cancers of the head and neck), it is also well recognized that other factors may play a role in this correlation, such as smoking and alcohol consumption, which are strongly associated with the heavy maté-consuming culture of the regions where these associations have been made.^{47, 486–488} Polycyclic aromatic hydrocarbons have also been found in maté of those regions, likely because it is often prepared by drying the leaves over smoky wood fires. The association may also be related to the temperature of the infusion when it is consumed, affecting the oral tissue, rather than a particular carcinogenic constituent.^{486–489} In fact, IARC published a 2016 review of maté and modified its classification to a Group 3 (not classifiable as to its carcinogenicity to humans) as long as it is consumed while “not very hot”.¹⁷⁸ Maté was previously classified as a Group 2A (probably carcinogenic to humans), but the association has now been found to be related to temperature of the consumed beverage and not maté itself.¹⁷⁸ *I. paraguariensis* has been shown in many studies to have potent antioxidant activity, and in vitro and animal studies have shown a protective effect of maté against cancer.^{47, 486}

To compare the composition of guayusa and maté infusions, samples of five lots of dried and milled leaves of each plant were analyzed by an independent laboratory by RUNA (now All Market Inc.) for nutrient content and characteristic plant metabolites. The infusions were prepared under identical conditions: by boiling 3 g of dried and milled leaves in 227 mL of water at 80°C for 10 minutes (representing the manner in which tea would be brewed at home).

**Table 16:** Comparison of constituents of *Ilex guayusa* and *Ilex paraguariensis* infusions

	Caffeine content of dried leaves ¹ (g/100g)	Caffeine content of infusion ² (mg/100mL)	Theobromine content of infusion ³ (mg/100 mL)	Chlorogenic Acid content of infusion ⁴ (mg/100 mL)	Protein ⁵ (g/100g) content of dried leaves (and g/100mL infusion in parentheses)	Carbohydrates ⁶ (g/100g) content of dried leaves (and g/100mL infusion in parentheses)	Fiber ⁷ (g/100g) content of dried leaves (and g/100mL infusion in parentheses)
<i>Ilex paraguariensis*</i>							
Maté Factor Dark Roast	0.84	8.56	1.60	22.45	11.6 (0.10)	26.7 (0.3)	45.8 (0.14)
Rosamonte Yerba Maté	1.13	9.42	1.52	25.88	9.4 (<0.10)	17.9 (0.2)	58.1 (0.08)
Guayaki San Mateo Yerba Maté	0.99	10.08	2.40	32.90	10.9 (<0.10)	13.7 (0.1)	57.7 (0.11)
Harney & Sons Yerba Maté Buds	1.42	14.46	1.55	43.37	10.0 (<0.10)	16.0 (0.1)	57.2 (0.11)
Taragui Yerba Maté	1.35	13.26	1.90	32.14	11.0 (<0.10)	17.7 (0.2)	57.3 (0.11)
<i>Ilex guayusa*</i>							
Black Guayusa buds	4.94	57.72	1.87	34.19	28.8 (0.13)	0.5 (0.2)	52.7 (0.12)
Green Guayusa	2.61	27.23	0.18	40.97	16.0 (<0.10)	15.4 (0.3)	50.0 (0.06)
Golden Guayusa	2.52	29.12	0.14	25.64	16.9 (0.12)	12.7 (0.2)	50.1 (0.08)
Green Guayusa Buds	2.08	19.84	1.99	72.42	22.8 (<0.10)	19.6 (0.2)	37.9 (0.13)
RUNA Traditional Guayusa	2.39	25.46	0.14	9.93	16.5 (<0.10)	11.7 (0.2)	54.4 (0.9)

*Theophylline content below detection limit of 0.0015% in all samples.

Analytical methods:

1. Caffeine in dried leaves, UHPLC, ISO 14502-2 (mod), expressed in % of dried leaves as received (extraction with 70% methanol).
2. Caffeine in aqueous infusion of dried leaves (as received) UHPLC, ISO 14502-2 (mod).
3. Theobromine in infusion of dried leaves (as received) UHPLC, ISO 14502-2 (mod).
4. Chlorogenic acid in infusion: calculated as 5-caffeylquinic acid, HPLC-UV, DIN 10767:2015-08, mod.
5. Protein, according to Kjeldahl (N x 6.25).
6. Carbohydrates, calculated.
7. Fiber, according to L.00.00-18, mod. enzymatic-gravimetric method.

While the leaf protein content of the analyzed samples of *I. paraguariensis* (range: 9.4–11.6 g/100 g) were lower than that of *I. guayusa* (range: 16.0–28.8 g/100 g), the protein content of the infusions was similar, and all values were lower than 0.13%. Fat levels of the dried leaves are not shown in the table but were 2.8–5 g/100 g for *I. paraguariensis* samples and 6.1–9.2 g/100 g for the *I. guayusa* samples. Fat levels in the aqueous infusions were <1.0 for all samples. Carbohydrate levels were reasonably similar, and overall, levels of macronutrients in infusions of both species were very low compared to recommended daily intakes.



The dried and milled leaves of *I. guayusa* contained more caffeine (2.08–4.94%) than the corresponding leaves of *I. paraguariensis* (0.84–1.42%), and caffeine content of the aqueous infusions of *I. guayusa* was correspondingly higher. The levels of caffeine varied between samples, similar to variations seen within different coffee or tea samples. The average theobromine concentration of the dried and milled leaves was 0.19% for *I. paraguariensis* and 0.07% for *I. guayusa*. Theophylline concentrations were tested but were below the limit of detection for all samples (0.0015%, w/v) and the data is not shown. The content of CA exhibited a considerable variance that is bigger within the different samples of each *Ilex* species than between the two *Ilex* species.

6.8.2 Toxicological Studies

de Andrade et al.(2012) studied acute and subchronic dosing effects of orally administered yerba maté dried aqueous extract (YMDE) in rats and rabbits.⁴⁸⁵ YMDE was characterized by RP-HPLC and calorimetric assay to have the following approximate composition: 30.5% total phenols, 4% CA, 1.9% gallic acid, 0.7% caffeine, 0.5% theobromine, and 2.2% saponins.

In the acute oral toxicity study, 6 rats/sex/group received a single dose of YMDE (2 g/kg bw) or water (control) by gavage.⁴⁸⁵ Rats were monitored shortly after dosing and once daily for 14 days. At the end of the study animals were sacrificed and examined macroscopically in situ. Acute dosing resulted in no mortalities, no changes in behavior, water or food intakes or macroscopic examination of organs (data not provided). Rats were active and presented with good weight gain throughout the study, therefore authors could not determine an LD₅₀.

Subchronic toxicity was investigated in Wistar rats and in New Zealand rabbits using a dose of 2 g/kg bw/day for 12 weeks.⁴⁸⁵ Rats groups were 5 animals/sex in the control group and 10/sex/group in the YMDE group. YMDE was administered orally to rats and rabbits. Intake of 2 g/kg/day of YMDE did not affect animal survival or clinical signs in rats or rabbits. An increase in MCHC in male and female rats was observed, possibly due to a non-significant decrease of MCV concomitant to increased MCH values in male and female rats. Platelet counts were increased in male and female rats (results were within reference range, or lower than those values reported in other studies), while there was a decrease in neutrophils counts in male rats (within reference range) and an increase in monocyte counts in female rats (within reference range for the species). With respect to biochemical parameters, an increase in urea and a reduction in iron levels were noted in female rats compared to controls. Male rats had increased activity of GGT (around 3-fold) and a decrease in ALP activity and triglycerides (the latter are generally opposite of the direction of concern). The authors noted that no significant changes in blood parameters or serum iron were noted in human studies within their laboratory after ingestion of 1



1 L daily infusion of maté tea for 40 or 90 days, suggesting the effects in rats may not be present in humans at a 1 L dose level. In rabbits, the majority of hematological and biochemical parameters remained unchanged except for an increased hematocrit in male and female rabbits (within reference range and considered clinically irrelevant) compared to controls. There was also a decline in serum iron levels in male rabbits (again, human studies at 1 L per day did not show this effect). There was a lack of histopathological changes in the stomach, kidney, liver and small intestine compared to controls in rats and rabbits. As there was only a single dose used in the study, dose-related changes cannot be assessed, and a NOAEL cannot be determined. However, the authors concluded that YMDE was overall safe for human studies.

Miranda et al. (2008) randomly assigned forty male Swiss mice to four groups and gave them a maté tea aqueous extract (containing 350 mg/g of phenolic compounds) at doses of 0, 0.5, 1.0 or 2.0 g/kg bw/day, for 60 days, in order to study its antioxidant activity and influence on DNA repair.⁴⁹⁰ Liver, kidney and bladder cells were isolated and DNA damage induced by H₂O₂ was investigated using the comet assay. The maté was reported to be non-genotoxic to the liver, kidney and bladder cells (levels of DNA damage were not different than the control group), and increased resistance of DNA to H₂O₂-induced DNA strand breaks and improved DNA repair after H₂O₂ challenge in liver cells, irrespective of the dose ingested.

6.8.3 Human Studies

A single-blind trial of 102 healthy individuals was conducted to study the impact of yerba maté on lipid levels.⁴⁹¹ Subjects ingested 330 mL, three times per day (about 1 liter) of green or roasted yerba maté infusions (50 or 20 mg/mL, respectively, reflecting the usual consumed pattern by the population) for 40 days, immediately before or during meals. Characterization of the maté was as follows:

Compound	Green yerba maté (µg/mL)	Roasted yerba maté (µg/mL)
Chlorogenic acid	804.1 ± 11.7	170.0 ± 4.5
Epicatechin	101.1 ± 2.9	34.07 ± 1.52
Gallocatechin	458.9 ± 8.1	47.4 ± 2.1
Caffeine	157.4 ± 1.5	109.9 ± 3.8
Theobromine	48.12 ± 1.38	26.98 ± 0.77
Theophylline	ND	ND

ND = not detected; data are expressed as mean ± SEM

Blood samples were collected before the study began, and after 20 and 40 days of maté consumption. Participants served as their own controls. Routine biochemical



and hematology parameters were measured, and blood pressure, body height and weight were measured at each visit. Four individuals reported adverse events such as irritation of the oral or stomach mucosa, insomnia or nausea and did not continue in the study; however, there was no control group for comparison of such events. There were no significant or clinically relevant differences between baseline and 20- or 40-day values of measured parameters after consumption of maté preparations (data not provided).

Some of the same authors performed a randomized clinical trial on 74 dyslipidemic volunteers that were divided into three groups: maté tea, dietary intervention, or both, for 90 days.⁴⁹² Maté consumption followed the same schedule as above. The ingestion of maté was not associated with adverse events in the participants, and it was associated with increased plasma and blood antioxidant protection independent of the dietary intervention.

Again, many of the same authors enlisted 29 individuals with type-2 diabetes and 29 subjects with pre-diabetes in a study. Subjects were divided into 3 groups; maté tea, dietary intervention, or both.⁴⁹³ Individuals drank maté on the same schedule as above for 60 days. Blood samples and food assessments were taken at baseline and after 20, 40, and 60 days of treatments. While the overall results showed some health benefits from maté consumption, eight individuals had minor adverse reactions associated with maté, such as insomnia, heartburn, and tachycardia.

A randomized, crossover study composed of 12 men looked at consumption of maté (200 mL prepared from 1 g of an instant maté product, taken three times per day) compared to water, over 11 days, with regard to effects related to exercise.⁴⁹⁴ Maté had a beneficial effect on strength recovery over 24 hours after exercise, and on blood antioxidant compounds. No adverse events were mentioned. Similarly, no adverse events were mentioned in a study where subjects with HIV took three grams of a soluble maté preparation, corresponding to 107 mg/g total phenols, for 15 days,⁴⁹⁵ nor in a randomized, double-blind, placebo-controlled study where 142 subjects with high blood viscosity were given maté tea or placebo (5 g/day) for 6 weeks.⁴⁹⁶

Santos et al (2005) assessed the effect of maté consumption during pregnancy on preterm and small for gestational (SGA) births using a cross-sectional study design.⁴⁹⁷ A total of 5189 single births that occurred at hospitals in Pelotas, Southern Brazil were analyzed. About 68% of the women reported being maté drinkers and 70% of those women were daily consumers (47.5% of the entire sample). Maté drinkers were more frequently smokers and consumers of alcohol and had a lower family income than their counterparts. In crude analysis, maté drinking was not associated with pre-term birth. While maté was initially significantly associated with higher incidence of SGA birth, after adjusting for potential confounding factors, the association disappeared. Local intake of maté in that region is on average



1800 mL per day; maté contains approximately 17 mg of caffeine per 100 mL maté.⁴⁹⁷ Thus, maté consumers drink about 300 mg of caffeine per day, which has been suggested as the upper limit of caffeine consumption during pregnancy.^{74, 86}

In summary, *Ilex paraguariensis* and *I. guayusa* are related species consisting of similar constituents, and with similar methods of preparation and consumption patterns. The long history of regular consumption of aqueous decoctions of yerba maté made from *I. paraguariensis* leaves and the scientific studies on consumption of this beverage corroboratively support the safety profile of RUNA® Concentrate.

6.8.4 *Ilex paraguariensis* (Yerba Maté) Regulatory Status

Ilex paraguariensis St. Hil. (maté) is listed in the U.S. Code of Federal Regulations (21 CFR 182.20) as one of many substances of which the essential oils, oleoresins (solvent-free), and natural extractives (including distillates) are generally recognized as safe for their intended use. The plant is also listed on various “old dietary ingredient” lists by trade associations (e.g., The Council for Responsible Nutrition (CRN), National Nutritional Foods Association (NNFA, now the Natural Products Association), and the United Natural Products Alliance (UNPA)), which suggests that it was sold regularly prior to 1994.

As mentioned in Part 6.1.5 above, in February 2017, the Food Safety Authority of Ireland (FSAI) received an application from Runa, LLC for an opinion on the substantial equivalence of aqueous extracts of the dried leaves of *I. guayusa* with aqueous extracts of *I. paraguariensis* (which is not considered a novel food as it was in the EU market prior to 1997). They showed that the two extracts are similar in terms of macronutrients, caffeine and CA levels. FSAI was satisfied from the information that the two are substantially equivalent.¹⁶⁵ Aqueous extracts of dried leaves of *I. guayusa* are now an authorized novel food in the European Union, under the food categories “herbal infusions” and “food supplements”. The maximum levels of use are stated as “in line with normal use in herbal infusions and food supplements of a similar aqueous extract of dried leave of *I. paraguariensis*”. The composition of the novel food is stated as 0.2–0.3 g/100 mL of carbohydrate, 19.8–57.7 mg/100 mL caffeine, 0.14–2.0 mg/100 mL theobromine, and 9.9–72.4 mg/100 mL CAs.¹⁶⁶

6.9 Allergenicity

No reports of allergic reactions to guayusa were found in the literature. RUNA® Concentrate does not contain or have added, and is manufactured in a facility free of, all eight major allergens (milk, egg, fish, Crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) identified, and required to be disclosed in labeling, in the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally,



RUNA® Concentrate does not contain cow milk proteins, chicken egg, sugars, peanuts, crab, shrimp or any derivatives or products of the aforementioned. RUNA® Concentrate contains less than 10 ppm of gluten and sulfites.

6.10 Past Sales and Reported Adverse Events

All Market Inc. (previously RUNA LLC) has supplied and put into commerce over 800,000 kg of guayusa extract/leaves since initial production and formulation into conventional foodstuffs in 2003. The company has had no adverse events, records or customer complaints associated with the use, ingestion, or intake of the guayusa extract or finished products. Contact information for the company is included on labeling of finished products.

Additionally, no FDA letters regarding concern for safety to companies that market products containing guayusa were located (searched May 1, 2019). A search of FDA's Center for Food Safety and Applied Nutrition Adverse Event Reporting System did not uncover any mention of guayusa products (searched May 1, 2019).

6.11 Other Guayusa Products in the Marketplace

A general Internet search as well as searches of the National Institutes of Health (NIH) Dietary Supplements Label Database and several large distributors of dietary supplements resulted in findings of other products containing guayusa, illustrating that this ingredient available in the U.S. marketplace. Despite this prevalence, we are unaware of any adverse events attributed to guayusa products. Examples of products containing guayusa are listed below in Table 17: In addition, foods (especially beverages such as coffee, tea and energy drinks) containing added caffeine can be found ubiquitously in markets throughout the United States (and the world) and are too numerous to list, although a comprehensive summary can be found in the review by Somogyi et al.⁵⁴

Table 17. Products Containing Guayusa in the U.S. Marketplace

Company	Product Name	Serving Size(s)
Mamma Chia	Chia Energy Beverages http://www.mammachia.com/blackberry-blast/ http://www.mammachia.com/cherry-charge/ http://www.mammachia.com/grape-power/ http://www.mammachia.com/raspberry-razz/	90 mg of caffeine from guayusa in 296 mL
Mountain Rose Herbs	Guayusa Tea https://www.mountainroseherbs.com/products/guayusa-tea/profile	Loose leaf tea for brewing
Guayusa Tea House	Guayusa Tea http://www.guayusatea.com/purchase-now/	Loose leaf tea for brewing



Garden of Flavor	<u>Cold Pressed Energy</u> http://www.gardenofflavor.com/cold-pressed-energy/	100 mg of caffeine from guayusa per 473 mL
BSN (Bio Engineered Supplements and Nutrition, Inc.)	N.O.-Xplode https://www.gobsn.com/en-us/product/noxplodexeedge	100 mg of guayusa leaf
Gaspari Nutrition	HyperAmino https://www.dsld.nlm.nih.gov/dsld/prdLabel.jsp?id=80255#prdDSF	Amount not provided on the label

6.12 Data and Information that is Inconsistent with the GRAS Conclusion

We have reviewed the available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with a conclusion that RUNA® Concentrate is reasonably certain to be safe under the conditions of its intended use.

6.13 Information that is Privileged or Confidential

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



Part 7: Supporting Data and Information

Literature searches for the safety assessment described in Part 6 of this GRAS notice were conducted through April 2019.

7.1 Data and Information that are *not* Generally Available

All of the information described in this GRAS notice is generally available.

7.2 References that are Generally Available

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February 18, 2020

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Re: Responses to GRN 883 questions

Dear Dr. Kolanos,

Please find our responses to your questions for GRN 883 below. The original FDA questions are in blue, and responses to the questions are in black.

- (QUESTION #1) On page 16 of the notice in Table 2 (specifications), the notifier noted in a footnote that no specified limits are established for Brix value, however, on page 17 in Table 3 (batch analyses), a range of 42 to 45°Bx is listed as the acceptable range. Please clarify if the range listed for the Brix parameter is considered a specification for the notified substance.
- (RESPONSE #1): The Brix value is not currently considered a specification or control point in the manufacturing process. We apologize for the confusion on the batch analysis table, which suggests that it could be a specification. The brix values stated as acceptable on the batch analysis table are just a range that is typically noted based on the experience of the manufacturer/extractor, but is not considered a true specification for the ingredient.
- (QUESTION #2) We noted contradictory statements in the notice. The following are examples only:
 - a. On page 60 of the notice, the notifier states, “3 mg/kg bw/day could potentially serve as a no concern level”; on page 68 the notifier states, “3 mg/kg bw tested in a dose-finding study and at which no adverse effects were observed in the majority of infants;” on the same page (68) the notifier states, “3 mg/kg bw/day derived for adults was considered to potentially serve as a basis to also derive no concern levels for children and adolescents.” Then, on page 76 the notifier states, “Limited data from short-term clinical trials suggested that caffeine intakes of 3 mg/kg bw/day or more may have adverse effects in children and adolescents.”
 - b. On page 77, the notifier states, “the review found that most prospective cohort studies have not found that coffee consumption is associated with significantly increased risk of heart disease or

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stroke” and then follows with a statement, “randomized controlled trials lasting up to 12 weeks have found that coffee consumption is associated with increases in several cardiovascular disease risk factors.”

- c. The notifier states in several places in the notices that the consumption of caffeine at levels 300-400 mg/person/day can be safe. On page 76, the notifier states that 3 mg/kg bw/day (=180 mg/person/day, assuming 60 kg body weight per person) can cause adverse effects. Please explain how 300-400 mg/person/day can be safe if 180 mg/person/day can cause adverse effects. (PARTIAL RESPONSE): We answered this in more detail below, however we also wanted to point out that the statement that 3 mg/kg bw/day can cause adverse events refers to acute (bolus) dosing in *children/adolescents*, while the 300–400 mg/person/day safe limit applies to chronic consumption in *adults*. We believe this is clear in the GRN submission, however please let us know if we otherwise are misunderstanding this point.

We realize that the information discussed in the notice was collected from different sources, but it is the notifier’s responsibility to write a coherent narrative. Please reconcile all contradictory statements (described above are examples only) in the caffeine safety section narrative (6.2 Safety of Caffeine) and make necessary changes in your response to FDA.

- (RESPONSE #2): The ILSI website for the Wykoff et al. 2017 systematic review on caffeine states that since 2003, caffeine has been the subject of over 10,000 papers (half of which include effects in humans) and over 800 reviews related to caffeine effects in humans (<https://ilsina.org/caffeine-systematic-review-2017/>). Thus, as FDA is aware, the literature on caffeine research is vast, and we attempted to put together a reasonably robust summary of the current caffeine safety literature from numerous sources in GRN 883, suggesting that moderate levels of caffeine intake levels in various populations are generally recognized as safe. As is easy to imagine, in trying to relay the “good, bad, and ugly” of this body of information, there are instances of research/reviews that have slightly contradictory (or what may appear to be contradictory) information or interpretations. Additionally, reviews published earlier had less data to rely on than more recent reviews. It is the totality of evidence that was taken into account by various scientific bodies and the notifier to determine levels deemed safe for various populations.

As FDA pointed out, with regard to children and adolescents, the GRN states that 3 mg/kg bw/day may be both a level of no concern, and at the same time there is a statement that some evidence suggests that a specific dose of 3 mg/kg bw/day could cause adverse effects in children and adolescents. The GRN cites Higdon and Frei, (2006) for this latter statement, which cites a study by Rapoport et al., (1981) from the Nawrot et al., (2003) paper, in which a single (acute/bolus) dose of 3 mg/kg bw caffeine in boys age 10.6 ± 2.5 years resulted in nervous and jittery feelings. While anxious feelings can be considered an adverse effect, they are considered reversible and are not known to result



in lasting health effects. Nawrot et al., points out that findings of altered behavior from caffeine, including anxiety, are difficult to compare between studies due to differences (and in some cases, inadequacies) in methodologies. After a review of the totality of the literature, Nawrot et al. considered a total consumption (from all sources) of 2.5 mg/kg bw/day as a cautious/conservative safe level of exposure in children that is unlikely to cause harmful effects. ILSI's 2017 systematic review on caffeine, which used the Nawrot/Health Canada safe levels for various populations as comparators, determined that the 2.5 mg/kg bw/day safe comparator level in children can still be considered safe, although they did not attempt to determine a possibly more updated safe limit. EFSA, on the other hand, in their 2015 opinion on caffeine safety, suggested that while their estimated safe level of *habitual* (not acute) consumption for adults of 5.7 mg/kg bw/day *may* also apply to children (as caffeine clearance is similar in adults and children), due to limited availability of data/studies on anxiety and behavioral effects in children, they proposed a level of no concern of 3 mg/kg bw/day in children. This is the same level that they proposed for *acute* (single-dose) exposure in adults. Regardless, **per GRN 883: The conservative caffeine exposure estimates (Part 3 of the notice), which take into account background caffeine consumption plus caffeine consumption from the RUNA® Concentrate intended uses, resulted in an estimated 90th percentile exposure of 0.8 mg/kg bw/day in children. This falls below both the 2.5 mg/kg bw/day and 3 mg/kg bw/day safe estimated use levels for children cited by different scientific bodies as discussed above. Additionally, as stated in the GRN, products containing RUNA® Concentrate are not intended to be intentionally marketed to children (or to be used in/as infant formula).**

As FDA also pointed out, with regard to caffeine and cardiovascular associations, Higdon et al., (2006) found that most prospective study evidence at the time of their review showed no increased association of caffeine and risk of heart disease or stroke. *However*, they also stated that randomized clinical trials suggest coffee consumption is associated with an increase in several cardiovascular disease risk factors. More specifically, these risk factors were small increases in blood pressure and increased serum homocysteine. Discussion of the associations/effects of caffeine on cardiovascular disease are scattered throughout subpart 6.2 in the GRN (due to the fact that large reviews/opinions are discussed first—many of which incorporated cardiovascular reviews—followed by sections on specific topics. Subpart 6.2.3.4 discusses effects of caffeine on cardiovascular disease, and states that while blood pressure increases (often of low magnitudes) are seen after acute coffee intake, especially in caffeine naïve individuals, tolerance appears to limit this effect as it is not generally seen in more habitual drinkers, and long term hypertension is not associated with moderate caffeine consumption levels. While hypertension is a known risk factor for cardiovascular disease, intermittent increases in blood pressure such as occurs with exercise are not, and hence the acute slight effects on blood pressure and not clearly clinically relevant. A review on caffeine and cardiovascular health by Turnbull et al., (2017) details the literature on caffeine/coffee and homocysteine. Generally, the caffeine levels associated with increases in homocysteine are higher than the 400 mg/day that is generally considered safe for adults, although in several studies a dose response has been seen at lower doses (starting as low as 89

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mg caffeine in one study). Yet Turnbull importantly points out that while plasma homocysteine has been identified as a cardiovascular disease risk factor, interventions that reduce plasma homocysteine don't show a reduction in heart disease, and thus the impact on cardiovascular risk is not clear, especially in light of the fact that moderate caffeine intake has not been shown to be associated with heart disease risk. **As relates to GRN 883, according to current studies and reviews, moderate levels of caffeine (400 mg/day) have not been associated with cardiovascular risk or cardiovascular effects in adults, with many citations for this research listed in the first paragraph of subpart 6.2.3.4. The conservative caffeine exposure estimates (part 3 of the GRN), which take into account background caffeine consumption as well as caffeine consumption from the RUNA® Concentrate intended uses, resulted in an estimated 90th percentile exposure of less than 400 mg/kg bw/day in adults. Thus RUNA® Concentrate, under the conditions of its intended use, is not expected to be associated with cardiovascular side effects.**

With regard to other statements that could be or may appear to be contradictions in subpart 6.2, we located the following:

- On page 93 of the GRN, it states that a 2011 meta-analysis on coffee and blood pressure and cardiovascular disease concluded that in hypertensive individuals, caffeine intake (200–300 mg/day) produces acute increases in both systolic (8 mmHg) and diastolic (6 mmHg) blood pressure for up to three hours after consumption, **similar to what has been shown in normotensive individuals**. As discussed above, caffeine is not associated with long-term hypertension or increased cardiovascular disease, and transient increases in blood pressure caused by exercise or from caffeine are not known to lead to long term adverse effects.
- While the Wikoff et al., (2017) systematic review supports a safe level of 300 mg/day of caffeine during pregnancy, EFSA suggests a more conservative 200 mg/day “based on prospective cohort studies showing a dose-dependent positive association between caffeine intakes during pregnancy and the risk of adverse birth weight-related outcomes (i.e. fetal growth retardation, small for gestational age) in the offspring.” This level (\leq 200 mg/day for pregnant women) was also considered reasonable in 2010 by the American College of Obstetricians and Gynecologists with regard to miscarriage or preterm birth. While Wikoff et al. also identified some studies suggesting adverse (but low magnitude) birth weight effects below 300 mg/day, they found that a *majority* of studies showed no such effect at 300 mg/day or higher. They found that the studies that more robustly evaluated small for gestational age or intrauterine growth restriction did not suggest a concern at 300 mg/day. Wikoff et al., also evaluated current data related to miscarriages and found a moderate to high level of support for 300 mg/day as a safe level in pregnancy that would not be expected to result in miscarriage or preterm births, except possibly in some subgroups with genetic susceptibility to caffeine. **As relates to GRN 883, the conservative caffeine exposure estimates (part 3 of the GRN), which take into account background caffeine consumption combined with caffeine consumption from the RUNA® Concentrate intended uses, resulted in an estimated 90th percentile exposure of less than 300 mg/day in women of childbearing age.** Of



note, the GRN exposure estimates using NHANES data only cover women of childbearing age without knowledge of pregnancy, and the exposure estimate was 224.6 mg per day for background plus RUNA® Concentrate intended uses. As discussed on page 29 of the GRN, Knight et al. (2004) reported that in their study of 10,712 individuals, pregnant women consumed about half of the caffeine as compared to non-pregnant women of reproductive age (90th percentile consumption during pregnancy was 157 mg/day versus 229–247 mg/day in reproductive aged non-pregnant women). Thus, while the exposure estimate from background plus RUNA® Concentrate intake is slightly higher than the 200 mg limit suggested by some EFSA, the Knight et al. data suggests that the GRN 883 exposure estimates during pregnancy would be well under 200 mg/day.

- The contradictory designations by IARC (that *coffee* was 1) possibly carcinogenic to the human urinary bladder (Group 2B) in 1991, and 2) not classifiable as to carcinogenicity to humans in 2016 (Group 3)), were explained via new information in the latter conclusion and a what was considered limited controlling for tobacco smoking (associated with coffee drinking and a risk factor for bladder cancer) in the earlier conclusion. This is further discussed in the GRN, both in the IARC section and on page 86 in the bladder cancer section.
- The GRN summary of Wikoff et al., (2017) states that some effects for physiologic endpoints for cardiovascular disease were noted in some studies at doses lower than 400 mg/day for adults and 2.5 mg/kg bw/day for children, and effects on anxiety have been shown to occur in some cases at doses lower than 400 mg/day. However, we believe that appropriate explanations as to why such levels were still considered safe by the authors is already present in the GRN (subpart 6.2.1.6), thus we will not repeat them here unless requested.
- The GRN summary of Higdon et al., (2006) states that limiting caffeine consumption to 300 mg/day may help prevent osteoporotic fractures in older adults. However, the more updated review by Wikoff et al., (2017) found that the majority of relevant studies support that 400 mg/day in healthy adults is not harmful with respect to bone marrow density, osteoporosis, and risk of fracture. Risk is especially low if calcium intake is adequate. Importantly, the exposure estimates in part 3 of the GRN suggest that caffeine exposure from background plus RUNA® Concentrate intended uses is expected to be less than 400 mg/day in adults.
- On page 95, it is mentioned that single large boluses of caffeine (≥ 250 mg) may exaggerate post-prandial hyperglycemia and hyperinsulinemia in diabetic individuals when sugar is consumed at the same time. The amounts of caffeine in a single serving in the RUNA® Concentrate intended use products are only 150 mg/serving (i.e. much lower than 250 mg, and thus are not expected to cause this response in diabetics).
- The GRN states that single doses of up to 200 mg (~3 mg/kg bw/day for 70 kg adult) are considered safe by EFSA. Yet single doses of 100 mg (about 1.4 mg/kg bw for a 70 kg adult) may increase sleep latency and reduce sleep duration in some adult individuals, particularly if consumed close to bedtime. As stated in the GRN on p.74, effects of caffeine on sleep are not necessarily considered as adverse—such effects highlight the difficulty of characterizing adversity versus



well known desirable and/or anticipated effects (as caffeine is often ingested to avoid sleepiness).

- (QUESTION #3): We noted that on pages 55-56 the notifier included in quotes a significant amount of text copied from OECD SIDS document. Using quotes and ascribing the source is not enough to avoid plagiarism when the section being copied is long. Please rewrite the section in quotes in your own words. Be aware of the rules to avoid plagiarism; there are many guidance practices including those provided by the U.S. Government, National Institutes of Health, etc.
- (RESPONSE #3): Thank you for informing us that plagiarism may still be an issue if a section is too long, despite the fact that we used quotations and referenced the information. The quoted text is a description of NTP studies on caffeine, and as a brief explanation as to why it was quoted initially, the quoted text was intended to show the reader what information was given versus what was missing due to the fact that quite a lot of information that is usually described in toxicology studies is missing. Thus, the thought was that it would be clearer and leave less room for many questions if the summary was directly quoted in this case. We apologize for this oversite.

The quoted text summarizes two 90-day toxicity studies, one in Fischer 344 rats and one in B6C3F1 mice, in which caffeine was administered via the drinking water at concentrations of 0, 188, 375, 750, 1500, and 3000 ppm (rats) and 0, 94, 188, 375, 750, and 1500 ppm (mice). Results in the rat study included a statistically significant decreased body weight gain compared to controls in the high-dose group only. The high-dose group also showed decreased water consumption compared to controls, while the opposite was true in the 375 and 750 ppm groups (which showed increased water consumption). No significant clinical signs were noted up to 1500 ppm, which suggests that there *were* signs noted in the high-dose (3000 ppm) group, yet none were described. There were no dose-related changes in clinical chemistry, although again, no details were given. The only gross or histopathological finding noted was a dose-dependent cellular enlargement in the salivary gland, which was considered a well-known adaptive effect from caffeine. The NOAEL was 1500 ppm (151 and 174 mg/kg bw/day in male and female rats, respectively).

Results of the mouse study also included a decrease in body weight compared to controls in some groups, however the effects were not dose-dependent and not seen in the high-dose group. As in the rat study, water consumption was decreased in the high-dose group mice (as well as in the second to highest dose) but was increased in the lower dose groups. The same adaptive change to the salivary glands as in the rat study was the only histopathological finding mentioned for the mice, and the NOAEL was considered the highest dose tested of 1500 mg/kg bw/day (167 and 179 mg/kg bw/day in male and female mice, respectively).



- (QUESTION #4): The notifier presents several publications in a manner that suggests these publications are the position papers of the institutions, i.e., the authors' affiliations are listed in the headings of several sections of the notice. Examples:

Section 6.2.2.2. Facultad de Medicina, Valencia, Spain/Cano-Marquina et al., 2013

Section 6.2.2.4. Cambridge University, Harvard University, University of Cantania/Grosso et al., 2017

To avoid misleading information, please provide revised headings for the relevant sections (include the publication reference only without the name of the institution).

- (RESPONSE #4): Please see the revised headings for the relevant sections below:

Subpart 6.2.2.1 “Linus Pauling Institute (LPI)/Higdon and Frei (2006)” should instead read: “Higdon and Frei (2006)”

Subpart 6.2.2.2 “Facultad de Medicina, Valencia, Spain/Cano-Marquina et al., (2013)” should instead read “Cano-Marquina et al., 2013”

Subpart 6.2.2.3 “Northern Ireland Centre for Food and Health/Pourshahidi et al. (2016)” should instead read “Pourshahidi et al., 2016”

Subpart 6.2.2.4 “Cambridge University, Harvard University, University of Cantania/Grosso et al. (2017)” should instead read “Grosso et al., 2017”

- (QUESTION #5): On page 126, the notifier describes a mouse study published by Zhang et al. (2014) and states that “the rats fed CA plus caffeine showed a decrease in body weight.” Please clarify whether the study was conducted with mice or rats.
- (RESPONSE #5): The study was reference #423 in the notice (Zheng G, Qiu Y, et al. Chlorogenic acid and caffeine in combination inhibit fat accumulation by regulating hepatic lipid metabolism-related enzymes in mice. *Br J Nutr.* 2014;112(6):1034-40). The study was performed in mice, thus the sentence should be corrected to instead read “the mice fed CA plus caffeine showed a decrease in body weight.”
- (QUESTION #6): On page 136, the notifier refers to “Teavino” We note that it should be “Teavigo.”



- (RESPONSE #6): Noted, thank you.
- (QUESTION #7): The notifier should consult the following publications and provide a brief, targeted narrative on the following aspects as suggested below.

Publications to consult:

- (a) Caffeine toxicity (<https://www.ncbi.nlm.nih.gov/books/NBK532910/>);
- (b) Temple, J. L., et al. 2017. The safety of ingested caffeine: a comprehensive review. *Front. Psychiatry*. 8:80. doi: 10.3389/fpsyg.2017.00080.
- (c) Wikoff, D., et al. 2017. Systematic review of the potential adverse effects of caffeine consumption in healthy adults, pregnant women, adolescents, and children. *Food Chem. Toxicol.* 109(Pt 1):585-648.
- (d) Wise, G., Negrin, A. 2019. A critical review of the composition and history of safe use of guayusa: a stimulant and antioxidant novel food. *Crit. Rev. Food. Sci. Nutr.* 1:1-12.

Aspects to be addressed in the response to FDA:

The notifier should consult the **first three publications** and address the following points:

- (QUESTION A): Address the pharmacokinetics and metabolism of caffeine in no more than 1-2 pages in your own words. Mention the caffeine-metabolizing enzymes (e.g., CYP1A2), the known metabolites, the half-life of caffeine, etc. The pharmacokinetics and metabolism discussion is scattered in the GRN; please consolidate this information in this section.
- (RESPONSE A): Indeed, there is pharmacokinetic (PK) information about caffeine in various locations of the GRN, although we would like to point out that there is also a dedicated section on caffeine PK (subpart 6.2.3.1) in the GRN notice as well. Regardless, we have compiled a new PK discussion here as directed by FDA based specifically on information from the three publications listed above. The citations of (a)–(c) are utilized in this communication.

The PK profile of caffeine, which is soluble in both water and lipids, is well established.(c) It is rapidly and nearly completely (~90%) absorbed in the stomach/small intestines, with peak plasma concentration occurring within two hours of ingestion.(a)(b) Absorption does not appear to be affected by gender or genetic background. Once absorbed, caffeine is widely distributed in body fluids (e.g. saliva, cerebrospinal fluid, umbilical cord and breast milk) and other tissues, and crosses the blood-brain barrier.(b) Caffeine is primarily metabolized in the liver via n-demethylation, acetylation, and oxidation reactions.(a) The CYP1A2 enzyme is the major contributor to caffeine metabolism, and its activity may be increased/decreased via various genetic variations/polymorphisms, circadian rhythms, xenobiotics (e.g. caffeine

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clearance increases with cigarette smoking and decreases with alcohol consumption), and/or health states of the liver (e.g. liver disease may decrease clearance).(a–c) Caffeine metabolism is also slowed by the presence of steroid hormones (e.g. during pregnancy, fetal stage, and oral contraceptive use), which increase caffeine’s half-life.(c) While the metabolites of caffeine are not discussed in the three references provided by FDA, they are described in subpart 6.2.3.1 of the GRN.

Much of the more recent research on the PK of caffeine is dedicated to studying the effects of various genetic alleles of caffeine metabolizing enzymes and receptors to which it binds, as is discussed in a subsequent response to an FDA question below. The overall half-life of caffeine is 3–10 hours in adults, and again depends on complex genetic and environmental interactions.(a)(b) While the half-life of caffeine in neonates is relatively high (65–130 hours), by six months of age (before the age at which consumption of RUNA® Concentrate containing products is expected), caffeine is eliminated at the same rate as that of adults.(b) Caffeine and its metabolites are excreted in the urine.

- (QUESTION B): Address the clinical findings of caffeine toxicity in normal adults in conditions of overdose in no more than 1-2 pages in your own words. You may cite the reference as “(see review by Wikoff et al., 2017 and references therein)”, or you may cite the individual references from Wikoff et al. (2017). These references are expected to be already covered by the 497 references in the current notice.
- (RESPONSE B): The adverse effects of caffeine overdoses in normal adults are considered related to the alkaloid’s various effects as an antagonist of adenosine receptors, inhibitor of phosphodiesterase, producer of renin and catecholamines, and sensitizer of dopamine receptors.(a)(b) According to Wikoff et al., (2017), the majority of overdoses occur from consumption of caffeine at high doses over a relatively short time frame, mainly in the form of powder or tablets, while the remainder have reportedly come from energy drinks, cola, coffees and teas.(c) A lethal dose is generally considered 10 g caffeine or greater.(a)(c) Note that the exposure estimates based on the RUNA® Concentrate intended uses in the GRN do not suggest that a high dose ingestion pattern will occur up to the 90th percentile consumer.

While death from caffeine overdoses are quite rare, determining serum caffeine concentrations after large ingestions and reducing them (e.g. by using hemodialysis or intralipid emulsion therapies) may be critical to prevent acute kidney injury, rhabdomyolysis, and/or cardiac arrest.(a) Clinical findings of caffeine toxicity may include nausea/vomiting (due to gastric irritation—vomiting aids in the prevention of toxic effects), fever, tachycardia (or bradycardia), hypertension (which may be followed by hypotension), rigid muscles, pupil



dilation, and neurological effects such as agitation, hallucinations, delusional thoughts, seizures, and hyper reflexes.(a)(b) Laboratory values may show an elevated lactate level (and subsequent anion gap metabolic acidosis), hypokalemia, hypocalcemia (although large amounts of calcium may be released from intracellular stores during extreme toxicity), hyponatremia, hyperglycemia, and altered myoglobin and creatine kinase levels. An electrocardiogram may show results of tachycardia, ST segment depressions, or T wave inversions.(a)(b)

- (QUESTION C): Address the inter-individual differences in caffeine metabolism, emphasizing on the adverse effects of caffeine in those individuals, in no more than 1-2 pages in your own words.
- (RESPONSE C): Inter-individual differences in caffeine metabolism and effects are often associated with genetic variation in metabolizing enzymes and the receptors to which caffeine binds. This is an active area of current caffeine research and is touched on in various sections of GRN 883, including more specifically subpart 6.2.3.10. Genetic variability in subjects is complex and likely accounts for variation in research study outcomes, and is by no means fully understood. Utilizing only information from the citations suggested by FDA, a brief discussion follows.

As stated above, the cytochrome p450 enzyme CYP1A2 is responsible for much of the metabolism of caffeine in the liver. This enzyme has a high amount of genetic variability between individuals, and individuals with decreased/slower activity of this enzyme have slower metabolism of, and hence increased sensitivity to, caffeine.(b)(c) Temple et al., (2017) suggests that at least 150 single-nucleotide polymorphisms can accelerate caffeine clearance.(b) CYP1A2*1K alleles are associated with decreased caffeine metabolism, while other alleles of CYP1A2 have been associated with increased patterns of caffeine consumption, as cited in Wikoff et al., (2017).(c)

Additionally, while not specifically related to caffeine metabolism, the adenosine receptor, on which caffeine acts and produces many of its physiological effects via various biochemical pathways, also has a number of variants that are known to affect the specific actions/effects of caffeine in humans.(b)(c) For example, small nucleotide polymorphisms in the ADORA2A (adenosine A2A receptor) gene have been found to affect a person's sensitivity to caffeine, including effects on sleep and levels of anxiety reaction to acute caffeine exposure.(b)(c) Wikoff et al. found evidence that consumer self-regulation and awareness of potential sensitivity to caffeine occurs and is important for avoiding caffeine-induced anxiety.(b)

Genetic variations that lead to increased caffeine sensitivity differences may then lead to inter-individual differences in any caffeine related health outcome (anxiety, effects on blood pressure, sleep, etc.). Yet individuals generally have awareness of their personal tolerance to caffeine

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through experience over time and moderate their intake accordingly. This is discussed (and research is cited) in subpart 6.2.3.10 of the GRN. This self-regulation effect is also demonstrated by the fact that caffeine consumption levels have remained stable in the U.S. despite many new caffeine beverage additions to the market (see GRN citation numbers 14–16 and 55–58). The majority of studies in the literature are assumed to have subjects representative of a large range of genetic differences, and safe level determinations by various scientific bodies are based on total subject populations.

- (QUESTION D): Bridge the entire information discussed above with the safety of your product. This should be simple to address because caffeine-sensitive individuals are expected to avoid your product (assuming that your product will be labeled to contain caffeine). For the caffeine-consuming population, the EDI of your product should be much less than the accepted safe level of caffeine consumption.
- (RESPONSE D): While the pharmacokinetics of caffeine are generally well-established, as discussed above, it is also established that genetic polymorphisms have significant effects on caffeine metabolism and overall effects in individuals. Safe levels discussed in the GRN have been determined by various scientific bodies, and are based on the population as a whole, with the understanding that there is a range of individual sensitivities. As is discussed above and cited in section 6.2.3.10 of GRN 883, and is also discussed in Wikoff et al., (2017), there is evidence that self-regulation of caffeine intake limits its overall consumption by sensitive individuals. As RUNA® Concentrate is expected to be labelled with regard to caffeine content, individuals who are sensitive are expected to avoid or limit consumption of RUNA® Concentrate/caffeine-containing products. This is supported by the number of studies showing that caffeine consumption levels have remained stable in the population (including children and adolescents) despite new caffeinated beverage additions to the market, as cited throughout the GRN.

As described above, the estimated exposure to caffeine from the RUNA® Concentrate intended uses plus background caffeine consumption are shown in part 3 of the GRN, and there are also many studies that suggest that caffeine consumption in adults, adolescents and children has remained stable over the last decade despite new caffeine products being added to the marketplace. The estimates fall below daily intake levels considered safe by various scientific bodies. Levels of caffeine per serving in each of the intended use categories are considered reasonable compared to caffeine levels per serving in other foods in the marketplace, and compared to levels generally considered safe for bolus dosing of caffeine. In conclusion, RUNA® Concentrate's intended uses are expected to be safe for humans.



- (QUESTION E): The reference (d) is related to your product. Please discuss the findings from this publication in no more than 2-3 pages in your own words that relates to the safety of your product. This reference is currently missing in the notice.
- (RESPONSE E): The review by Wise et al. was published online August 1st, 2019, after the literature searches were completed for GRN 883 and very close to its submission date to FDA, thus discussion of it was not included in the original submission. The review discusses the recent large international interest in *Ilex guayusa* leaf consumption, most specifically in the form of tea. The authors used the EU novel food assessment framework to analyze the literature surrounding the safety of guayusa for human consumption.

The paper covers the taxonomy, cultivation and processing, ethnobotany, composition, antioxidant profile, toxicology, and history and patterns of safe use of the guayusa plant, much of which is also covered in our GRN 883, and will not be repeated here unless requested. The authors concluded that the current knowledge of the composition of the plant suggests that it is similar to, and no more of a safety concern with respect to consumption, than that of *Camellia sinensis* (green/black tea) or the related *Ilex paraguariensis* (yerba maté).

The authors discuss the broad history of use of guayusa in/as beverages, without known side effects. They specifically cite a study on the safety of consumption in Ecuador (population of 14.5 million), which was assessed by analyzing three years of data from provincial hospital admissions, national disease register, national toxicology call center, and the national food safety authority. There were no findings related to guayusa consumption, other than a single call center report of hyperactivity and insomnia after its consumption. The lack of any data on adverse effects of the plant despite wide-spread consumption helps support the history of safe use of this plant, and ultimately RUNA® Concentrate.

Some of the gaps in the literature that the authors identified include a need for further research to understand accumulation of metals/heavy metals in the plant across different growing conditions, as well as various determining factors affecting the caffeine content of the plant, as leaf concentrations in the literature vary quite widely. The subject of GRN 883 (RUNA® Concentrate) is not expected to be affected by these variation factors, as it has specifications limiting both total and various specific heavy metals as well as caffeine concentration (Table 2 in the GRN).

Wise et al. also discussed that the “brief resting period” commonly occurring after harvest of guayusa leaves (similar in length to that for green tea rather than more highly fermented teas such as black or yerba maté) limits risk of microbial contamination during processing. The clear microbial specifications for RUNA® Concentrate additionally alleviates concerns in this realm (Table 2 of the GRN). As is also discussed in the GRN, Wise et al. authors mention that the roasting



and smoking that normally takes place during yerba maté processing is linked to the formation of compounds that may have negative health impacts. Traditionally, and in the case of RUNA® Concentrate manufacturing, no roasting or smoking steps are utilized, and thus any health hazards related to the formation of such compounds are not expected. Lastly, the authors suggest that risk of pesticide residue contamination is minimal due to the organic agriculture practices that are generally used in growing this plant. Regardless, the raw leaf material utilized in every batch of the RUNA® Concentrate manufacturing process undergoes pesticide evaluation, and batches would be rejected if they were to ever exceed the specified tolerances.

As in GRN 883, the Wise et al., authors suggest that consumption patterns for guayusa tea will likely mimic and substitute for those of other teas. A toxicology study that is not mentioned in GRN 883 was cited by the authors, in which the lethal concentration for an aqueous extract of guayusa was determined to be >10,000 mg/mL in brine shrimp, which does not suggest any safety concerns. Overall, this very recent review does not suggest any additional safety issues, and overall corroborates the safety of *Ilex guayusa* and thus RUNA® Concentrate consumption.

We hope that these responses are adequate with regard to your questions. Please don't hesitate to let us know if there are any further questions or comments during your GRN evaluation process. We will be happy to discuss and/or provide any additional written responses.

Sincerely,

(b) (6)

A large gray rectangular box with a thin black border, positioned below the '(b) (6)' text. It is used to redact a signature.

Amy Clewell, ND, DABT
VP Scientific and Regulatory Affairs