Notice to US Food and Drug Administration of the Conclusion that the Intended Use of an Aqueous Guayusa Leaf Extract (Gyusa.g™) is Generally Recognized as Safe

Submitted by the Notifier:

Applied Food Sciences, Inc.
8708 South Congress Ave, Ste B-290
Austin, TX 78745

Prepared by the Agent of the Notifier:

AIBMR Life Sciences, Inc.
2800 E. Madison, Suite 202
Seattle WA 98112

June 10, 2019

June 10, 2019

GRN 869

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 Applied Food Sciences, Inc. (the notifier), the undersigned, Amy Clewell, submits, for FDA review, the enclosed notice that the aqueous *Ilex guayusa* leaf extract, Gyusa.g™, is GRAS for use in specified 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)
Vice President, Scientific and Regulatory Affairs
AIBMR Life Sciences, Inc. ("AIBMR")

Table of Contents

Figures and Tables

Table 13	Total (Aggregate) Exposure to Caffeine from Background Sources by Caffeine Consumers Relative to Body Weight Using NHANES 2013–14 and FNDDS data (mg/kg bw/day)
Table 14	Total (Aggregate) Absolute Exposure to Caffeine from Background Sources Plus Proposed Uses of Gyusa.g™ by Caffeine Consumers Using NHANES 2013–14 data (mg/day)
Table 15	Total (Aggregate) Exposure to Caffeine from All Background Sources Plus Proposed Uses of Gyusa.g™ by Caffeine Consumers Relative to Body Weight Using NHANES 2013–14 data (mg/kg bw/day)
Table 16	Comparison of Exposure to Caffeine in Children from Background Sources to that from Background Plus Gyusa.g™'s Proposed Use Categories Using NHANES 2013–14 data
Table 17	Summary of Gyusa.g™ Exposure Estimates for the Total Population (Ages 2+)
Table 18	Analysis of GC test article compared to typical batches of Gyusa.g™
Table 19	Summary of Mean Clinical Chemistry Values—90-day Guayusa Concentrate Study
Table 20	Summary of Effects of Guayusa Concentrate and Caffeine
Table 21	Composition Comparison of Green and Roasted Coffee Beans[2] and Gyusa.g™—Dry Basis
Table 22	Composition Comparison of Roasted Coffee Beans and Gyusa.g™—Per Serving
Table 23	Comparison of Individual Chlorogenic Acids in Gyusa.g™ and Various Coffee Roasts
Table 24	Major Conclusions on Caffeine Safety by Scientific and/or Regulatory Organizations
Table 25	Comparison of CoffeeBerry® Extracts and Gyusa.g™
Table 26	Margin of Safety Calculations for Chlorogenic Acids from Gyusa.g™ based on the CoffeeBerry® Ethanol Extract 90-day Feeding Study
Table 27	Estimated Exposure Levels of Other Components of Gyusa.g™
Table 28	Chlorogenic Acid Composition of *Ilex paraguariensis* compared to Gyusa.g™
Table 29	Products Containing Guayusa in the U.S. Marketplace

Part 1: Signed Statements and Certification

1.1 Submission of GRAS Notice

Applied Food Sciences, Inc. (hereinafter called "AFS") (the notifier) is submitting a new GRAS notice in accordance with 21 CFR Part 170, Subpart E, regarding the conclusion that its aqueous *Ilex guayusa* leaf extract, Gyusa.g™, 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

Christine Fields
Vice President, Scientific Affairs
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1.3 Name of the Substance

The common or usual name for the ingredient is aqueous *Ilex guayusa* leaf extract (Gyusa.g™).

1.4 Intended Conditions of Use

AFS' Gyusa.g™ is intended to be added into energy bars, energy drinks, ready to drink tea beverages, carbonated drinks, coffee-like beverages and enhanced waters, at addition levels based on maximum caffeine concentrations of 60–125 mg per serving as described in detail in Part 3. It is not intended for use in foods where standards of identity would preclude their use, infant formula, or any products that would require additional regulatory review by USDA. The extract is also not intended to be used in beverages containing alcohol or to be marketed to consumers for use in highly concentrated caffeine forms, nor in beverages intentionally marketed to young children.

1.5 Statutory Basis for GRAS Conclusion

The GRAS status conclusion for Gyusa.g™ based on its intended conditions use, as stated in Part 1.4 of this report, has been made based on scientific procedures.

1.6 Not Subject to Premarket approval

Gyusa.g™ is GRAS for its intended conditions of use, stated in Part 1.4 of this report, and, therefore, such uses are 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 Applied Food Sciences, Inc., 8708 South Congress Ave, Ste B-290, Austin, TX 78745, or will be sent to FDA upon request.

1.8 Exemption from Disclosure under the Freedom of Information Act

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

1.9 Certification of Completion

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

June 10, 2019

Chris Fields
Vice President, Scientific Affairs
Applied Food Sciences, Inc.
Notifier

Date

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

2.1 Identification

AFS' Gyusa.g™ is an aqueous spray-dried extract 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.[3,4] 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*.[4-8] 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.[4] *I. guayusa* plant constituents include the methylxanthines caffeine and theobromine, phenols, tannins, reductive sugars, steroids, terpenes, carotenoids, flavonoids, and quinones.[1, 4, 9-11]

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).[11] The caffeine concentrations in the more concentrated Gyusa.g™ is 3.5–8.5%, with a maximum caffeine addition level of 125 mg/serving, as discussed in Part 3. 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 $C_8H_{10}N_4O_2$ and a molecular mass of 194.19 g/mol.[12] 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), *I. 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,[13-16] 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 per espresso serving).[17-20]

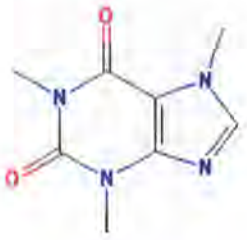


Figure 1. Chemical Structure of Caffeine[21]

Chlorogenic acids (CAs, hydroxycinnamic acid derivatives) are phenolic compounds found in relatively high levels in guayusa leaves.[9] While Gyusa.g™ contains CAs (a typical batch contains ~8.2% CAs as shown in Part 2), it is not standardized to CA content. Phenolic compounds are secondary plant metabolites known to exhibit antioxidant activities[22-24] and have been associated with a host of beneficial effects largely attributed to their inherent antioxidant potentials.[25-27]

Hydroxycinnamic acid derivatives are the major subclass of plant phenolic acid compounds.[28-30] 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.[31] 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.[32-34]

In its classical singular form, CA refers to 5-*O*-caffeoylquinic acid (5-CQA), although it is still often called 3-caffeoylquinic 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 chlorogenic acids, has been reviewed in the literature.[35, 36] 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.[20, 35-37] 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[27, 30, 38]; 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.[30, 39] These include dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), diferuloylquinic acids (diFQA), *p*-coumaroylquinic acids (pCoQA), caffeoylferuloylquinic acids (CFQA), dimethoxycinnamoyl-caffeoylquinic acids (dimCQAs) and others.[40] The major CAs in green/roasted coffee beans are 5-CQA, 3-CQA and 4-CQA (all three have a molecular formula of $C_{16}H_{18}O_9$ and a molecular weight of 354.311 g/mol), with lower amounts of FQAs and diCQAs (see Figure 2 below). As shown later in this report, the same CAs are present in guayusa. It should be noted that caffeic acid (i.e., 3,4-dihydroxy-cinnamic acid) is not detected in the finished Gyusa.g™ product using ultra-high performance liquid chromatography (UHPLC). The composition of the individual CAs with regard to R-group substitutions are also shown for clarity in Table 1 below.

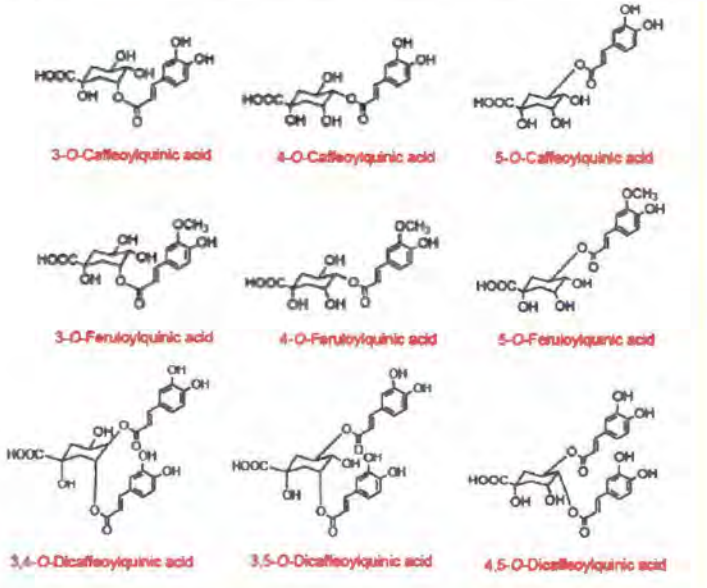
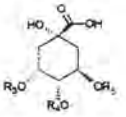


Figure 2. Chemical Structures (IUPAC nomenclature) of Major Chlorogenic Acids in Green Coffee Beans (figure borrowed in part with permission from del Rio et al., 2010)[41]

Table 1. R-group Substitutions of Quinic Acid in Chlorogenic Acids (table borrowed in part from Kremr et al., 2016; Structure of (-)Quinic Acid Shown in Top Row)[37]



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

Theobromine and theophylline are other naturally occurring methylxanthines, the former of which has been shown to be present to some degree in the guayusa plant.[42-45] Testing of Gyusa.g™ consistently shows that it does not contain theobromine or theophylline.

Garcia-Ruiz et al. (2017) characterized the polyphenols found in guayusa.[9] 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.[10] 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.[11, 46]

2.2 Manufacturing

2.2.1 Manufacturing Overview

The manufacturing process for Gyusa.g™ begins with the harvesting of fresh guayusa leaves from growers in Central America. When the leaves reach the factory, they are held in a pre-drying area for 20 hours, which reduces their moisture content by approximately 40%. Batches of leaves are then shipped to a manufacturing facility where an industrial dryer dries the leaves at 58 °C for 5 hours in order to reduce the moisture content to <8%. The leaves are milled and pulverized to reduce their particle size and are then sorted into five different size grades. 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 55 kg of dried milled guayusa. The milled ground leaves are then shipped to AFS for extraction.

Gyusa.g™ is produced from the dried guayusa leaves. The water-soluble fraction of aqueous leaf extract (manufactured with a process comparable to brewing tea on a large scale) is transferred to a multi-stage evaporator to increase the final concentration of caffeine and remove the water content in the guayusa leaf slurry. The concentrated extract is filtered to remove any insoluble solids and then spray-dried (without a carrier), resulting in a light brown water-soluble powder that is filtered again to obtain uniform particle size and ensure a clean finished dry powder with a water content less than 3% wt/wt. The final powder is packed in polytetrafluoroethylene (PTFE) bags and sealed in food-grade plastic drums. A flow chart diagram of Gyusa.g™ production is shown below in Figure 3.

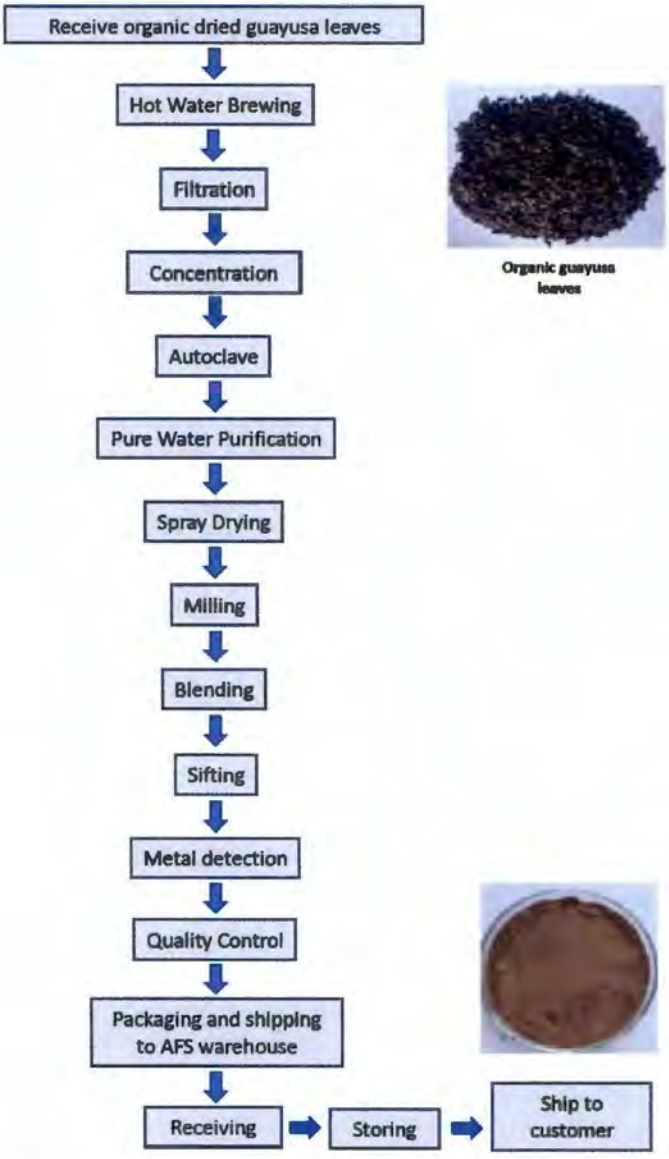


Figure 3. Manufacturing Flowchart for Gyusa.g™

2.2.2 Good Manufacturing Practice

Production of Gyusa.g™ complies with laws and governmental regulations of the US FDA and the European Community and their member states as well as with other standards such as Ph. Eur, USP, FCC, HACCP and WHO. These apply to production unit operations, biotechnological processing aids, utilities, and quality control and assurance procedures. Independent, third party auditors (NSF and SGS)

are used to assess the Food Safety Programs at the production facilities 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 guidelines as set forth in the current 21 CFR §110 and §117.

2.2.3 Raw Materials

Raw materials used in the production of Gyusa.g™ are of appropriate food grade and/or conform to 21 CFR §110.80(a)(2)–(4) requirements. No material of human or animal origin is used in the manufacturing process. Gyusa.g™ is not manufactured from genetically modified plant material and are not produced using irradiation or ethylene oxide treatments.

2.3 Specifications

The specifications for the food-grade Gyusa.g™ product, along with the specification methods are listed in Table 2 below.

Table 2. Gyusa.g™ Specifications

Tested Parameters	Specification	Method
Characteristics		
Appearance	Brown-greenish powder	Visual
Loss on Drying	<5%	USP <731>
Caffeine Content	6 ± 2.5% (3.5–8.5%)	FCC 10^{12} (HPLC)
Solubility	>95.0% w/w	Internal AFS SOP 0.4-008, for 0.50 w/w % solution
Heavy Metals		
Lead	<0.25 ppm	AOAC 2013.06 (ICP-MS)
Arsenic	<0.15 ppm	AOAC 2013.06 (ICP-MS)
Cadmium	<2.5 µg/g	AOAC 2013.06 (ICP-MS)
Mercury	<1.5 µg/g	AOAC 2013.06 (ICP-MS)
Total Heavy Metals	<20 ppm	AOAC 2013.06 (ICP-MS)
Microbiological Tests		
Escherichia coli	Negative/25 g	USP <62>
Total Coliform	<10 MPN/g	AOAC 966.24, BAM
Salmonella	Negative/25 g	USP <62>
Staphylococcus aureus	Negative/25 g	USP <62>
Yeast & Mold	<100 colonies/g	USP <61>
Aerobic Total Plate Count	$<10^4$ CFU/g	AOAC 990.12, BAM

Abbreviations: AFS, Applied Food Sciences, Inc.; AOAC, Association of Analytical Communities; BAM, FDA's Bacteriological Analytical Manual; CFU, Colony forming unit; ICP-MC, HPLC, High performance liquid chromatography; Inductively coupled plasma mass spectrometry; LOD, Loss on drying; MPN, Most probable number; UPS, United States Pharmacopeia.

2.3.1 Batch Analysis

Production conformity and consistency of Gyusa.g™ 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 physical/chemical composition, heavy metals, and microbial analyses.

Table 3. Gyusa.g™ Batch Analyses

Test Items	Specification	F12455	F12662	F13020
Characteristics				
Appearance	Brown-greenish powder	Brown-greenish powder	Brown-greenish powder	Brown-greenish powder
Loss on Drying	<5%	2.48%	2.20%	2.34%
Caffeine Content	6 ± 2.5%	5.25%	5.05%	5.75%
Appearance	Brown-greenish powder	Brown-greenish powder	Brown-greenish powder	Brown-greenish powder
Solubility	>95.0 w/w %	98.67 w/w %	98.77 w/w %	98.97 w/w %
Heavy Metals				
Lead	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm
Arsenic	<0.15 ppm	< LQL*	< LQL*	< LQL*
Cadmium	<2.5 µg/g	< LQL*	< LQL*	< LQL*
Mercury	<1.5 µg/g	< LQL*	< LQL*	< LQL*
Total Heavy Metals	<20 ppm	< LQL*	< LQL*	< LQL*
Microbiological Tests				
E. coli	Negative/25 g	Negative/25 g	Negative/25 g	Negative/25 g
Total Coliform	<10 CFU/g	<10 CFU/g	<10 CFU/g	<10 CFU/g
Salmonella	Negative/25 g	Negative/25 g	Negative/25 g	Negative/25 g
Staphylococcus aureus	Negative/25 g	Negative/25 g	Negative/25 g	Negative/25 g
Yeast & Mold	<100 colonies/g	80 colonies/g	90 colonies/g	70 colonies/g
Aerobic Total Plate Count	$<10^4$ CFU/g	1000 CFU/g	1000 CFU/g	1000 CFU/g

*LQL = less than lower quantifiable limit (LQL for lead = 0.002 ppm; mercury = 0.008 ppm; cadmium = 0.004 ppm; arsenic = 0.004 ppm).

2.3.2 Residual Solvent Analysis

In the production of Gyusa.g™, the only solvent used is water; thus, residual solvent tests are not performed.

2.3.3 Residual Pesticide Analysis

In accordance with standard operating procedures, AFS performs 3rd party pesticide testing of every fresh harvest of guayusa leaves raw material. Occasional batch testing for pesticide residues is also performed based on volume and standard deviation calculations. Pesticide tolerance limit specifications for coffee / coffee

beans in 40 CFR Part 180 are utilized as the most notably similar products to guayusa.

2.3.4 Additional Product Analysis

Analytical results of typical batches of Gyusa.g™ for CAs and overall nutritional composition are shown in the two tables below. Note that while not shown in a table format, UHPLC/UV analysis of Gyusa.g™ for catechins, theobromine and theophylline was also performed. Theobromine and theophylline were not detected in the extract, while catechin compounds were "barely" detected.

Table 4. Analysis of individual Chlorogenic Acids Present in a Typical Batch of Gyusa.g™

Analyte*	Gyusa.g™ Results (%)*
3-CQA + 5-CQA	1.9
3,4-diCQA + 3,5-diCQA + 4,5-diCQA	3.6
Minor/other CQAs including 4-CQA	2.7
Total Chlorogenic Acids	8.2

*Chlorogenic acid and related compounds are quantified based on 3-CQA (5-CQA IUPAC) and are reported as percentage of the whole product

Table 5. Gyusa.g™ Typical Nutritional Analysis

Label Analytes	Results per 100 g
Calories	385.6
Calories from fat	<1.0
Total Fat (g)	0
Saturated Fat	0
Trans Fat	0
Cholesterol	0
Total Carbohydrate (g)	78.8*
Dietary Fiber (g)	< 1.0
Sugars (g)	2.93
Protein (g)	18.5
Total Vitamin D (μg)	<1.0
Sodium (mg)	124
Potassium (mg)	531
Iron (mg)	<0.47
Calcium (mg)	67
Ash (%)	1.46

*Consists of polysaccharides, cellulose, hexoses (e.g. glucose, galactose), 6-deoxyhexoses (e.g. rhamnose) and pentoses (e.g arabinose, xylose), etc.

2.3.5 Shelf-Life Stability

Gyusa.g™ production lots were evaluated in a shelf-life stability study as shown in the table below. The finished products are packed in 5 kg double lined food grade polyethylene bags in 25 kg HDPE drums. The recommended storage conditions are ambient, low moisture in original packaging. The production samples were packed in sealed plastic bags and were stored at room temperature in an enclosed ambient condition cabinet.

Table 6. Gyusa.g™ Stability Study

	F12455 (manufactured February 2016)	F12662 (manufactured July 2016)	F13020 (manufactured January 2017)
Caffeine (%)			
DOM	5.25	5.05	5.75
2/12/17	5.35	4.78	5.77
8/15/18	5.29	5.22	6.10
Mean average delta from DOM	0.04	0.17	0.35
Moisture (LOD) (%)			
DOM	2.48	2.20	2.34
8/15/18	2.44	2.12	2.50
Mean difference	0.02	0.04	0.07

Abbreviations: DOM, date of manufacture; LOD, loss on drying

Powdered forms of caffeine tend to be stable for years.[47] The stability study results shown above suggest that after 19–37 months of storage, Gyusa.g™ levels of caffeine and moisture aligned with product specifications. Recommended shelf-life is suggested at 30 months from date of manufacture until parameters are further investigated.

2.4 Physical or Technical Effect

Gyusa.g™ is intended to be used as natural antioxidant and caffeine delivery ingredient. The antioxidant effects of the extract are not expected to impact safety. The physical effects of caffeine are well known and are discussed in Part 6 of this report. The addition levels necessary for flavoring and effects on, for example, alertness or physical performance, vary based on individual sensitivities. The maximum intended caffeine addition levels for this extract are considered safe as detailed in Part 6. In summary, at the levels of intended use, Gyusa.g™ is not intended to produce any physical or other technical effects that are relevant to its the safety as an ingredient; data and information regarding the quantity of caffeine,

and, hence, the quantities of Gyusa.g™ that would be required to produce an effect that bears on safety are discussed in Part 6.

Part 3: Dietary Exposure

3.1 Intended Use

Gyusa.g™, manufactured in accordance with cGMP, is intended to be used as ingredient in energy bars and drinks, ready to drink tea beverages, carbonated drinks, coffee-like beverages, and enhanced water beverages, at addition levels based on maximum caffeine concentrations of 60–125 mg per serving as shown in the tables below. The extract is not intended for use in foods where standards of identity would preclude their use, infant formula, or any products that would require additional regulatory review by USDA. The extract is also not intended to be used in beverages containing alcohol and is not intended to be marketed to consumers for use in highly concentrated caffeine forms, nor in beverages intentionally marketed to young children. A summary of the intended uses for Gyusa.g™ is shown in Table 7 below:

Table 7. Intended Use Categories and Addition Levels for Gyusa.g™ in Terms of Total Extract and Caffeine Levels

Food category (usual serving size)	Maximum caffeine addition levels		Gyusa.g™ addition level (range, based on the specification range for caffeine concentration; 3.5–8.5%)	
	as maximum mg/g or mg/mL (ppm)	as maximum mg/usual serving	mg/g or mg/mL	mg/usual serving
Energy Bars (50 g)	1.4 (1400)	70	16–40	824–2000
Energy Drinks (360 mL)	0.347 (347)	125	4.1–9.9	1470–3570
RTD Tea* (360 mL)	0.263 (263)	95	3.1–7.5	1118–2710
Carbonated Drinks (360 mL)	0.2 (200)	72	2.4–5.7	847–2057
Coffee-like Beverages (360 mL)	0.263 (263)	95	3.1–7.5	1118–2710
Enhanced Waters (240 mL)	0.25 (250)	60	2.9–7.1	706–1710

RTD: Ready to drink
*May include kombucha

3.1.1 Energy Bars

Energy bars are reasonably popular in the current food market. The intended use of Gyusa.g™ will result in a maximum level of 70 mg of caffeine per usual 50 g serving size of an "energy bar", which is similar to levels in some of the other energy bars on the market now. For example, according to the Caffeine Informer website (https://www.caffeineinformer.com/caffeine-content), Clif Peanut Toffee Buzz bars® contain 50 mg caffeine per bar (from green tea extract) and Awake bars contain 50–110 mg caffeine per bar. Verb Energy Co® bars contain approximately 100 mg caffeine per bar (https://www.verbenergybar.com/). Probar Base® Chocolate Bliss and Coffee Crunch contain 55 mg of caffeine per bar (from yerba maté) (http://theprobar.com/protein-caffeine-new-base-protein-bar-flavors/). Powerbar® (Coconut flavor) contains 33 mg of caffeine per bar (https://www.powerbar.eu/en_GB/products/energize). While not in the energy bar category, for comparison, a 28 g chocolate bar has been reported to contain 11 to 115 mg of caffeine.[48] On some occasions energy bars might be consumed in a substitutive manner with regard to chocolate bars. The addition of Gyusa.g™ will result in approximately 68–164 mg of CAs per 50 g bar (based on a typical batch analysis resulting in 8.2% CAs); CAs are not known to be typical ingredients of other bars in the marketplace; thus, a comparison of levels is not possible or relevant. However, this CA addition level is equal to or less than that found in typical servings of coffee or espresso.[19, 20, 30, 49-52]

3.1.2 Energy Drinks

Energy drinks are currently ubiquitous in the U.S. 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 Gyusa.g™ to energy beverages will give a maximum delivery of 125 mg of caffeine per 360 mL serving, or a maximum concentration of 0.347 mg/mL (347 ppm) caffeine. The caffeine concentration from the intended use is similar to that found in the more common energy beverages in the marketplace and is significantly lower than that found in others. For example Red Bull contains 80 mg caffeine/8.3 oz. (326 ppm) and Monster Energy, Rockstar and Java Monster contain 160 mg/16 oz. (338 ppm), while other energy drinks contain up to 280 mg of caffeine per serving, or up to a concentration of 500 ppm.[18, 48] Aside from caffeine, energy drinks may also contain additional ingredients such as fruits or vegetable juices/flavors (e.g. coconut water), sweeteners, herbal teas, and nutrients. The addition of Gyusa.g™ will result in approximately 121–293 mg of CAs per 360 mL serving (based on a typical batch analysis resulting in 8.2% CAs). CAs are not known to be typical ingredients in energy beverages in the marketplace; thus, a comparison of levels is

not possible or relevant. However, this CA addition level is equal to or less than that found in typical servings of coffee or espresso.[19, 20, 30, 49-52]

3.1.3 Ready to Drink Tea Beverages

Bottled ready to drink tea beverages are becoming quite popular in the U.S. marketplace as well. The intended use of Gyusa.g™ in ready to drink tea beverages may also include fermented tea beverages such as kombucha, as long as the alcohol by volume content in such beverages remains below 0.5% (the level considered "non-alcoholic" by FDA and the Alcohol and Tobacco Tax and Trade Bureau).[53] The intended addition level of Gyusa.g™ to ready to drink tea-like beverages is up to a maximum of 95 mg caffeine per 360 mL tea beverage serving, or a maximum concentration of 263 ppm caffeine. This caffeine concentration is intended to replace/substitute for caffeine from other tea sources, and the level is similar to that in a number of tea beverages currently sold in the U.S. market as cited by Somogyi et al.[48] (e.g., Starbucks Tazo Chai Latte Grande (100 mg caffeine/16 oz. = 211 ppm), Pacific Chai (100 mg caffeine/12 oz. = 282 ppm), generic black tea (up to 74 mg caffeine/8 oz. = 312 ppm), and oolong tea (up to 64 mg caffeine/8 oz. = 270 ppm)). The addition of Gyusa.g™ will result in approximately 92–222 mg of CAs per 360 mL serving (based on a typical batch analysis resulting in 8.2% CAs). CAs are not known to be typical prominent ingredients of ready to drink tea beverages in the marketplace; thus, comparisons to other products cannot be made. However, maté tea beverages, consumed regularly in South American countries and gaining some popularity in the U.S. can contain high concentrations of CQAs and diCQAs (65.6–575.5 mg/mL and 105.3–460.2 mg/100 mL, respectively), with intake of CAs from these beverages calculated to be 512.5–1779.7 mg per day.[54] Additionally, the 187 mg/serving addition level intended use for Gyusa.g™ is equal to or less than levels found in typical servings of coffee or espresso.[19, 20, 30, 49-52]

3.1.4 Carbonated Drinks

The intended use level for Gyusa.g™ in carbonated beverages will result in caffeine concentrations that are allowable and GRAS pursuant to 21 CFR §182.1180 for cola-type beverages (i.e., up to 200 ppm, or 72 mg per 12 oz./360 mL serving). The caffeine from Gyusa.g™ is intended to substitute for other caffeine sources used in carbonated beverages. The addition of Gyusa.g™ will result in approximately 69–169 mg of CAs per 360 mL serving (based on a typical batch analysis resulting in 8.2% CAs). CAs are not known to be typical ingredients of carbonated beverages in the marketplace; thus, comparisons to other products cannot be made and are not relevant. However, this CA addition level is equal to or less than that found in typical servings of coffee or espresso.[19, 20, 30, 49-52]

3.1.5 Coffee-like Beverages

In coffee-like beverages, caffeine from Gyusa.g™ is intended to replace caffeine that normally comes from roasted coffee beans and is consumed in brewed coffee. The coffee-like beverages may take the form, for example, of ready to drink cappuccino-like beverages. The maximum intended addition level of the extract will result in up to 95 mg caffeine per 12 oz. serving, or a maximum concentration of 263 ppm caffeine. This maximum caffeine level tends to be typical or lower than that found in many of the coffee beverages on the market, as reported by Somogyi et al. (e.g., Starbucks Tall Americano coffee and Starbucks Grande brewed coffee contain 330 mg/16 oz. = 696 ppm).[48] The addition of Gyusa.g™ will result in approximately 92–222 mg of CAs per 360 mL (12 oz.) serving (based on a typical batch analysis resulting in 8.2% CAs), which is within the range and is expected to be substitutive for normal levels reported in other coffee beverages in the marketplace. For example, a single serving of brewed coffee and/or an espresso beverage may contain from 15 mg to 675 mg CAs.[19, 20, 30, 49-52] Espresso beverages from various locations were recently analyzed and found to contain 24–422 mg of CAs per single serving.[19]

3.1.6 Enhanced Water

The enhanced water category refers to non-carbonated beverages that may not readily fit into the other intended use beverage categories. While typically fortified/enhanced waters do not contain caffeine, the category was still used as a surrogate for exposure estimates in order to be conservative. In reality, these beverages, with the addition of caffeine, could possibly be classified as energy drinks or ready to drink tea beverages, and will likely be consumed as a substitute for other beverages that contain caffeine, although the maximum intended caffeine concentration identified for this category is slightly lower. The intended addition level of Gyusa.g™ to these enhanced waters will give a maximum delivery of 60 mg of caffeine per 240 mL serving, or a maximum concentration of 250 ppm caffeine. The addition of Gyusa.g™ will result in approximately 58–140 mg of CAs per 240 mL serving (based on a typical batch analysis resulting in 8.2% CAs). CAs are not known to be typical ingredients of other enhanced waters in the marketplace; thus, a comparison of levels is not possible or relevant. However, this CA addition level is equal to or less than that found in typical servings of coffee or espresso.[19, 20, 30, 49-52]

3.2 Exposure Estimates for *Ilex guayusa* Leaf Extracts

3.2.1 Exposure to Gyusa.g™ using NHANES data

Exposure to Gyusa.g™ 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 data available at the time of this writing (2013–2014) was analyzed using Creme Food Safety software 3.6 (www.cremeglobal.com). These data were obtained from 7,574 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 were utilized in the assessment and were assigned the relevant intended use concentrations. Note that because there are no or few caffeinated examples of enhanced waters and bars, for example, the assessment utilized consumption of various "non-caffeinated" products as surrogates. This is a very conservative approach as it assumes that caffeinated products would be consumed in similar amounts/patterns as non-caffeinated products and, as such, would not be substitutive but would rather lead to added caffeine intake. In reality, it is considered likely that these products will be consumed as a replacement for other products containing caffeine due to consistent data indicating that caffeine consumption remains stable in the U.S. population despite many new caffeinated products being added to the market.[14-16, 55-58] Food codes that specifically state "decaffeinated" in their title were not utilized in the assessment, but food codes that are not explicitly named to not contain caffeine were utilized.

Creme Food Safety software is a probabilistic modeling tool that uses high-performance computing to predict intake (including total aggregate exposure) of food groups and/or individual food ingredients. Creme Food Safety performs calculations using large-scale food consumption data sets. It bases the calculated estimates on each individual's body weight from the survey, as opposed to averaged body weights. In other words, tabulated results for absolute exposure (mg/day) and exposure relative to body weight (mg/kg bw/day) cannot be compared using a standard (e.g., 70 kg) weight factor. Calculations also incorporated the NHANES assigned sample weights for each individual in the survey, which measure the number of people in the population represented by that specific subject and help to ensure that the results statistically represent the entire U.S. population. Sample weights for NHANES participants incorporate adjustments for unequal selection probabilities and certain types of non-response, as well as an adjustment to independent estimates of population sizes for specific age, sex, and race/ethnicity categories. The data are shown for "food consumers" (which includes only data from individuals who reported consuming one or more food/beverage categories

intended to contain the ingredients 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).[59] RSE values greater than 25–30% are often considered reasonable cut-offs by which to consider a value unreliable.[59, 60] For the purpose of this GRAS conclusion, 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 (i.e., Daily Averages) do not necessarily adequately represent individual usual long-term intake due to the large amount of random error. This is because the data 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.[61] These lifetime data are considered the most relevant data, as GRAS 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.

Exposure estimates for Gyusa.g™ based on the maximum levels of the extract required to achieve the maximum caffeine use levels stated in Tables 7 are shown below in Tables 8 and 9. Note that while the assessments shown in later subparts (related to exposures to caffeine and CAs from Gyusa.g™) were given in the context of background consumption levels of those constituents, guayusa leaf is not considered a commonly consumed food/food ingredient in the U.S., and thus, background consumption levels were not considered relevant and were not addressed in the assessments of exposure to the total extract in this Subpart.

Table 8. Total (Aggregate) Absolute Exposure to Gyusa.g™ by Proposed Use Food Consumers Using a 100% Presence Probability Factor and NHANES 2013–14 data (mg/day)

Population Group	Age in yrs	N (% of total)	Absolute Gyusa.g™ consumption Daily Average (mg/day)				90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/day)
			Mean	Mean std err	90th%	90th% std err		
Children	2–12	851 (52.3)	1390.0	64.8	3009.9	161.8	5.4	2218.5
Adolescents	13–18	667 (80.0)	3204.32	176.8	6623.2	666.4	10.1	5582.3
Adults	19–49	2109 (86.8)	5579.3	136.8	10888.7	400.3	3.7	10070.1
Adults	50+	1950 (91.2)	5684.9	210.0	10800	372.9	3.5	9961.9
Women of Reproductive Age	14–44	1233 (84.1)	4433.6	156.3	9375.6	497.5	5.3	7920.0
Total Population	2+	5577 (82.8)	5048.0	103.5	10381.6	253.5	2.4	9249.0

Creme run #313

Table 9. Total (Aggregate) Exposure to Gyusa.g™ by Proposed Use Food Consumers Relative to Body Weight Using a 100% Presence Probability Factor and NHANES 2013–14 data (mg/kg bw/day)

Population Group	Age in yrs	Gyusa.g™ consumption relative to body weight Daily Average (mg/kg/bw/day)					90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/kg bw/day)
		N (% of total)	Mean	Mean std err	90th%	90th% std err		
Children	2–12	851 (52.3)	46.6	2.09	97.7	8.7	8.9	71.2
Adolescents	13–18	667 (80.0)	46.9	2.6	88.7	10.2	11.5	82.0
Adults	19–49	2109 (86.8)	69.6	1.7	135.9	4.8	3.5	126.5
Adults	50+	1950 (91.2)	71.2	2.9	134.1	6.9	5.1	124.1
Women of Reproductive Age	14–44	1233 (84.1)	60.8	2.1	125.8	5.3	4.2	108.4
Total	2+	5577 (82.9)	66.3	1.4	129.1	3.8	2.9	117.3

Creme run #313

The exposure analyses above suggest that a majority (approximately 82.8%) of the total population (ages 2+) may be exposed to Gyusa.g™ from the stated intended uses. The 90th percentile lifetime exposure estimates for the total population were 9249.0 mg per day and 117.3 mg/kg bw/day.

These results are considered extremely conservative as they assume that 100% of the products from the relevant food codes in the marketplace will contain Gyusa.g™

at the respective maximum addition levels. While food labels will list the extract as an ingredient and may even highlight it in marketing, it is assumed that many consumers will not always realize that it is present in the food, as consumers may be searching out a caffeinated product but not necessary a product containing caffeine from guayusa. In other words, the Gyusa.g™ in products may be an "invisible" ingredient to many consumers in terms of a caffeine source, which decreases the chance that only food products that contain it will be chosen by those consumers daily throughout a lifetime. Additionally, there will be cost and market share limitations of adding the extract to foods in general, making it even less likely that an individual would consume them in all of the intended use food groups daily.

Thus, in order to calculate a slightly more realistic exposure estimation for Gyusa.g™ from the proposed food uses, an additional Creme exposure assessment was performed that assumed a presence probability of 20% Gyusa.g™ in all of the proposed food categories. The 20% presence probability factor was intended to represent an approximate 20% market share of the ingredient in foods from each of the intended use categories, which is still considered a highly conservative, yet more realistic, assumption. The maximum addition level for each ingredient in the food categories was still utilized. The resulting exposures to Gyusa.g™ by consumers using the 20% presence probability factor are shown in Tables 10 and 11 below:

Table 10. Total (Aggregate) Absolute Exposure to Gyusa.g™ by Proposed Use Food Consumers Using a 20% Presence Probability Factor and NHANES 2013–14 data (mg/day)

Population Group	Age in yrs	N (% of total)	Absolute Gyusa.g™ consumption Daily Average (mg/day)				90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/day)
			Mean	Mean std err	90th%	90th% std err		
Children	2–12	271 (16.6)	838.0	50.2	1551.2	101.2	6.5	1142.1*
Adolescents	13–18	270 (33.2)	1663.7	113.4	2959.5	288.8	9.8	2959.5**
Adults	19–49	1063 (45.1)	2189.7	74.7	4200.3	282.1	6.7	2981.4
Adults	50+	1037 (51.5)	2034.3	93.6	4050.0	336.5	8.3	3281.0
Women of Reproductive Age	14–44	567 (39.9)	1948.6	104.9	3938.6	448.8	11.4	3938.6*
Total Population	2+	2641 (42.3)	2011.6	54.6	4050.0	180.7	4.5	3116.2

Creme run #316

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

**Creme Failure 12 "Negative variance of Usual Intakes (too few people with multiple observations). Daily Average used instead.

Table 11. Total (Aggregate) Exposure to Gyusa.g™ by Proposed Use Food Consumers Relative to Body Weight Using a 20% Presence Probability Factor and NHANES 2013–14 data (mg/kg bw/day)

Population Group	Age in yrs	Gyusa.g™ consumption relative to body weight Daily Average (mg/kg/bw/day)					90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/kg bw/day)
		N (% of total)	Mean	Mean std err	90th%	90th% std err		
Children	2–12	271 (16.6)	29.6	2.0	54.3	5.7	10.5	43.0
Adolescents	13–18	270 (33.2)	24.2	1.6	42.3	5.5	13.0	42.3*
Adults	19–49	1063 (45.1)	26.8	0.9	48.8	2.5	5.1	34.8
Adults	50+	1037 (51.5)	25.4	1.3	52.3	3.7	7.1	44.1**
Women of Reproductive Age	14–44	567 (39.9)	26.3	1.4	47.1	4.9	10.4	47.1*
Total	2+	2641 (42.3)	26.2	0.7	50.0	2.2	4.4	39.7

Creme run #316
*Creme Failure 12 "Negative variance of Usual Intakes (too few people with multiple observations). Daily Average used instead.
**Creme Warning -32 "Fourth moment of Usual intakes less than 3.0. Data can still be used.

Using the 20 percent presence probability factor in the assessments, the exposure estimates decreased by approximately a third compared to the assessments at 100% presence probability. These results are considered more realistic but still quite conservative. The results suggest that 41–42% of the population (ages 2+) may be exposed to Gyusa.g™ from one or more of the intended use categories. The 90th percentile lifetime exposure estimates for the total population were 3116.2 mg/day and 39.7 mg/kg bw/day.

3.2.2 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.[3, 4, 6, 7, 62-64] 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.[4, 62, 65] 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.[4] 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 society, and is considered the most commonly used plant species in the culture.[63] 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.[4] 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.[6] 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.[6]

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).[4, 63] 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).[3] 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.[3] 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.[3] 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.[3, 4]

Several other species of Ilex are consumed by humans in the form of herbal teas in various parts of the world.[66] *I. paraguariensis* is consumed as yerba maté tea.[5] Guayusa tea preparations and drink consumption patterns resemble that of yerba maté,[6] although *I. paraguariensis* has a comparably lower concentration of caffeine (0.78–1.25%).[5] *I. ambigua* is also known to contain caffeine.[3, 4] *I. vomitoria,* native to North America, was also consumed as yaupon tea by Native Americans and European colonists,[66-68]and *I. kudingcha, I. latifolia, I. cornuta* and *I. pentagona,* are consumed as Chinese Kudingcha tea.[8, 11, 66, 69, 70] 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.*[5, 11]

3.2.3 Summary of *Ilex guayusa* Leaf Extract Exposures

In summary, exposure to Gyusa.g™ based on its intended uses was evaluated using Creme Global software. Using a 20% presence probability factor, lifetime exposures at the 90th percentile were estimated at 3116.2 mg/day and 39.7 mg/kg bw/day. 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 tea in China).

3.3 Caffeine Dietary Exposure Estimates

3.3.1 Caffeine Exposure Estimates based on Intended Uses

Gyusa.g™ contains 3.5–8.5% caffeine by weight. 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.[14-16, 55-57] The food products that will contain caffeine from Gyusa.g™ are expected to generally replace consumption of other similar caffeinated products available in the marketplace. Thus, exposure to caffeine from products containing Gyusa.g™ is expected to be mainly 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 products on the market.

While caffeinated energy bars may appear to be a novel food category, in fact there are a number of such products already in the marketplace (as summarized in Subpart 3.1.1 above). Chocolate bars, which may be considered a separate category from energy bars, are certainly not novel and also contain caffeine, and consumption of chocolate bars might logically be replaced with energy bar consumption as well by some individuals. 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).[48] Thus energy bars are not expected to carry a large weight with regard to overall caffeine exposure, and are expected to be substitutive for consumption of other energy bars (and again, possibly chocolate bars).

While the enhanced water beverage products were treated as novel caffeine containing beverages with regard to caffeine exposure assessments, the product labels will state that they contain caffeine, and thus, they are arguably likely to be consumed as a substitute for other caffeinated beverages such as teas or energy drinks. Nevertheless, these food codes were considered as surrogates for the NHANES data exposure analysis (instead of considering tea or energy beverage

surrogates) in order to be conservative, which likely resulted in an additive exposure estimate with regard to caffeine as opposed to a substitutive exposure.

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). The FNDDS database 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 from the background diet was then derived using analysis by Creme software and is shown in the tables below. Tables 12 and 13 show background caffeine consumption for the U.S. population (absolute and relative to body weight, respectively), while Tables 14 and 15 show the estimated exposure to caffeine from Gyusa.g™'s intended use categories combined with intake from background caffeine sources and using 100% presence probability of the extract. As mentioned previously, while food codes for specifically decaffeinated products were not utilized in the analysis, food codes that do not specify decaffeination but likely don't normally contain caffeine (such as herbal teas and various carbonated beverages) were utilized in order to be conservative.

Table 12. Total (Aggregate) Absolute Exposure to Caffeine from Background Sources by Caffeine Consumers Using NHANES 2013–14 and FNDDS data (mg/day)

Population Group	Age in yrs	N (% of total)	Absolute caffeine consumption Daily Average (mg/day)				90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/day)
			Mean	Mean std err	90th%	90th% std err		
Children	2–12	1237 (78.9)	13.7	10.6	34.1	3.6	10.6	27.9
Adolescents	13–18	711 (84.7)	55.4	19.3	128.5	24.8	19.3	109.0
Adults	19–49	2171 (90.1)	149.9	4.1	319.4	13.0	4.1	316.3
Adults	50+	2058 (96.2)	181.7	4.2	384.8	16.0	4.2	375.7*
Women of Reproductive Age	14–44	1287 (88.5)	108.3	6.7	241.3	16.2	6.7	222.7
Total Population	2+	6177 (90.2)	137.4	2.6	320.1	8.2	2.6	298.8*

Creme run #204

*Creme Warning -2048: "Number of days per person should be constant for a Foods calculation", Lifetime data may still be used

Table 13. Total (Aggregate) Exposure to Caffeine from Background Sources by Caffeine Consumers Relative to Body Weight Using NHANES 2013–14 and FNDDS data (mg/kg bw/day)

Population Group	Age in yrs	Caffeine consumption relative to body weight Daily Average (mg/kg/bw/day)					90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/kg bw/day)
		N (% of total)	Mean	Mean std err	90th%	90th% std err		
Children	2–12	1237 (78.9)	0.5	0.03	1.2	0.1	8.3	0.9
Adolescents	13–18	711 (84.7)	0.8	0.01	1.8	0.2	11.1	1.6
Adults	19–49	2171 (90.1)	1.9	0.1	4.1	0.2	4.9	4.0
Adults	50+	2058 (96.2)	2.3	0.1	5.0	0.2	4.0	4.8*
Women of Reproductive Age	14–44	1287 (88.5)	1.5	0.1	3.5	0.2	5.7	3.2
Total	2+	6177 (90.2)	1.8	0.04	4.2	0.1	2.4	3.8*

Creme run #204
*Creme Warning -2048 "Number of days per person should be constant for a Foods calculation" Lifetime data may still be used.

Table 14. Total (Aggregate) Absolute Exposure to Caffeine from Background Sources Plus Proposed Uses of Gyusa.g™ by Caffeine Consumers Using NHANES 2013–14 data (mg/day)

Population Group	Age in yrs	N (% of total)	Absolute caffeine consumption Daily Average (mg/day)				90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/day)
			Mean	Mean std err	90th%	90th% std err		
Children	2–12	1340 (84.3)	33.4	1.7	83.8	5.9	7.0	66.2
Adolescents	13–18	758 (88.5)	104.3	6.0	211.3	22.6	10.7	189.6
Adults	19–49	2249 (92.9)	185.9	4.7	374.5	13.0	3.5	358.9
Adults	50+	2111 (97.9)	194.1	7.02	374.1	12.8	3.4	358.0*
Women of Reproductive Age	14–44	1344 (91.5)	145.5	5.43	319.1	17.9	5.6	280.4
Total Population	2+	6458 (93.1)	162.7	3.4	349.6	8.9	2.5	333.0*

Creme run #312
*Creme Warning -2048 "Number of days per person should be constant for a Foods calculation" Lifetime data may still be used

Table 15. Total (Aggregate) Exposure to Caffeine from All Background Sources Plus Proposed Uses of Gyusa.g™ by Caffeine Consumers Relative to Body Weight Using NHANES 2013–14 data (mg/kg bw/day)

Population Group	Age in yrs	Caffeine consumption relative to body weight Daily Average (mg/kg/bw/day)					90th% RSE Value	Lifetime 90th% Exposure Estimates (mg/kg bw/day)
		N (% of total)	Mean	Mean std err	90th%	90th% std err		
Children	2–12	1340 (84.3)	1.14	0.06	2.61	0.18	6.9	2.09
Adolescents	13–18	758 (88.5)	1.53	0.09	3.08	0.27	8.8	2.76
Adults	19–49	2249 (92.9)	2.32	0.06	4.74	0.16	3.4	4.48
Adults	50+	2111 (97.9)	2.43	0.10	4.71	0.21	4.5	4.52*
Women of Reproductive Age	14–44	1344 (91.5)	2.00	0.07	4.30	0.19	4.4	3.85
Total	2+	6458 (93.1)	2.15	0.05	4.44	0.11	2.5	4.17*

Creme run #312

*Creme Warning -2048 "Number of days per person should be constant for a Foods calculation" Lifetime data may still be used.

The exposure estimates above suggest that over 90% of the population is exposed to caffeine on a regular basis. The total population of consumers (age 2+) is exposed to approximately 298.8 mg/day (3.8 mg/kg bw/day) of caffeine from the background diet at the 90th percentile for lifetime exposure (Tables 12 and 13). Women of reproductive age consume approximately 222.7 mg/day (3.2 mg/kg bw/day), and children consume 27.9 mg/day (0.9 mg/kg bw/day) from the background diet at the 90th percentile. None of the population sub-groups evaluated consumed over 400 mg per day (the safe consumption level of caffeine determined for adults)[71, 72] using the 90th percentile lifetime estimates.

Caffeine exposure estimates from the intended uses of Gyusa.g™ (i.e., energy bars, energy drinks, coffee-like beverages, ready to drink teas, carbonated soft drinks and enhanced water beverages) combined with background caffeine concentrations (caffeine intended use concentrations in some cases replaced USDA estimated caffeine concentrations for the intended use categories) are shown above in Tables 14 and 15. Concentrations of caffeine in products such as fortified/enhanced water and some carbonated beverage food codes were previously "zero" and were changed to the intended use addition level. USDA previously did assign caffeine concentrations to some of the "bar" food codes, but those concentrations were replaced by the maximum intended use concentration of Gyusa.g™ for the assessment. Again, the analysis assumed that 100% of the intended use food categories contain Gyusa.g™ at the highest concentration, which is considered an extremely conservative assumption.

The resulting aggregate caffeine exposure estimates for Gyusa.g™ intended uses combined with background intake resulted in levels still below those considered safe for the various population groups analyzed. The estimations suggest that the total population of consumers will be exposed to approximately 333 mg/day (4.17 mg/kg bw/day) of caffeine at the lifetime 90th percentile from intended uses combined with background caffeine exposure.

Caffeine aggregate exposure in children was estimated to increase to 66.2 mg/day (2.09 mg/kg bw/day), compared to background estimates of 27.9 mg/day (0.9 mg/kg bw/day). In looking deeper into the exposure data for children from the individual intended use categories (see Table 16), the increase over background levels at the 90th percentile appears to be related to consumption of energy bars, tea beverages, carbonated soft drinks, and enhanced water beverages. The breakdown of exposures by food group in this table cannot be added up to derive a total exposure because the 90th percentile consumers of one food category are not generally the same individuals that comprise the 90th percentile consumers of another food category; Creme software looks at the actual aggregate data from the NHANES subjects to derive the total aggregate exposure, which is listed in Tables 12–15.

Table 16. Comparison of Exposure to Caffeine in Children from Background Sources to that from Background Plus Gyusa.g™'s Proposed Use Categories Using NHANES 2013–14 data

Intended Use Food Category	90th Percentile Daily Average Consumption as mg/day (mg/kg bw/day)	
	Background Caffeine Exposure	Estimated New Caffeine Exposure from Background Plus Intended Use Categories
Bars* / Energy Bars	1.4 (0.1)	56.0 (1.6)
Energy Drinks	53.9 (1.6)	64.5 (1.9)
RTD Tea	47.2 (1.5)	97.8 (2.9)
Carbonated Soft-drinks	44.6 (1.7)	87.4 (2.6)
Coffee-like Beverages	75.7 (2.2)	67.4 (1.8)
Enhanced Water	0 (0)	90.0 (2.7)

Creme runs #204 and #312

*Note that NHANES surrogate food codes for the energy bar Gyusa.g™ application included non-caffeinated nutrition bars

It should be noted that the products containing Gyusa.g™ will not intentionally be marketed to young children. The products are expected to be clearly labeled with

caffeine content; thus, it is expected that many parents will avoid giving the products to their children for that reason.

With regard to energy bar consumption by children as shown in the above table, the estimate of exposure from Gyusa.g™ was based on consumption of many non-caffeine containing surrogate food categories, including nutrition bars, by NHANES participants. While they were chosen as the most reasonable surrogates that were available for comparison, the consumption patterns in children of these non-caffeine containing bars likely leads to a large overestimation of consumption estimates from Gyusa.g™. Additionally, bars generally come in relatively expensive, finite packaging per serving (unlike snack-type foods such as chips, which often come in larger bags from which multiple servings can be unknowingly consumed at a single sitting). The energy bars will not be intentionally marketed to young children and will be labeled as containing caffeine; they are expected to be consumed intentionally by adults for the purpose of gaining energy, likely in place of a caffeine-containing beverage. Overall it is expected that consumption will actually be much lower than the data shows for children for the reasons stated above.

While the caffeine exposure from carbonated soft drinks appears to have gone up in children with the addition of the intended use caffeine concentrations as compared to background, this is likely because all soft drink food codes (including those with lower and/or zero caffeine levels assigned by FNDDS) were changed for the assessment to the maximum intended use concentration of Gyusa.g™, which is an extremely conservative estimation. It is nearly impossible that this scenario would occur in real life; it is more likely that *I. guayusa* leaf extract beverages will be only a relatively small segment of the total marketplace. Regardless, the estimates were intended to be conservative, and the caffeine addition levels for the carbonated beverages are, again, the GRAS allowable level for caffeine (i.e., the caffeine level is already GRAS for this purpose pursuant to 21 CFR §182.1180). The resulting caffeine exposure in children from the enhanced water category is nearly identical to that from the carbonated soft drinks. The enhanced water will also be marketed to adults and labeled as containing caffeine. It is again unlikely that parents will regularly give the caffeine containing beverages to their children over other non-caffeinated fortified waters that the exposure data relied upon.

Importantly, it should be noted that, despite the reasoning above that utilizes the facts that the products will not be intentionally marketed to children and that many surrogate codes for foods that do not normally contain caffeine were used in the exposure analyses, the conservative caffeine exposure estimate for all of the proposed uses combined with background levels was 2.06 mg/kg bw/day in children, which still falls below the 2.5 mg/kg bw/day that is considered reasonably safe for children by various scientific bodies.[71, 72] Also important is the fact that caffeine consumption has remained stable in the U.S. population in recent years despite many new caffeinated products being added to the market.[14-16, 55-58]

Exposure to caffeine from the intended uses plus background by women of reproductive age was estimated at 280.4 mg/day (3.85 mg/kg bw/day), which was also a slight increase from the background-only caffeine exposure of 222.7 mg/day (3.2 mg/kg bw/day). Again, part of this increase may be due to the fact that surrogate food categories that do not usually contain caffeine were utilized. Nevertheless, the exposure estimates for this population still fell below safe consumption estimates for pregnant women of <300 mg/day as published by Nawrot in 2003 and Wikoff in 2017.[71, 72]

Exposure to caffeine in adults 50 years and older was estimated to actually decrease with the intended use additions compared to background levels alone (358.0 mg/day (4.52 mg/kg bw/day) versus background exposure levels of 375.7 mg/day (4.8 mg/kg bw/day)), likely due to a decreased concentration of caffeine in food categories consumed by this age group in the assessment.

3.3.2 Published Caffeine Exposure Estimates

In addition to the background exposure estimates based on NHANES data above, a number of studies of 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).[13] 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).[13] 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.[48] 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.[73]

While the age groups assessed were different, the 90th percentile results were lower in the 2013–2014 NHANES Creme analysis (tables above) as compared to Mitchell et al.[13] (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)[13] to data collected using a method that assigned caffeine values to beverages using a more general category-specific methodology.[56] 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.[55] 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 Mitchell[13] and Ahluwalia[55] 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 128.5 mg/day and 1.8 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.[74] 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.[57] 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 12 and 13 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.[14] 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.[75] They found that while use of these products remains low overall in the U.S. population (2.7% of the population using NHANES 2007–2010 data), sales increased by 60% over the four year period from 2008 to 2012 while emergency room visits associated with energy drink consumption doubled over the four year period from 2007 to 2011. The highest usage was by males aged 19–30 years.

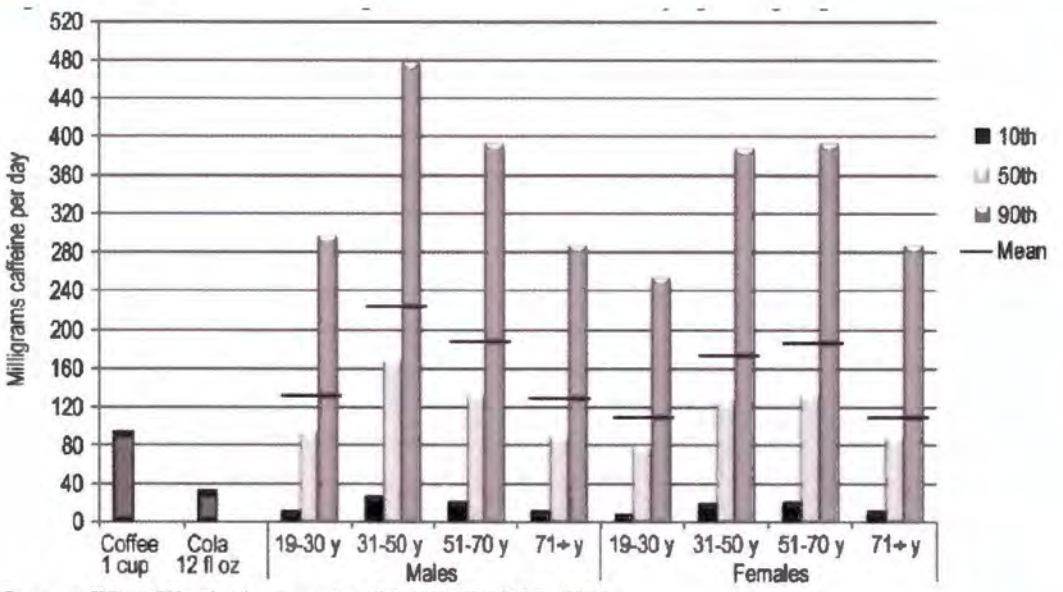
Similar to many of the above investigations, Tran et al. (2016) studied caffeine intake in teens, young adults and adults using NHANES data (2003–2012).[15] Eighty-five percent consumed caffeine (84% via beverages). The percentage remained constant despite new caffeine sources being added to the market. 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 over-estimates 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.[16] 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.

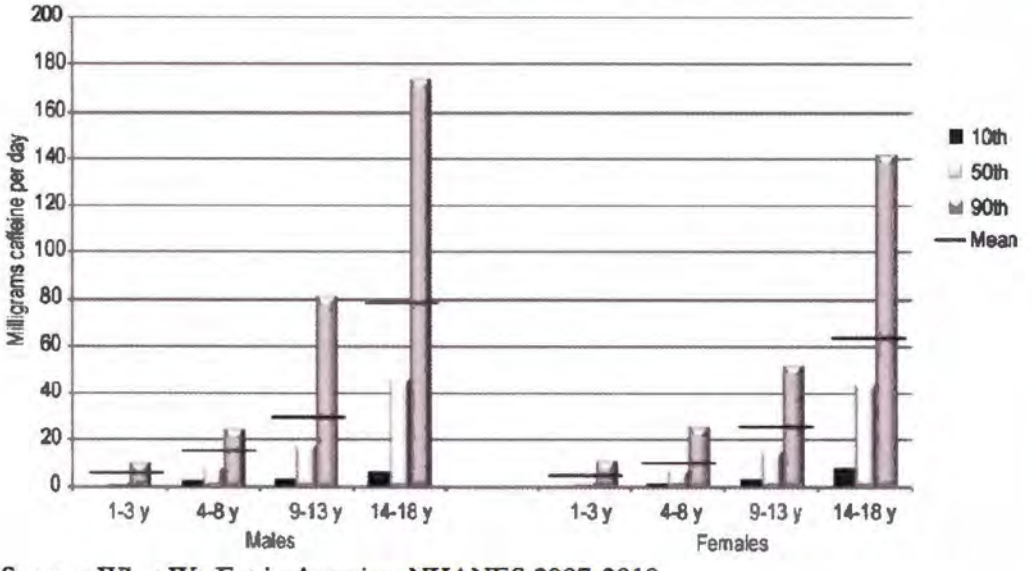
Chen et al. (2014) reported on pre-pregnancy caffeine consumption and changes during pregnancy, based on data from the National Birth Defects Prevention Study (October 1997–December 2007).[76] Of the 8,488 control women analyzed (controls in this large study were mothers of babies without birth defects—this 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.

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 4 and 5 below (which were directly borrowed from the report).[77] 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 Canada[71] 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 4. Mean and Percentiles of Usual Caffeine Intake by Age/Sex Groups; Adults (graph borrowed from DGAC report)[77]



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

Figure 5. Mean and Percentiles of Usual Caffeine Intake by Age/Sex Groups; Children and Adolescents (graph borrowed from DGAC report)[77]

3.3.3 Summary of Caffeine Dietary Exposure Estimates

In summary, caffeine exposure estimates for the U.S. population from the background diet, and background diet plus intended uses of Gyusa.g™ were performed using Creme analysis of NHANES 2013–2014 data. Background diet caffeine exposure from published studies were also summarized.

The results from the 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).[71, 72] Results from other recently published caffeine exposure estimates showed similar results to those from the 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/day[77]). 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.

3.4 Chlorogenic Acids Exposure Estimates

3.4.1 Chlorogenic Acids Exposure Estimates based on Intended Uses

While Gyusa.g™ is not standardized to levels of CAs, a typical batch analysis suggests that CAs and related compounds comprise approximately 8.2% of the extract (see section 2.3.4), which is similar to its concentration of caffeine (maximum of 8.5%). Numerous foods consumed by humans contain CAs, and ubiquitously consumed coffee beans are especially rich in these substances.[32-34] The content of CAs in raw green coffee beans can be as high as 14 g/100 g by dry weight[33, 34]; this value varies depending upon species, demography, agricultural practices, harvesting practices, and method of analysis.[78] Average CA levels in raw coffee beans range from 4.1–7.9 g/100 g for *C. arabica* and 6.1–11.3/100 g for *C. robusta*.[32]

The Gyusa.g™ intended use addition levels per serving of the various food categories shown in Table 7 range from 706–3570 mg/serving. Using the results of a typical batch analysis shown in Table 4 showing ~8.2% CAs, the range of CAs per serving from Gyusa.g™ is ~58–293 mg/serving. As discussed in Part 3.1, this

range is within the levels found in a serving of coffee. For example, a single serving of brewed coffee and/or an espresso beverage may contain from 15 mg to 675 mg CAs.[19, 20, 30, 49-52] Espresso beverages from various locations were recently analyzed and found to contain 24–422 mg of CAs per single serving.[19] As coffee-like beverages are one of the intended uses for the ingredient, and due to the substitutive nature of caffeinated beverages for each other, the CAs from Gyusa.g™ are expected to be at least partially substitutive for those from coffee.

Tables 8–11 show the estimated aggregate exposure to Gyusa.g™ for the total population from its intended uses is 9249 mg/day (117.3 mg/kg bw/day) using a 100% presence probability factor, and 3116.2 mg/day (39.7 mg/kg bw/day) using a 20% presence probability factor. At 8.2% CAs, these data suggest an exposure to 758 mg CAs/day (9.6 mg/kg bw/day) from Gyusa.g™ at 100% presence probability, and 256 mg/day (3.3 mg/kg bw/day) at 20% presence probability. Again, some of this exposure is likely to be substitutive for background exposure from coffee beverages. As there is no specification for CAs for the Gyusa.g™ ingredient, the levels of CAs may naturally vary and these exposure estimates are intended to be very general for discussion purposes.

3.4.2 Published Chlorogenic Acids Exposure Estimates

Instant roasted coffee (caffeinated and decaffeinated) have been reported to have approximately 30–40 mg of CAs per gram.[78, 79] A single cup of brewed coffee contains anywhere from 15 mg to 675 mg CAs.[20, 49-52] Espresso beverages from various locations were recently analyzed and found to contain 24–422 mg of CAs per single serving.[19] Daily intake of CAs by coffee drinkers is considered to be in the range 500–1000 mg.[30, 31, 79-81]

CAs are also widely prevalent in other fruits and vegetables at much lower levels compared to coffee beans[82-86] 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.[87] 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).[30, 82, 88] CAs are also present in whole grain flours such as corn and barley (0.08 g/kg DW).[89]

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.[90] 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.[91] 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).[91] 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).[92] Average caffeic acid derivative intake (including CAs) was found to be 417 ± 325 mg/day in Finnish adults[93]; 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.[94] The individual phenolic compounds with the highest intake were CAs. Mean phenolic acid consumption by individuals in Sao Paulo, Brazil was determined to be approximately 284.8 mg/day with nearly all being from hydroxycinnamic acids.[95] 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.[96] 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.[54]

3.4.3 Summary of Chlorogenic Acids Exposure Estimates

In summary, while Gyusa.g™ does not have a specification range for CAs, exposure estimates to CAs from Gyusa.g™ were estimated using the typical batch analysis result of 8.2% CAs. The range of CAs per serving from Gyusa.g™ is expected to be ~58–293 mg/serving. This range is within the levels found in a serving of coffee. Aggregate exposure to CAs from Gyusa.g™ was estimated at 256–758 mg CAs/day (3.3–9.6 mg/kg bw/day), with the assumption that these exposures will be partially substitutive for those from coffee consumption.

Background diet exposure estimates to CAs from published studies were also summarized. The published data shows that the mean daily intake of CAs is approximately 500 mg/day in various populations around the globe, and the vast majority is from coffee consumption. FDA recognizes that consumption at the 90th percentile is usually approximately 2 times the mean,[97] thus the mean data from the published studies suggests 90th percentile intakes maybe approximately 500–1000 mg/day.

3.5 Summary of Exposure Estimates

In summary, based on its intended uses, exposure estimates for Gyusa.g™ were calculated using Creme Global software, along with estimated exposure to the caffeine and CAs components of the extract, as detailed in the Subparts above. The composite results are shown in Table 17 below.

Table 17. Summary of Gyusa.g™ Exposure Estimates for the Total Population (Ages 2+)

Extract or Extract Component and Presence Probability	Maximum exposure estimates for the total population (ages 2+)	
	Gyusa.g™	
	mg/day	mg/kg bw/day
Gyusa.g™ (100% PP)	9249.0	117.3
Gyusa.g™ (20% PP)	3116.2	39.7
Caffeine (in addition to background)	333.0	4.17
CAs* (100% PP)	758	9.6
CAs* (20% PP)	256	3.3

PP=presence probability;
*Gyusa.g™ is not standardized to CAs, general exposure levels were derived using Gyusa.g™ exposures.

Part 4: Self-limiting Levels of Use

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

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

The GRAS conclusion for Gyusa.g™ 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 Safety of Guayusa and Guayusa Extracts

6.1.1 Bioavailability and Metabolism related to 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) published a randomized, double-blind, three-period crossover clinical trial that investigated both the safety and pharmacokinetics of AmaTea® (a hydroethanolic extract of guayusa, described in the paper as 20% caffeine and 30% polyphenols by weight) and a green coffee extract (JAVA.g, described as 30% caffeine and 40% polyphenols by weight) in 12 healthy adult males ages 21–34.[98] The study was funded by AFS, Inc., the proponent of this GRAS conclusion. At each visit, subjects received one of three caffeine sources: AmaTea®, JAVA.g, 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}, AUC_{0-4}, and $AUC_{0-\infty}$ were bioequivalent for each test article.[98]

6.1.2 Toxicology Studies on Guayusa

A set of toxicology studies on an aqueous *I. guayusa* leaf extract, performed according to Good Laboratory Practice (GLP) where applicable, was published by Kapp et al. in 2016.[1] The published studies are summarized in the sections below. The test article in the studies was "Guayusa Concentrate" (GC; provided by RUNA, LLC), which was prepared by adding dried guayusa leaves to purified water (1.3–1.6:1), followed by brewing for 2–4 hours, cooling and storing. Chemical analysis of the GC test article, as shown in the Kapp et al. paper, is compared to the composition of Gyusa.g™ in Table 18 below.

Table 18. Analysis of GC test article compared to typical batches of Gyusa.g™ (information for GC borrowed in part from Kapp et al., 2016)[1]

Parameters from Kapp et al., 2016[1]	GC[1]	Gyusa.g™
	%	%
Moisture	66.41	< 3
Ash	4.9	~1.5
Protein	7.0	~18.5
Total sugars	3.5	~2.9
Total fat	0.39	<1.0
Dietary fiber	3.8	<1.0
Cholesterol	Not determined*	ND*
Caffeine	3.6	6 ± 2.5
Theobromine	0.03	ND
Chlorogenic acids	5.2	4 ± 4.5
Total polyphenols	1.0	5
Catechin (C)	0.2	ND
Isoflavones	0.08	ND
Epicatechin (EC)	0.0179	ND
Epicatechin gallate (ECG)	0.0199	ND
Epigallocatechin gallate (EGCG) (EGCG)	0.00876	ND
Epigallocatecin (EGC)	0.111	ND
Kaempferol	Trace	ND
Naringin	Trace	ND

*Reporting limit = 1.0 mg/100 g
ND, not detected during analysis

As shown in the table, while GC and Gyusa.g™ are different in that the former is a liquid and the latter is a powder, the concentrations of the key constituents are otherwise similar (caffeine and CAs). Additionally, as published in Kapp et al., 2016,[1] 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 GC lots tested.

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.[1] It followed US FDA GLP regulations, and was based on ICH[99] and US FDA Redbook guidelines,[100] 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, methylmethane-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 assay 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.[1] The assay was performed according to US FDA Redbook[101] and OECD 473[102] 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 doses 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,[103] to determine the potential of GC to produce toxicity following a single oral dosing in rats.[1] 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 LD_{50} 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 LD_{50} values ranging from 200–400 mg/kg.[104]

6.1.2.4 Fourteen-Day Range Finding Study in Rats

A 14-day range finding study was performed according to OECD 407[105] and FDA Redbook[106] guidelines for the purpose of setting dose levels for the 90-day study.[1] Seven groups of five males and five females each (vehicle control group, three GC dose groups (1200, 2500, and 5000 mg/kg/d); and three equivalent caffeine reference control groups (36, 75, and 150 mg/kg/d)) 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. 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/d had evidence of salivation and hypoactivity. Dose-dependent hypoactivity was also observed in the intermediate (75 mg/kg/d) and high-dose (150 mg/kg/d) caffeine groups. Statistically significant dose-dependent reductions in body weights were noted in both sexes; 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 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).[1] The study was performed according to OECD 408[107] and US FDA Redbook 2000, IV.C.4[106] 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 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, 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. Additional tissues were preserved if signs of toxicity or target organ involvement was observed. Selected organs and tissues from all dose groups were evaluated histologically.

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 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. As there were no other significant findings, atrophy of the thymus was considered secondary to morbidity. 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 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 19. 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.

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.

Table 19. Summary of Mean Clinical Chemistry Values—90-day Guayusa Concentrate Study (table borrowed from Kapp et al.)[1]

	Test substance dose levels, mg/kg/d								Caffeine dose level, mg/kg/d	
	0		1,200		2,500		5,000		150	
Parameter (unit)	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
BILI (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; BILI, 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.

[a]$P < 0.05$.

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, 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 20 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 were caused by the caffeine in the GC.

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.[104, 108-112] 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).[104] Gans et al. reported that caffeine (and theobromine) seem to produce a biphasic effect on appetite such that caffeine stimulates appetite at lower concentrations and inhibits appetite at higher dietary concentrations.[109]

Table 20. Summary of Effects of Guayusa Concentrate and Caffeine (Table borrowed from Kapp et al.[1])

	Test substance dose levels, mg/kg/d										Caffeine dose level, mg/kg/d	
	Laboratory historical control range		0		1,200		2,500		5,000		150	
Quantitative effects	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	0.092[c]	0.057	0.079[c]	0.049[c]
Hypoactivity	-	-	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Increased salivation	-	-	0/10	0/10	0/10	0/10	0/10	0/10	8/9	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/µL)	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/µL)	0.29-1.55	91.3-247.1	212	170	216	173	198	172	225	189	226	211[a]
Increased WBC (10^3/µL)	7.64-22.09	2.41-14.79	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[b]	96[c]
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
Increase BILI (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 TRIG (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	3.3	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	13.6	16.2	23.3	23.2[a]
Decreased urinary protein (mg/dL)	44-1,330	11 - 620	143	78	139	57	94	65	199	35[a]	117	20[b]
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	6.87[c]	3.72[c]	6.18[c]	4.05[c]	5.09[c]	2.91[c]	4.84[c]	2.70[c]
Decreased RETP fat pad weight (g)	-	-	15.0	8.06	6.87[c]	3.30[c]	6.62[c]	3.68[c]	4.78[c]	2.44[c]	5.11[c]	1.97[c]

Abbreviations: ALB, albumin; ALKP, alkaline phosphatase; ALT, serum alanine aminotransferase; APTT, active partial thromboplastin time; ARET, absolute reticulocyte count; AST, serum aspartate aminotransferase; BILI, total bilirubin; BW, body weight; CHOL, cholesterol; CREA, blood creatinine; IPHS, inorganic phosphorus; K, potassium; MCHC, Mean corpuscular hemoglobin concentration; PT, prothrombin time; RETP, Retroperitoneal; SD, standard deviation; TRIG, triglycerides; WBC, total white blood cell; -, data not applicable.
[a] $P < 0.05$.
[b] $P < 0.01$.

When administering 0.5% dietary caffeine (approximately 250 mg/kg bw/day using the Lehman method[113]) to male rats for 7–8 weeks, they observed decreased food intake and decreased body weight gain in males as well as decreased thymus gland weights.[109] The higher caffeine dose could be the reason for the food intake decrease that was noted in the Gans study but not the current experiment. Differences in male and female responses to caffeine in humans have been noted and have been hypothesized to be at least partly related to steroid hormone levels.[114, 115]

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,[116] and after gavage of 1 mg/kg and 9 mg/kg caffeine in rat pups.[117]

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.[104, 118-120] 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.[104] Such sympathomimetic effects of caffeine on the salivary glands are known to be reversible.[104] 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).[121] Salivation may also be an indicator of stress.[122] 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 and, thus, were not considered to be of toxicological concern.

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.[123, 124] 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, and fell 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.[125-128] 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.[104] 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.[129-133] Ruhl and Everhart found that adults at high risk for liver injury, consumption of coffee and especially caffeine was associated with lower risk of elevated ALT.[131] 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).[134] Similar significant inverse associations with coffee intake were observed for serum AST and ALT in males and less strongly in females.[135-137]

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; 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.[108, 110, 119, 128, 138] 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.[128] 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.[110] There have been mixed results with regard to the effects of caffeine and coffee on cholesterol/lipids in humans.[108, 119, 138] 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.[111, 139] 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 secondary to treatment-related stress.[109, 122] With regard to organ weights, the significant differences in absolute and relative gonadal and retroperitoneal absolute and relative fat weights in males and females from all GC treatment groups and in male and female caffeine controls were considered to be related to caffeine administration. This has been shown in other published studies.[108, 111, 140, 141] In humans and rodents, caffeine ingestion elevates the metabolic rate and increases the oxidation of fat via lipolysis and release of catecholamines.[142-144] 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.[111] 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.[140] 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.[108] 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.[145]

The absolute or relative reduction in the thymus, spleen or epididymitis weights, along with absolute and relative increases in adrenal gland weights are interpreted to be secondary to treatment-related stress,[122] 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 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 consuming 10.5 g/day of caffeine (by gavage all at one time) by a 70 kg human, or approximately 53 cups of coffee all at once, at approximately 200 mg of caffeine per cup. 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 in a later section, safe caffeine consumption levels for humans have been agreed up by numerous scientific and/or regulatory organizations.

There were no findings considered of toxicological concern that were otherwise attributable to GC. Thus repeated administration by gavage of 1200, 2500 and 5000 mg/kg bw/day of GC 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.

Caffeine NOAELs reported by NTP when administered via drinking water were 1500 ppm; equivalent to 151 mg/kg bw/d for male rats and 174 mg/kg bw/d for female rats and 167 mg/kg bw/d for male mice and 179 mg/kg bw/d for female mice (which are similar to the 150 mg/kg bw/day level of caffeine in the GC study high dose group).[104] The summary of the studies in the NTP report was as follows. "(b) (6)

(b) (6)

[redacted]

NOAELs are generally much lower when a substance such as caffeine is administered via gavage as compared to administration in the food or water supply. Such factors must be considered in any risk assessment process for caffeine, because under normal conditions of consumption, humans cannot/do not attain serum blood levels comparable to those associated with the threshold for adverse effects from caffeine exposure in rats when bolus dosing is studied.[146]

6.1.3 Human Studies on Guayusa

As previously described in the pharmacokinetic section (subpart 6.1.1), AmaTea® and a green coffee extract (JAVA.g) were studied by Krieger et al. in a double-blind, randomized, crossover, clinical trial sponsored by AFS.[98] 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 on Guayusa

Swanston-Flatt et al. 1989 studied the effects of individual plant-derived preparations, including guayusa, and their effects on blood sugar regulation in mice.[147] 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 Composition of Gyusa.g™ compared to Coffee

As discussed above, coffee has been consumed daily by a large percentage of the population for a very long time, and researchers have demonstrated strong associations between reportable health benefits and the consumption of coffee.[32, 34, 148, 149] The beneficial properties of coffee are generally attributed to either caffeine or its antioxidant capacity and CAs content[150, 151] (note that the roasting process generally causes degradation of CAs[152-156]). Gyusa.g™ contains some similar constituents to roasted coffee beans used to make coffee beverages, and individual constituent levels (including individual CA levels) in a serving of Gyusa.g™ versus a serving of coffee will be compared in several tables below in order to demonstrate that the history of safe consumption of these constituents from coffee generally lends support to the safety of their consumption from Gyusa.g™.

Comparisons of the general composition of green and roasted coffee beans to Gyusa.g™ are shown in Tables 21 and 22 below. The green and roasted bean data was borrowed from the World Health Organization, International Agency for Research on Cancer (WHO-IARC) 1991 monograph on the evaluation of coffee

with regard to carcinogenic risk to humans (WHO-IARC cited other published work for the data). Using the composition data from the monograph and AFS' data on the Gyusa.g™, a comparison was made with regard to the amount of a compound expected to be in a serving of coffee and a serving of Gyusa.g™. Ten (10) and 16 grams of roasted beans were used for the serving of coffee calculations, which were based on 1.3 grams of beans per ounce of water and 8–12 ounces of fluid (considered typical coffee serving sizes (for example, 12 ounces is the published reference amount customarily consumed for coffee pursuant to 21 CFR §101.12 and is the serving size of a medium ("Tall") coffee at Starbucks). A commonly used standard recipe for coffee brewing is a bit stronger (1.67 grams of beans per ounce of water, or two tablespoons per 6 ounces water)[157, 158]; however, 1.3 grams was used to be conservative and account for individuals who drink weaker coffee. Thus 10–16 grams is expected to cover a reasonable range of typical coffee bean serving sizes. In general, the data shows that relatively similar amounts of caffeine and CAs will be present in a serving of Gyusa.g™ and a 10 g serving of coffee.

Table 21. Composition Comparison of Green and Roasted Coffee Beans[2] and Gyusa.g™—Dry Basis

Component	Green Coffee (Dry Basis, %) (WHO/IARC, 1991)[2]		Medium-roasted Coffee			Gyusa.g™
			Dry Basis, %[2]		% extractable with water at 100 °C (WHO/IARC, 1991)[2]	
	Arab	Rob	Arab	Rob		Dry Basis, %[§§]
Alkaloids (caffeine)	1.2	2.2	1.3	2.4	75–100	8.5
Trigonelline	1.0	0.7	1.0	0.7	85–100	
Minerals	4.2	4.4	4.5	4.7	90	1.46
Acids						
Total CAs	6.9[§]	10.4[§]	3.3[§]	4.8[§]	100	8.2
Aliphatic (volatiles)	1.0	1.0	1.6	1.6	100	
Sugars	8.1	4.4	0.3	0.3	100	2.93
Polysaccharides/ Lignin/Pectin/Fiber	50.0	55.0	38.0	42.0	10	78.8
Proteinaceous compounds	11.5	11.8	10.0	10.0	15–20	18.5
Lipids	16.0	10.0	17.0	11.0	1	0
Caramelized/ condensed products (e.g., melanoidins) by difference	–	–	23.0	22.5	20–25	0
Volatile substances other than acids	–	–	0.1	0.1	40–80	0

[§]Sum of total CAs, and quinic acid data from WHO-IARC publication

[§§]Results are the average of testing three batches of Gyusa.g™ (AFS; unpublished data on file)

Table 22. Composition Comparison of Roasted Coffee Beans and Gyusa.g™—Per Serving

Component	Medium-roasted Coffee		Gyusa.g™
	Per 10 g Serving* (mg)	Per 16 g Serving* (mg)	Per Serving** (mg)
Alkaloids (caffeine)	97.5–240	156–384	60–125
Trigonelline	60–100	96–160	
Minerals	405–423	648–677	24.9–52.1
Acids Total CAs Aliphatic (volatiles)	 330–480 160	 528–768 256	 <57.9–378.2***
Sugars	30	48	50–105
Polysaccharides	330–370	528–592	47
Lignin	0	0	
Pectin	0–300	0–480	
Proteinaceous compounds	150–200	240–320	316–660
Lipids	11–17	18–27	0
Caramelized/ condensed products (e.g., melanoidins) by difference	450–575	720–920	0
Volatile substances other than acids	4–8	6–13	0

*Calculated using the minimum % extractable of the minimum dry basis value and the maximum % extractable of the maximum dry basis value (refer to Table 21), to get the full range.

**Data from Table 7 and/or calculated based on minimum intended use mg/serving (for enhanced waters) and the maximum intended use serving mg/serving (for energy drinks), which was 706–3570 mg/serving for Gyusa.g™

***As previously discussed, there are no specifications for CAs in Gyusa.g™; levels were calculated using results of a typical batch analysis (8.2% CAs).

In reviewing the data in the tables above in more detail, it is important to note that the analyses for the green/roasted coffee and Gyusa.g™ were performed in different laboratories, and the true composition of coffee will vary depending on the growing conditions, method of analysis, species, roasting process and actual serving size. However, the tables allow for a general comparison of constituent levels. Maximum levels of caffeine and CAs in a serving of Gyusa.g™ falls within the range of levels in a serving of coffee. Trigonelline, found in coffee and a number of other edible plants,[2] is not a constituent of Gyusa.g™ (it is generally found in the lipid fraction of coffee beans).[81] Minerals are lower in a serving of Gyusa.g™ compared to a serving of coffee, while sugar, lipids and protein levels vary depending upon the extract; these constituents are ubiquitous in the diet and the small levels found in a serving of Gyusa.g™ are not expected to impact levels already consumed in the diet. Lignins and pectins (components of plant cell walls and considered dietary fiber)[159] are present at low levels in coffee and are also ubiquitous in the diet. Caramelized products and volatile substances that present during the roasting of coffee beans are not found in Gyusa.g™. In summary, aside from some differences in ratios of constituents, levels of key compounds are similar in servings of Gyusa.g™ and coffee.

A comparison of the breakdown of individual CAs found in Gyusa.g™ to those found in a 10–16 g serving of coffee beans exposed to various roasting conditions (as published by Farah et al., 2005),[160] are shown in Table 23 below. The intent behind the table is again to give a general sense of individual CA levels for comparison; as with Table 22, the analyses were performed in different laboratories and exact levels of the individual CAs in coffees will vary depending on the species, growing conditions, exact roasting conditions, method of analysis, and the wide range of actual serving sizes. Gyusa.g™ data are reported as averages of analyzed samples. As previously discussed, various CA isomers are combined in the table due to confusion in the literature with regard to nomenclature and for comparison purposes.

Table 23. Comparison of Individual Chlorogenic Acids in Gyusa.g™ and Various Coffee Roasts[78, 160]

CAs (g/100 g or %)	Gyusa.g™		mg per 10–16 g** of Various Coffee Roasts (Farah, 2005)[160]					
	% Dry basis	Per serving* (mg)	Green	Very Light	Light	Light Medium	Dark Medium	Dark
3-CQA + 5-CQA	1.9	13–69	429–686	431–690	298–477	157–251	66–106	38–61
4-CQA + 3-FQA + 5-FQA*§	2.7	19–98	85–136	163–261	129–206	79–126	38–61	20–33
3,4-diCQA + 3,5-diCQA + 4,5-diCQA	3.6	25–127	99–159	70–112	40–64	17–27	4.7–7.5	1.9–3.1
Total	8.2	58–293	613–981	664–1063	467–747	253–404	109–175	60–97

*Data calculated based on minimum intended use mg/serving (for enhanced waters) and the maximum intended use serving mg/serving (for energy drinks), which was 706–3570 mg/serving for Gyusa.g™.

**Calculated by taking the mean of the three data points in the publication, quantified using 5-CQA (IUPAC) with correction for molar absorbance coefficients. Note that roasting green coffee beans causes degradation of CAs; the degree of degradation is generally proportional to the intensity of the roasting conditions.[152-156]

*§For Gyusa.g™ data, this includes minor/other CQAs, so more than just 3- and 4-FQA (3-, 4-, and 5-pCoQAs, 4-FQA, 3,4-diFQA, 3,5-diFQA, dimCQAs, and other very minor constituents).

Table 23 above gives a general comparison of the individual CAs in a serving of Gyusa.g™ to that in a serving of green/roasted coffee beans. Overall, the levels of total and individual CAs in a serving of Gyusa.g™ are reasonably similar to the levels found in a serving of various roasts of coffee.

In summary, the different compositional comparisons between a serving of Gyusa.g™ and a serving of coffee shown in the tables above demonstrate that the key constituent levels are reasonably similar, which lends supports to the safety of consuming a serving of Gyusa.g™ as compared to consuming a serving of coffee. Servings of Gyusa.g™ and coffee are likely to be substitutive for each other by consumers, due to the nature of caffeine beverages being substitutive for each other.

Numerous reviews of clinical data suggest that moderate levels of coffee consumption are safe, as will be detailed in Subpart 6.2 on the safety of caffeine.

6.1.6 Summary of Guayusa Safety

Overall, the safety studies on GC discussed in this subpart do not suggest any genotoxicity or other toxicological concerns with regard to ingestion of *I. guayusa* leaf extract compounds, other than those caused by very high levels of caffeine. While the GC test article in the Kapp et al., publication is not identical to Gyusa.g™ (as shown in Table 18), the toxicological studies in the paper are still strongly supportive of its safety. The fact that the NOAEL was the highest dose tested in the 90-day study (when findings considered related to caffeine exposure are dismissed) give a high degree of support with regard to safety of guayusa extractable components. The key constituent levels are very similar for GC and Gyusa.g™, and are reasonably similar to those levels in coffee on a per serving basis, adding additional support to their safety based on the long history of consumption and safety assessments related to coffee in the literature.

6.2 Safety of Caffeine

The major safety conclusions of this subpart include:

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 caffeine for use in cola-type beverages up to the level of 0.02% (200 ppm) caffeine, or approximately 0.2 mg/mL (~ 47 mg per 8 oz.), pursuant to 21 CFR §182.1180; this is the maximum intended caffeine addition level from Gyusa.g™ for carbonated beverages.
4. 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.
5. The fact that caffeine consumption patterns have remained relatively consistent (or even declined) over the years despite the introduction of various new caffeinated beverages.[14-16, 55-57]

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 A_1 and A_2 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).[81, 161-165] It has flavoring capabilities due to its bitter taste.[12] Caffeine is thought to function as a natural herbicide and insect repellent in plants.[166, 167] 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.[168]

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 Gyusa.g™.

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 24 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 24. 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)[161]	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.[71]	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 a human carcinogen at doses ≤500 mg/day. Reproductive-aged women should consume ≤300 mg caffeine per day (equivalent to ~4.6 mg/kg bw/day for a 65-kg person) while children should consume ≤2.5 mg/kg bw/day. Based upon the results from the Nawrot et al. publication,[71] 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.[169]
European Food Safety Authority (EFSA)[123]	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)[77, 170]	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.[2, 171, 172]	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.[72]	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.[161] 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 reproduction 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 $t_{½}$ 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.[71, 169] As an aside, this has been considered one of the most extensive reviews on caffeine safety for many years, and is frequently cited.[71]

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 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 caffeine doses ≥500 mg/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 below[171-173]). 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 a doses ≤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 populations[169]:

- 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.[174] In 2015, EFSA released its scientific opinion on the safety of caffeine,[123] 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.[170] 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.[77, 170]

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).[77, 170]

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.[77, 170]

6.2.1.5 International Agency for Research on Cancer (1991, 2018)/Loomis et al. (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.[175]

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)*".[2] 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 signification 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).[171, 172] 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., 2003[71] caffeine safety review to determine if the conclusions reached by Nawrot/Health Canada were still supported by the literature published since that time.[72] 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 Relevant 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.[51] 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[72, 123] 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.[176] 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.[177] 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 there are no diterpenes in Gyusa.g™) 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, diterpenes are not found in Gyusa.g™).

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.[178] 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 (diterpenes are not found in Gyusa.g™). 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[72, 123]). 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,[179] but the two are otherwise identical molecules with the same chemical structure and are not expected to behave differently in the body. To emphasize this, AFS very recently performed a prospective, randomized, double-blinded, two-period, crossover, pharmacokinetic trial comparing a botanically sourced caffeine (from a green coffee extract (GCE)) to a synthetic USP control caffeine product.[180] The study was performed in accordance with all applicable Good Clinical Practice guidelines. One of the goals

of the study was to determine if the caffeine from GCE would have the same relative absorption and pharmacokinetic characteristics as synthetic caffeine. Sixteen healthy male subjects, aged 18 to 45, were enrolled in the study and were randomly assigned to take a single dose of product 1 (approximately 60 mg and 238 mg of botanically sourced caffeine and CAs, respectively, derived from 480 mg GCE) 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. Blood samples were collected for analysis at 1 hour prior to dosing, and at approximately 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 hours post dosing. Blood serum was analyzed for caffeine, and the pharmacokinetic results are shown in the table below:

Parameters	GCE caffeine	Synthetic caffeine
C_{max} (ug/mL)	1.91 ± 0.876	2.09 ± 1.49
Median T_{max} (hours)	0.75	0.63
$AUC_{0\text{-}4h}$ (h·µg/mL)	6.35 ±3.34	6.99 ± 5.45
GMRs* (%)	97.77	98.33

*GMRs= geometric least-square mean ratios of Cmax and $AUC_{0\text{-}4h}$ between GCE and synthetic caffeine products

In conclusion, caffeine from GCE and synthetic caffeine resulted in peak concentrations and systemic exposures that were bioequivalent (i.e., not statistically significantly different). Additionally, there were no treatment-related adverse events reported by any subject or noted by any of the research staff during the study. Similarly, White et al. (2016) showed that caffeine (160 mg) absorption and exposure from different beverages (coffee and energy drinks) was similar irrespective of the beverage temperature or rate of consumption in 24 healthy subjects (ages 18–30).[181]

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.[71, 161] 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 into bodily fluids.[2, 71]

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.[51, 71, 182-185] Paraxanthine may be further metabolized to methylxanthines and methyluric acids.[71, 184] 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 deformylated nonenzymatically to form 5-acetylamino-6-amino-3-methyluracil.[51] Only small amounts of caffeine are excreted in the urine unchanged.[71] 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.[71]

Orally ingested caffeine has an elimination half-life in humans ($t_{½}$) of 3–7 hours, which can be influenced by factors such as sex, age, oral contraceptives, pregnancy and smoking.[71] 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.[71] 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.[186] 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.[186]

More recent pharmacokinetic studies on caffeine have focused on how individuals' genetic makeups lead to inter-individual differences in how caffeine is metabolized and excreted. Polymorphisms 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.[72] For example, in an randomized controlled trial of 114 healthy ADORA2A 1976T>C polymorphism (rs5751876) carriers, an intervention of 300 mg caffeine citrate (equivalent to 150 mg freebase caffeine) or placebo revealed that female ADORA2A 1976TT risk genotype carriers showed an impaired inhibition of the startle response compared to male ADORA2A 1976TT homozygotes.[187] Similarly, genomic variations in CYP1A2 alleles are associated with different patterns of caffeine metabolism.[72, 185, 188-191]

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.[184] 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,[192] the large multi-ethnic, prospective, population-based Northern Manhattan Study,[193] a large Japanese cohort study,[194] large cohorts of diabetic men and women,[195] as well as others.[196, 197] 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.[198] 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).[199] The pooled relative risk was similar whether ≥2–4 cups/day or ≥5–9 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.[200] 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.[201] 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.[202]

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.[171, 172] 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.[203] 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.[204] 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.[205] 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.[206]

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.[207-211] A Danish case-control study suggested a modest decreased risk of ovarian cancer was associated with coffee and caffeine consumption.[212]

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.[213]

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.[214] The large prospective Netherlands Cohort Study on Diet and Cancer found no significant association with coffee consumption and epithelial ovarian cancer in postmenopausal women.[215] 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.[216] 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.[171, 172]

Coffee consumption has been highly correlated with smoking habits (smoking is a known risk factor for bladder cancer).[171, 172, 217] In a prospective study in Japanese men and women, no significant association was found between caffeine consumption and overall bladder cancer risk.[218] 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.[173] Similarly, Yu et al. found that coffee consumption may have an inverse association with bladder cancer in their 2011 meta-analysis.[206]

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[171, 172] and a number of other reviews of the literature.[203, 204, 206] 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.[219] Another large prospective analysis found no associations between coffee, tea, or caffeine and breast cancer risk in women living in France.[220] 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.[221] 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.[192] 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.[222]

Analysis of data from the prospective Women's Health Study revealed no overall association between caffeine consumption and breast cancer risk,[223] 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.[224] 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.[225] 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.[226] 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.[227]

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.[171, 172, 203, 204] Hepatoprotective effects of coffee components, including caffeine, against liver fibrosis have been noted in a number of studies.[228] 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.[229] 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.[230] 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.[231]

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.[232] A number of recent meta-analyses found that coffee intake may be inversely associated with incidence of malignant melanoma and basal cell cancer development.[202, 233-235] 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.[236] 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.[237] 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.[238]

Endometrial Cancer

Recent large reviews of the literature have consistently reported inverse associations between endometrial cancer and coffee/caffeine consumption. [171, 172, 178, 206] 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.[239] 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/m^2.[240] 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.[241] 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).[242] 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.[243] A 2015 meta-analysis of 13 published studies concluded that coffee and caffeine intake might significantly reduce the incidence of endometrial cancer, and these effects may be modified by BMI and history of hormone therapy.[244]

Colorectal Cancer

Large reviews of the cancer literature have found no negative effects, and a potential protective effect, of coffee on colorectal cancer.[203, 204, 206] 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.[245] 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.[246] 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.[245] 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.[247]

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)[248] 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)[249] 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., 2011[249] 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.[250] 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.[251] 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.[72, 123] 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.[72] 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.[171-173, 203, 252, 253] 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.[254] A 2017 meta-analysis found no significant association between coffee consumption and renal cell carcinoma.[255] 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).[256]

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.[72, 77, 81, 170, 176, 177, 200, 205, 257-259] 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.[72] Pourshahidi et al.'s review of the literature[177] 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.[260] 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.[261]

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.[257] They found that this effect appears to be due to the content of diterpene oils (which are removed in filtered coffee, and are also not found in Gyusa.g™) 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.[257] 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.[262]

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.[263] Analysis of data from the Danish Diet, Cancer and Health study found no association between caffeine intake and risk of atrial fibrillation or flutter.[264] 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.[265] 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.[266] 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.[267] 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).[268] 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.[269] 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.[270] 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.[271] 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.[272, 273]

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.[274] 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.[275] 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.[276] A large prospective study found no evidence that caffeine consumption increases the risk of coronary heart disease in men or women.[277] 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.[198] 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.[195]

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, as has been determined in a number of recent comprehensive reviews of the literature.[77, 170, 176, 177, 205, 258, 278, 279] Caffeine appears to reduce insulin sensitivity in skeletal muscle and may also reduce hepatic glucose output,[164] although acute/short-term findings in some studies can seem to contrast to these longer term associations[280, 281] Caffeine also can improve glucose tolerance, insulin sensitivity, and insulin secretion from pancreatic β-cells. [77, 170, 176, 177, 205, 258, 278, 279] Individual genetic polymorphisms (in, for example, CYP1A2) likely play a role in glycemic (and other) effects.[191]

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,[205] and a French women cohort study.[282] Bhupathiraju and colleagues (2014) followed subjects in the Nurses' Health Studies and the Health Professionals Follow-up Study[283] 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.[284] 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 caffeine on insulin sensitivity in healthy human populations (i.e., without diabetes).[285] 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.[286] 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).[286] 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.[287] 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.[286] Genetic polymorphisms likely also play a role in individual glycemic responses in the presence of caffeine.[191]

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.[288] 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[289, 290], spontaneous abortions[290], stillbirths[290], and small for gestational age findings.[290] 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.[289, 290] 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).[289]

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.[146, 291] 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.[292]

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.[293]

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).[72, 77, 289, 290] 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.[72, 77, 146, 291] 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.[72] 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.[72] 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.[72, 123] 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.[294] 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.[295] 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.[296]

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.[297] 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.[298] 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.[299]

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.[298, 299] 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.[300, 301] 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.[300] 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.[301] 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.[302] Bioactive coffee constituents, including caffeine, may modulate parameters of neuro-inflammation, which may be a mechanism for effects on mood.[303]

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.[77, 298, 304, 305] 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.[306, 307] Caffeine doses of 3–5 mg/kg bw/day have been found to be neuroprotective in both epidemiological and preclinical studies.[308]

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.[304, 305, 307, 308] 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.[309, 310] Regardless, moderate coffee/caffeine consumption appears safe in populations at risk for cognitive deficits.[305] 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.[311]

Published reviews of the literature have found that coffee/caffeine may be associated with a decreased risk of Parkinson's disease.[77, 81, 170, 176, 177, 307, 308, 312] 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 A_2 receptors.[81, 308] A 2015 meta-analysis on Parkinson's disease risk found a linear dose-relationship for decreased disease risk with tea and caffeine consumption.[313] 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.[314]

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.[315]

While it was concluded in a 2003 review that large doses (at that time, $\geq$ 250 mg) of caffeine have an acute diuretic action,[315] a 2015 meta-analysis cast doubt on this conclusion.[316] 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 paper[317] 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.[318] 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.[319] 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.[320-322]

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.[185] 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.[71, 72] This is also demonstrated by the fact that caffeine consumption levels have remained stable despite new caffeinated beverage additions to the market.[14-16, 55, 57, 58, 74]

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.[72, 185, 188-190]

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. This is Gyusa.g™'s intended use level with regard to caffeine in carbonated soft drinks.
- 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.[323] 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.[323] 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.[324]

Several federal workshops occurred in 2013 with the aims of gathering information about caffeine and energy drinks and identifying critical data gaps.[18, 325-327] 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.[328]

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.[329] 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.[330]

The EU released subsequent reports related to the safety of energy drinks in 2003 and 2009.[330, 331] 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,[332] 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."[333]

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.[123] 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.[334]

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."[335] 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).[71] 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.[18] 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,[336] 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.[336-340] In April 2018 FDA released a guidance for industry on highly concentrated caffeine in dietary supplements.[341, 342] 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 AFS Gyusa.g™ is not intended for use in beverages containing alcohol and are 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.[32] 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 Gyusa.g™ 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 Gyusa.g™ evaluated in the current GRAS 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

Chlorogenic acids are components of guayusa leaves and Gyusa.g™, although the extract is not standardized to these substances. The intended uses for Gyusa.g™ were estimated to result in levels of approximately 58–293 mg CAs per serving of Gyusa.g™, depending upon the intended use food category. 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, as shown in subpart 6.1.5.

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. A discussion of the pharmacokinetic profile of CAs, suggesting that they are rapidly absorbed, metabolized, and eliminated from the body.
2. CAs from CoffeeBerry® ethanol extract are substantially similar to CAs in Gyusa.g™; 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 Gyusa.g™.
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).[41, 81, 343-355] 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.[353] 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.[349, 353-355] 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.[356] 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.[41, 343]

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.[81, 350, 357] 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.[350, 351, 352] 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.[357] Metabolic pathways for the CAs (after ingestion of coffee) are shown in Figure 6 below.

AFS conducted a double-blind crossover study, in part, to determine the bioavailability of CAs from their green coffee extract.[180] 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
$AUC_{0\text{-}4h}$ (h·ng/mL)	27.3	16.1	15.7

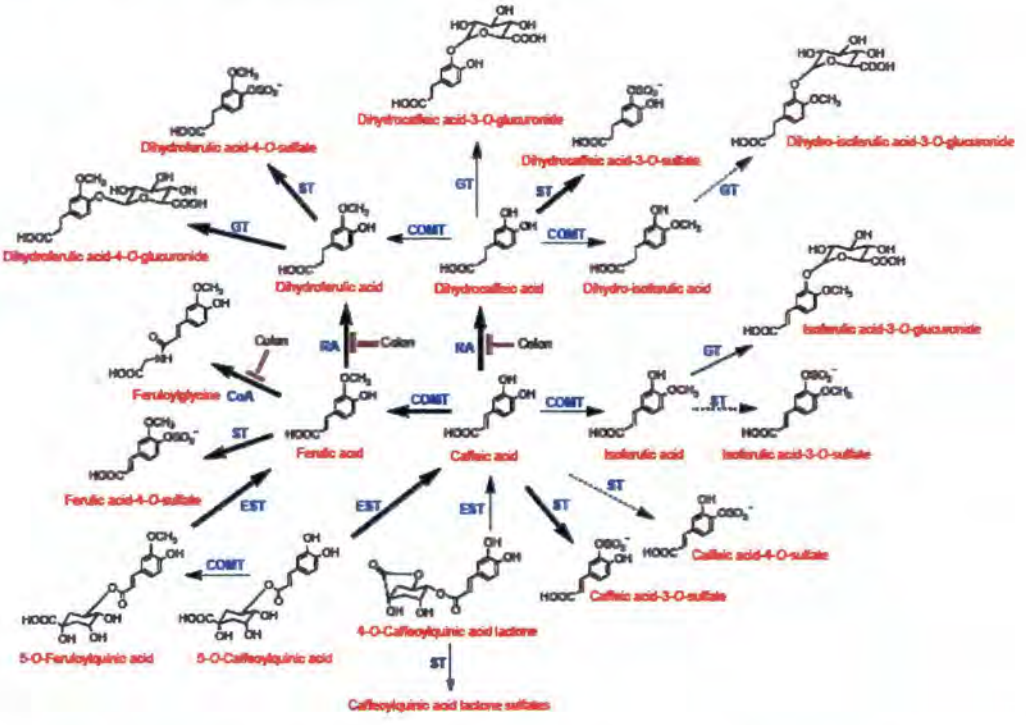


Figure 6. Metabolism of Chlorogenic Acids Following Ingestion of Coffee by Human Volunteers (borrowed with permission from del Rio et al., 2010).[41] Note that while only 5-*O*-CQA and 5-*O*-FQA are illustrated, their respective 3- and 4-isomers would be metabolized in a similar manner. COMT=catechol-*O*-methyltransferase, 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.

In summary, CQA compounds from green coffee extract were bioavailable and specifically, the 3-CQA (5-CQA IUPAC) compound was present and absorbed (as measured by C_{max}) in the greatest concentration. No treatment related adverse events occurred in the study.

Farah et al. (2008) also found the bioavailability of CAs from 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.[352] 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
$AUC_{0\text{-}4h}$ (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 dihydrocaffeic acids; together they represented approximately 85% of the phenolic compounds identified in the urine. The apparent bioavailability for CA compounds/metabolites 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 ± 17% absorption while the absorption of ingested pure caffeic acid was nearly complete at 95 ± 4%.[351] 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.[358] 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 bioavailability of intact CQAs from the small intestine but high bioavailability of CA gastrointestinal (gut flora) metabolites.[359, 360] 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.[38] 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).[361]

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 a study on green coffee extract, the CQA compounds were bioavailable. Similarly, CAs from another green coffee extract were shown to be bioavailable, although inter-individual variation in pharmacokinetic values was 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).[362] 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® and Gyusa.g™

A comparison of the CoffeeBerry® test articles and Gyusa.g™ is shown in Table 25 below based on available data in the Heimbach et al. publication, as well as data from AFS on Gyusa.g™ and on the total level of CAs in the CoffeeBerry® ethanol extract.

Table 25. Comparison of CoffeeBerry® Extracts[362] and Gyusa.g™

Parameter	CoffeeBerry® Whole Powder	CoffeeBerry® Water Extract	CoffeeBerry® Ethanol Extract	Gyusa.g
Appearance	Tan/Brown Powder	Brown Powder	Brown Powder	Brown-greenish powder
Extraction Solvent	—	Water	Water: Ethanol	Water
Solids	≥ 90%	96%	90%	>97%
Solubility in water	Partially	100%	100%	>95%
***Total Phenolic Acids (described as chlorogenic acid**	≥ 2%	5%	35–40%	6.3%

(CA), and caffeic, quinic and ferulic acids)				
Total Phenolic Acids (All CA isomers)	n/a	n/a	45–65%**	8.2%
Caffeine	0.7–1.0%	≤ 1.0%	0.6–9.08%	3.5–8.5%

*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.[363]
**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
ORAC = Oxygen Radical Absorption Capacity

As shown in Table 25 above, Heimbach et al. reported the total phenolic acid content for CoffeeBerry® ethanol extract (which include only the major CAs and caffeic, quinic and ferulic acids) as 35–40%, which is similar to the major CAs found in Gyusa.g™ (see Table 4). 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.[363] 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 Gyusa.g™, 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).[362]

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).[362] 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.[362] 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.[362]

6.1.2.3 Summary of CoffeeBerry® studies

In summary, the CoffeeBerry® studies are considered relevant to the safety evaluation of Gyusa.g™ due to the content of CAs. While ratios of various individual CA compounds differ slightly in guayusa compared to coffee products, the major compounds are the same and levels are reasonably similar (see Table 18). 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 estimated typical exposure of CAs from Gyusa.g™ 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 26 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%,[362] AFS additionally analyzed a purchased sample of the CoffeeBerry® ethanol extract, and determined a minimum of 45% total CAs (see Table 25), and a more detailed publication on the composition of CoffeeBerry® ethanol extract by Mullen et al. (2011) determined a content of 42% CAs.[363] If these higher percentages were used to calculate the NOAEL for the CAs, they would provide even higher margins of safety.

Table 26. Margin of Safety Calculations for Chlorogenic Acids from Gyusa.g™ based on the CoffeeBerry® Ethanol Extract 90-day Feeding Study

Estimated 90th Percentile Lifetime Exposure to CAs from Gyusa.g™ (Part 3.4.1)	CAs from Gyusa.g™ Margin of Safety (NOAEL/EDI) Assuming reasonable similarity of CAs in CoffeeBerry® ethanol extract to CAs in Gyusa.g™ (NOAEL = 1206 mg/kg bw/day CAs)
3.3–9.6 mg/kg bw/day	125–365

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.[364] 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 findings[365]) 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 humans.[366])

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.[367] 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.[368-370]) 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[368, 369]). 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., 1981[370]). 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).[365] 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).[113]

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.[371] 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,[113] 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.[372] 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).[367] 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.[373] 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.[374, 375] 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 show to be total reversed by the time fetuses allowed to deliver were 6 days old.[376]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 7855 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.[377] 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.[72, 77, 146, 289-291] 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.[72]

Overall, large reviews, including the 2017 ILSI/Wikoff et al. systematic review[72] have 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 of caffeine in pregnancy). As the exposure estimates in Part 3 of this dossier suggest, women of reproductive age will consume less than 300 mg/day of caffeine from background sources plus Gyusa.g™'s intended uses, and the exposure levels of caffeine and CAs from Gyusa.g™ is relatively similar to that in coffee from which much data on safe caffeine consumption levels was derived. Therefore, Gyusa.g™ is expected to be safe at its intended use levels with regard to reproduction and developmental toxicity endpoints.

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.[378-385] 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.[386] 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),[79, 387] 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.[79] 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).[388, 389] 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.[390] 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.[391] 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).[392] 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.[393] 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).[394] 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.[395] 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.[396] 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.[397] 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.[398] 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.[399] 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 Svetol™) for 12 weeks. None of the studies reported any signs of abnormal or toxicologically concerning outcomes.[79, 389, 391-393, 398, 400]

6.3.6 Chlorogenic Acids Possible Modes of Action

This dossier is related to the safety, as opposed to the efficacy, of Gyusa.g™, and neither the extract 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.[148, 401, 402] 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.[403] 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.[404] 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 uses of the Gyusa.g™ are 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.[279, 405] 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-kB 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.[279, 405] The antioxidant and glucose modulation actions of CAs may also be hepatoprotective, by suppressing liver fibrogenesis and counteracting steatogenesis.[228]

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.[79] 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.[406] 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-phospate 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.[378] 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.[407] 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.[408] 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.[409] 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.[387] Glucose-6-phosphate hydrolysis was measured in the presence of Svetol™ (a green coffee bean alcohol-extract standardized to 45–50% CA (equal amounts of 3-, 4- and 5-CQA[79, 387]) or CAs in intact human liver microsomes. Svetol™ significantly inhibited hydrolysis of glucose-6-phospate 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-phospate hydrolysis.

CA may also have an inhibiting effect on complex carbohydrate-hydrolyzing enzymes, which in turn can decrease absorption of carbohydrates after food intake.[410] For example, CA was found to inhibit α-amylase and α-glucosidase in vitro in a dose-dependent manner (2–8 μg/mL), although the effect was less than that of caffeic acid.[411]

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.[405, 412]

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.[413] 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 approximately192 hours of incubation at mM concentrations of CA.[412] 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.[148, 404, 414-421] 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.[420, 422] 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.[423-427] Acute effects on left ventricular polarization do not appear to occur with either caffeine or CAs.[428] Hydroxyhydroquinones, a byproduct of coffee roasting, may also mitigate beneficial endothelial effects from ingestion of CAs.[429] 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.[430]

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.[414] 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).[404] 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.[415] 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.[416] 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 to CAs from the intended uses of Gyusa.g™ are not expected to be outside of levels already consumed by 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,[431-435] ischemia and reperfusion, [436] neuronal toxicity and cell death,[437] endothelial dysfunction,[438] and myocardial infarctions.[439]

Additionally, CAs significantly reduced the frequencies of micronucleated polychromatic erythrocytes induced by whole body exposure to λ-radiation,[440] inhibited duck hepatitis B virus when given to ducklings orally at a dose of 100 mg/kg bw/day, twice per day,[441] and has shown chemoprotective potential in rats and hamsters.[442-445] 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.[432]

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 (^{55}Fe and ^{59}Fe) 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.[446] 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 ^{55}Fe and ^{59}Fe 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 (^{59}Fe radio-labeled iron citrate) absorption and subsequent tissue distribution.[447] 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.[448] 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.[449] 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.[450] The absorption of zinc (^{67}Zn) 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 (^{65}Cu) 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.[451] 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.[452] 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 sulfhydryl 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.[453, 454] 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.[455] 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.[456] 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.[446-450, 457] 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.[458] Similarly, while diets high in fiber and phytates are known to reduce zinc absorption, zinc deficiencies are uncommon in

healthy individuals.[459] 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 Gyusa.g™ are not expected to substantially increase consumption of CAs at the 90th percentile as compared to consumption by coffee drinkers as shown in Part 3, which, as stated by Hurrell et al.,[448] is not associated with prevalence of anemia according to NHANES II data. Thus, consumption of Gyusa.g™ 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 reasonably similar levels of individual CA compounds as are found in Gyusa.g™ on a per serving basis, 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/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 Gyusa.g™ from its intended uses. 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 Safety of Other Components of *Ilex guayusa* Leaf Extract

As mentioned previously and shown in Table 5, other constituents of Gyusa.g™ include carbohydrates/sugars, minerals, and protein. These substances are present in the extract at relatively low levels and are so ubiquitous in foods that consumption of them from Gyusa.g™ 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. Levels of macro- and micronutrient components in Gyusa.g™ with comments related to their recommended Daily Values to put the exposures into perspective are shown in Table 27 below.

Table 27. Estimated Exposure Levels of Other Components of Gyusa.g™ (based on Component Concentrations in Table 5 and Gyusa.g™'s 90th Percentile Lifetime Exposure estimate for the Total Population shown in Tables 17*)

	Gyusa.g™		Comments to put the 90th percentile exposure levels into perspective
	% of extract	Approx. mg/day	
Total Fatty Acids	0	0	The Daily Value** for fat is 65,000 mg/day
Total Carbohydrate	78.8	2456	The Daily Value** for carbohydrates is 300,000 mg/day
Calcium	0.067	2.1	The Daily Value** for calcium is 1000 mg/day
Sodium	0.124	3.9	The Daily Value** for sodium is 2400 mg/day
Other Elements	1.46	45.5	The Daily Values** for the individual elements Mg and K, for example, are 400 and 3500 mg/day, respectively
Protein	18.5	576	The Daily Value** for protein is 50,000 mg/day

*Based on 20% presence probability exposures of 3116.2 mg/day for Gyusa.g™
**Daily values are based on a 2000 calorie diet

6.5 *Ilex paraguariensis* (Yerba Maté)

6.5.1 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,[4] *I. paraguariensis* is more often found in the Southern region of South America, in Argentina, Brazil, Paraguay and Uruguay.[460, 461] 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.[278, 460-462] 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.[461] 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),[461] and the plant and maté beverage have penetrated the U.S. and European markets as well.[48, 461, 462]

Heck et al. conducted a scientific review of yerba maté (*I. paraguariensis*) in 2007.[462] 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.[462]

While some early observational studies 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 has been recognized that other factors play a role in this correlation, such as smoking and alcohol consumption, which are strongly associated with the heavy maté-consuming cultures of the regions where these associations have been made.[461-464] 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 also appears to be related to the temperature of the infusion when it is consumed, affecting the oral tissue, rather than a particular carcinogenic constituent.[461, 463-465] In fact, IARC published a 2016 review of maté and modified its previous Group 2A classification (probably carcinogenic to humans) to a Group 3 (not classifiable as to its carcinogenicity to humans) as long as it is consumed while "not very hot".[171, 172] Maté hot beverage's previous Group 2A classification was based entirely on epidemiological case-control studies, and the association has now been determined to be related to temperature of the consumed beverage and not maté itself.[171] *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.[461, 462]

Pursuant to 21 CFR §182.20, essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *I. paraguariensis* St. Hil. (maté) are GRAS in the United States for their intended use as natural flavors. 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.

Prepared maté beverages were shown in one publication to contain a wide concentration range of CAs (0.2–7.4 mg per mL of fresh tea, equivalent to the large range of approximately 4.8–1776 mg per 240 mL serving).[466] Marques et al. evaluated methanolic extracts and plant infusions (0.5%; 1 g dried plant material per 190 mL of 95 °C water to make a standard consumption serving) of green and toasted *I. paraguariensis* for individual CA compounds using HPLC-UV analysis. The results are compared with the Gyusa.g™ CA individual compound results in Table 28 below:[467]

Table 28. Chlorogenic Acid Composition of *Ilex paraguariensis* (data in table borrowed from Marques et al. 2009)[467] compared to Gyusa.g™ (data borrowed from Table 23)

CAs (g/100 g or %)	Gyusa.g™		*Ilex paraguariensis* (Marques, 2009)[467]			
			Green *Ilex paraguariensis*		Toasted *Ilex paraguariensis*	
	% Dry basis	Per serving* (mg)	% dry basis, methanolic extraction	per 200 mL serving	% dry basis, methanolic extraction	per 200 mL serving

				(0.5% infusion) (mg)		(0.5% infusion) (mg)
3-CQA + 5-CQA	1.9	13–69	4.0	36.5	1.0	39.1
4-CQA + 3-FQA + 5-FQA*§	2.7	19–98	1.5	11.5	0.5	11.4
3,4-diCQA + 3,5-diCQA + 4,5-diCQA	3.6	25–127	4.2	49.8	0.5	45.1
Total	8.2	58–293	9.7	97.8	2.0	95.6

*Data calculated based on minimum intended use mg/serving (for enhanced waters) and the maximum intended use serving mg/serving (for energy drinks), which were 706–3570 mg/serving for Gyusa.g™

*§For the Gyusa.g™ data, this includes minor/other CQAs, so more than just FQAs (3-, 4-, and 5-pCoQAs, 3,4-diFQA, 3,5-diFQA, dimCQAs, and other very minor constituents)

As noted in the table above, *I. paraguariensis* contains significant levels of the same CAs that are found in Gyusa.g™, at slightly different ratios and levels (note that the per serving data from the publication was from a 200 mL serving, which would be a very small serving by U.S. standards). The 200 mL infusion was shown to contain approximately 100 mg of CAs, which, as is true for the levels in Gyusa.g™ per serving, is within the range of CAs in a serving of coffee.[19, 20, 30, 49-52]

6.5.2 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.[460] 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.[460] 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 LD_{50}.

Subchronic toxicity was investigated in Wistar rats and in New Zealand rabbits using a dose of 2 g/kg bw/day for 12 weeks.[460] 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 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.[468] Liver, kidney and bladder cells were isolated and DNA damage induced by H_2O_2 was investigated using the comet assay. The maté was reported to be non-genotoxic to the liver, kidney and bladder cells, and increased resistance of DNA to H_2O_2-induced DNA strand breaks and improved DNA repair after H_2O_2 challenge in liver cells, irrespective of the dose ingested.

6.5.3 Human Studies

A single-blind trial of 102 healthy individuals was conducted to study the impact of yerba maté on lipid levels.[469] Subjects ingested of 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 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.[470] 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.[471] 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.[472] 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,[473] 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.[474]

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.[475] 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.[475] Thus, maté consumers drink about 300 mg of caffeine per day, which has been suggested as the upper limit of caffeine consumption during pregnancy.[71, 72]

In summary, *I. 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 Gyusa.g™.

6.6 Allergenicity

No reports of allergic reactions to guayusa were found in our investigations. Gyusa.g™ 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) required to be disclosed in labeling, pursuant to the Food Allergen Labeling and Consumer Protection Act (FALCPA). Additionally, Gyusa.g™ does not contain sugars, or any derivatives or products of the aforementioned. Gyusa.g™ contains less than 10 ppm of gluten and sulfites.

6.7 Past Sales and Reported Adverse Events

No FDA letters regarding concern for safety to companies that market products containing guayusa were located. A search of MedWatch, FDA's adverse event reporting program, and FDA's Recalls, Market Withdrawals, & Safety Alerts search engine did not uncover any mention of guayusa products. Applied Food Sciences Inc. has a strict surveillance standard operating procedure for recording, analyzing and reporting adverse events related to their products once they go to market.

6.8 Similar 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 29: 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.[48]

Table 29. Products Containing Guayusa in the U.S. Marketplace

Company	Product Name	Serving Size(s)
RUNA	Dried tea leaves and various ready to drink tea beverages made with guayusa http://runa.org/	12 oz. beverages made of brewed guayusa mixed with other juices and flavorings
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	Cold Pressed Energy 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
BNF	Cura Hibiscus Mint Yerba Mate https://www.bnfkombucha.com/nutrition-facts	Guayusa extract (one of multiple ingredients)
Kombucha Town	Guayusa Mint http://www.kombuchatown.com/our-products	Guayusa mint tea (one of multiple ingredients)
GT's Living Foods	Alive Adaptogenic Tea, Guayusa Turmeric https://gtslivingfoods.com/offering/alive/guayusa-turmeric/	Guayusa tea (one of multiple ingredients)
Four Sigmatic	Mushroom Focus Shot https://us.foursigmatic.com/products/mushroom-focus-shots	Organic guayusa leaf extract, 200 mg

Juice Press	Guayusa Tea https://juicepress.com/shop/specialty-drinks/guayusa-tea/	Guayusa leaves
Liv Energy	LIV https://www.drink-liv.ch/ https://www.amazon.com/Organic-Guayusa-Infusion-Drink-Sugarcane/dp/B07CHWXF4Z	Guayusa leaves
Nightwatch	Nightwatch Focus and Power https://www.nightwatchdrink.com/	Guayusa leaves
Barista Underground	Rishi Organic Guayusa Cacao Tea https://www.baristaunderground.com/products/rishi-organic-guayusa-cacao-tea?variant=12495043756143&utm_medium=cpc&utm_source=google&utm_campaign=Google%20Shopping&gclid=CJHmnJS-1eICFYK3wAod6x8LJA	Organic guayusa

6.9 Current 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 chlorogenic acid levels. FSAI was satisfied from the information that the two are substantially equivalent.[476] 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 leave 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.[477]

6.10 Basis for the GRAS Conclusion

Applied Food Sciences' aqueous *Ilex guayusa* leaf extract (Gyusa.g™) has been the subject of a thorough safety assessment as described above. The totality of evidence supporting the safety of Gyusa.g™ is comprised of data and information that establish its safety under the conditions of intended use and data and information that is corroborative of safety. The general availability and general acceptance, throughout the scientific community of qualified experts, of the data and information that establish the safety of Gyusa.g™ under the conditions of its intended use establish its general recognition. Together, the establishment of safety based on scientific procedures and its general recognition form the basis for the conclusion of GRAS status of Gyusa.g™ for its intended use.

6.10.1 Data and Information that Establish Safety

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

- The establishment of identity of Gyusa.g™ as a dried aqueous *Ilex guayusa* leaf extract with specifications for caffeine (3.5–8.5%) and containing other extractable major and minor material from the plant such as CAs, carbohydrates, protein, minerals, moisture, fatty acids, and small organic acids.
- The methods of manufacture, specifications, and batch analyses of the extract, demonstrating the safe production and the high quality control standards for the ingredient.
- The comprehensive consumer exposure analyses for the extract, as well as for its caffeine exposure (Part 3), demonstrating safe intake levels (Part 6), which are described further below.
- The safety profile of guayusa and guayusa extracts
    - A bacterial reverse mutation test and an in vitro mammalian chromosomal aberration test on an aqueous guayusa leaf extract published by Kapp et al.,[1] establishing the lack of in vitro genotoxic potential at limit doses.
    - A 90-day repeated-dose oral toxicity study in rats on an aqueous guayusa leaf extract,[1] establishing a lack of adverse effects and/or target organs (other than those attributed to caffeine intake, which has established safe levels of use for humans) with a 90-day NOAEL of 5000 mg/kg bw/day, the highest dose tested.
- The safety profile of individual constituents of Gyusa.g™:
    - Caffeine:

- Numerous toxicological safety reviews including those by authoritative bodies, concluding that human 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. The estimated exposure levels for caffeine from Gyusa.g™, including exposure from background, are below these established safe limits.
- The pharmacokinetic profile of caffeine suggesting rapid absorption, metabolism, and elimination from the body.
- The GRAS status of caffeine for use in cola-type beverages up to the level of 0.02% (200 ppm) caffeine, or approximately 0.2 mg/mL (~ 47 mg per 8 oz.), pursuant to 21 CFR §182.1180; which is the maximum intended caffeine addition level from Gyusa.g™ in carbonated beverages.
- The fact that caffeine consumption patterns have remained relatively consistent (or even declined) over time despite the introduction of various new caffeinated products into the marketplace.

- CAs:
  - The pharmacokinetic profile of CAs suggesting rapid absorption, metabolism, and elimination from the body.
  - The similarity of CAs from CoffeeBerry® ethanol extract to CAs in guayusa, and a 90-day feeding study NOAEL for CoffeeBerry® ethanol extract CAs providing a margin of safety of greater than 100 for the CAs in a typical batch of Gyusa.g™.
  - 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.
- Minor components:
  - The long ubiquitous history of consumption of other components of Gyusa.g™ (minerals, carbohydrates, protein) as macro- and micronutrient constituents of the diet suggest that these constituents are safe.

Gyusa.g™, manufactured in accordance with GMP, are intended to be used as an ingredient in energy bars, energy drinks, ready to drink tea beverages, carbonated drinks, coffee-like beverages, and enhanced waters, at addition levels based on maximum caffeine concentrations, which are essentially equivalent to caffeine concentrations already found in these product types in the U.S. market. Exceptions include energy bars (although some energy bar products in the marketplace do contain caffeine) and enhanced water (which will likely also be consumed in a substitutive manner to other caffeine containing beverages but are treated separately in this GRAS conclusion with regard to intended use and exposure estimations in order to be conservative). The exposure to caffeine from these uses is expected to be generally substitutive in the population, based on literature suggesting that caffeine consumption has not increased in the population despite many new caffeine products entering the marketplace. Similarly, the maximum addition levels per serving of CAs are comparable to those found in a cup of coffee.

Studying the toxicity of guayusa in animal models with incorporation of adequate dosing uncertainty factors is challenging due to its relatively high concentration of caffeine. The guayusa leaf extract dose groups in the 90-day gavage study by Kapp et al. contained caffeine levels of 36, 75 and 150 mg/kg bw/day. A positive control group administered 150 mg/kg bw/day of a reference caffeine for comparison was thus included. While the guayusa extract was associated with a number of significant effects on various parameters compared to the negative control group, the findings generally mimicked those seen in the caffeine control group and were attributed to the known effects of caffeine as discussed in detail in Part 6 of this report. Note that based strictly on weight comparisons, the 150 mg/kg dose of caffeine is equivalent to approximately 10.5 g/day for a 70 kg human, or consumption of approximately 53 cups of coffee at once (with 200 mg/caffeine per cup) per day, which is obviously extremely high. The NOAEL for the guayusa extract (outside of caffeine effects) was considered the highest dose tested, at 5000 mg/kg bw/day.

NHANES 2013–2014 data was analyzed using Creme software to determine total exposure to Gyusa.g™ based on its proposed uses in foods. Because the addition of guayusa extract to the intended use food categories is novel in most cases, both a 100% presence probability factor and a more realistic 20% presence probability factor were used in the calculations. The latter was intended to represent an approximate 20% market share (still considered highly conservative) in each of the proposed food categories. The estimated 90th percentile aggregate exposure to Gyusa.g™ for the total population (2+) from its intended uses is 9249 mg/day (117.3 mg/kg bw/day) using a 100% presence probability factor, while the still conservative 20% presence probability estimated exposure was 3116.2 mg/day (39.7 mg/kg bw/day). The Guayusa Concentrate (GC) aqueous extract used in the Kapp et al. publication cannot be directly compared to Gyusa.g™ in that GC was a liquid extract (~66% moisture), while Gyusa.g™ is a spray-dried extracts with <

5% moisture. Although GC and Gyusa.g™ are both aqueous extracts with fairly similar amounts of caffeine and CAs per typical batch analysis. Thus it is not appropriate to calculate a traditional margin of safety from the NOEAL of the GC study and the estimated daily intakes of AFS's Gyusa.g™ except as applicable to the exposures to caffeine and CAs from GC as, the caffeine:CAs ratios are relatively similar among the ingredients. Furthermore, to the extent that GC contains other similar constituents of guayusa as found in guayusa extracts, this study is also relevant qualitatively to the overall safety of Gyusa.g™ as a whole ingredient, especially because the NOAEL for GC was the highest dose tested (aside from findings attributed to caffeine), with testing at higher dose levels or longer durations likely unreasonable due to the effects of caffeine and the limitations of gavage dosing in rats. Additionally, the genotoxicity studies on GC did not reveal any concerns at limit doses; the extract was negative in both a bacterial reverse mutation test and an in vitro chromosomal aberration test in human lymphocytes.

The safety of the caffeine component of Gyusa.g™ has been evaluated extensively by various scientific and government bodies. The NHANES based caffeine exposure estimates reported within this report, as well as other published caffeine exposure estimates discussed suggest that background caffeine consumption by adults, children and reproductive aged women fall generally below the 400 mg/day, 2.5 mg/kg bw/day and 300 mg/day levels generally considered safe, respectively.[71, 72] Additionally, caffeine consumption has remained relatively consistent (or even declined) over the years despite the introduction of various new caffeinated products into the marketplace, which suggests that Gyusa.g™'s intended uses will not lead to increases in caffeine consumption in the population.[14-16, 55-57]

NHANES 2013–2014 data was also used, along with USDA FNDDS concentration data related to caffeine, to estimate caffeine consumption in the U.S. population from all background sources in addition to the proposed uses of Gyusa.g™ using Creme software. The total population estimates were 333.0 mg/day (4.17 mg/kg bw/day) of caffeine at the lifetime 90th percentile from intended uses combined with background caffeine exposure, compared to 298.8 mg/day (3.8 mg/kg bw/day) from the background diet; all results fell below the 400 mg/day estimate that is generally considered safe for consumption.

Caffeine aggregate exposure in children was estimated to increase to 66.2 mg/day (2.09 mg/kg bw/day), from background estimates of 27.9 mg/day (0.9 mg/kg bw/day). In looking deeper into the exposure data for children from the individual intended use categories, the increase over background levels at the 90th percentile appears to be related to consumption of energy bars, tea beverages, carbonated soft drinks, and enhanced water beverages and was based on the use of many surrogate NHANES food codes that do not normally contain caffeine; thus, when they were assigned a caffeine composition, exposures increased. The products containing Gyusa.g™ will not intentionally be marketed to young children. The products are expected to be clearly labeled with caffeine content; thus, it is expected that many

parents will avoid giving the products to their children for that reason. The caffeine addition levels for the carbonated beverages are the GRAS allowable level for caffeine pursuant to 21 CFR §182.1180. Importantly, the caffeine exposure estimate for all of the proposed uses combined with background levels of 2.09 mg/kg bw/day still fell below the 2.5 mg/kg bw/day that is considered reasonably safe for children by various scientific bodies.[71, 72] Also again important is the fact that caffeine consumption has remained stable in U.S. children in recent years despite many new caffeinated products being added to the market.[14-16, 55-58]

Exposure to caffeine from the intended uses plus background by women of reproductive age was estimated at 280.4 mg/day (3.85 mg/kg bw/day), which was also a slight increase from the background-only estimated caffeine exposure of 222.7 mg/day (3.2 mg/kg bw/day). Again, part of this increase was likely due to the fact that surrogate food categories that do not usually contain caffeine were utilized. Nevertheless, the exposure estimates for this population still fell below safe consumption estimates for pregnant women of <300 mg/day as published by Nawrot in 2003 and Wikoff in 2017.[71, 72]

Several published background caffeine exposure estimates (e.g., Mitchell 2014[13] and Fulgoni, 2015[57]) found that exposure at the 90th percentile in certain subpopulations did exceed 400 mg/day and/or 2.5 mg/kg bw/day. The sample sizes for consumption of some beverage categories were too low to accurately estimate a 90th percentile value for children in the Mitchell study; thus, it is unclear how accurate the estimates truly are.[13] It should also be noted that some organizations (including EFSA and the Linus Pauling Institute)[51, 123] have suggested that an upper limit of 3 mg/kg bw/day caffeine (instead of 2.5 mg/kg bw/day) may be reasonable for children. EFSA suggested that this exposure amount might be reasonable 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. While ILSI examined safety of caffeine in children in their 2017 systematic review,[72] they did not attempt to determine a novel safe level but instead used the levels considered to be safe by Nawrot/Health Canada, namely 2.5 mg/kg bw/day for children[71] as comparators to data from more recent studies. The review found that 2.5 mg/kg bw/day was still reasonably safe for children, but the authors did not comment on whether higher levels might be safe as well.

With regard to adults, while caffeine consumption is considered to be self-limiting in nature in individuals based on caffeine's stimulating properties,[71, 72, 185] there are also individuals who intentionally seek out consumption of high levels of caffeine for its stimulating properties. While the 90th percentile exposure levels in some adult populations creep above 400 mg/day, they still fall below the upper limit of levels that were considered moderate by the Institute of Medicine in 2001, for example (600 mg in military personnel).[161] Individual genetic polymorphisms likely play a

strong role in differing caffeine consumption patterns throughout the population.[72, 185, 188-190]

Gyusa.g™ is intended to be added to products at levels considered to be reasonable in terms of caffeine exposure per serving (for example, EFSA concluded in 2015 that single doses of caffeine up to 200 mg are not a concern with regard to safety for adults[123]) and that are, for the most part, comparable to those found in other similar products sold in the marketplace. The naming of a product as an "energy drink", coffee, or tea, generally suggests the presence of caffeine, which is expected to aid consumers in making individual choices about caffeine consumption. Total caffeine levels should be disclosed on the labeling of final products containing I Gyusa.g™, and products containing this ingredient should follow relevant voluntary guidelines for caffeine labeling such as those established by the Council for Responsible Nutrition or the American Beverage Association (e.g., for energy drink labeling: "this product is not intended or recommended for children, pregnant or nursing women, and/or those sensitive to caffeine").[18]

Exposure to the CAs component of Gyusa.g™ was also estimated in Part 3 of this report. As discussed in Subpart 3.4.1, the range of CAs per serving from Gyusa.g™ for the various intended use food categories is ~58–293 mg/serving. Additionally discussed in that Subpart, estimated aggregate exposure to Gyusa.g™ for the total population from its intended uses suggest an exposure to 758 mg CAs/day (9.6 mg/kg bw/day) from Gyusa.g™ at 100% presence probability, and 256 mg/day (3.3 mg/kg bw/day) at 20% presence probability. While Gyusa.g™ does not have a specification for CAs, the ratio of CAs to caffeine in a typical batch of Gyusa.g™ is approximately 1.0, thus it is expected that exposure levels of CAs may be similar to exposure levels of caffeine (i.e. <400 mg/day for adults).

As is detailed, while CAs are present in numerous foods in the diet (e.g., apples, potatoes, plums, carrots, maté), the majority of consumption of CAs comes from coffee (over >70–90% in studies). A single serving of brewed coffee and/or an espresso beverage may contain from 15 mg to 675 mg CAs.[19, 20, 30, 49-52] Espresso beverages from various locations were recently analyzed and found to contain 24–422 mg of CAs per single serving.[19] The composition of the individual CAs in Gyusa.g™ were compared to those in coffee on a per serving basis in this report and are considered reasonably similar. A clinical trial reported no adverse effects from consumption of green coffee bean CAs at a dose of at least 750 mg per day for 12 weeks.

A margin of safety range for the estimated typical exposure of CAs from Gyusa.g™ based on the CoffeeBerry® ethanol extract 90-day study NOAEL was calculated in Subpart 6.1.2.3 to be 125–365, which is greater than the usual expected margin of safety for a food ingredient of 100 (21 CFR §170.22). Average intake levels for CAs in published studies in various populations around the world are approximately 500 mg daily (by doubling this average, we can estimate that exposure maybe 1000

mg/day at the 90th percentile).[90-92, 94] As coffee-like beverages are one of the intended use categories, and due to the substitutive nature of caffeinated beverages in general, it is expected that the exposure to CAs from Gyusa.g™ will be at least partially substitutive for CAs from the diet. Thus in general, consumption of Gyusa.g™ CAs from its intended uses is considered reasonably certain to be safe.

6.10.2 Data and Information that is Corroborative of Safety

Additional information that is considered corroborative of safety for Gyusa.g™ includes:

- An acute oral toxicity study of GC in female rats resulted in an LD_{50} of greater than 5000 mg/kg bw.
- A long history of daily human consumption of guayusa leaf decoctions in South American regions without known adverse health effects.
- The safety of the closely related *I. paraguariensis* plant, the leaves of which are also used to make a tea beverage (yerba maté), which is widely consumed without known toxicological issues, and the natural extractives of which are considered GRAS per 21 CFR §182.20. The two plants contain many of the same constituents (including CAs) and have been considered substantially equivalent by at least one EU regulatory body (Ireland). Aqueous extracts of dried leaves of *Ilex guayusa* are now an authorized novel food in the European Union with maximum levels of use stated as "in line with normal use in herbal infusions and food supplements of a similar aqueous extract of dried leave 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.[477]
- That a number of other *Ilex* species are also consumed as teas around the world without any known safety concerns; *I. vomitoria,* native to North America, was consumed as yaupon tea by Native Americans and European colonists,[67, 68] and *I. kudingcha, I. latifolia, I. cornuta* and *I. pentagona,* are consumed as Chinese Kudingcha tea.[8, 11, 69, 70]

6.10.3 Safety Conclusion

In summary, exposure to Gyusa.g™, as well as its two major constituents (CAs and caffeine) and other more minor constituents, combined with the data and information that establish safety as outlined above, provides reasonable certainty of

no harm to consumers from exposure to AFS Gyusa.g™ under the conditions of its intended use.

6.10.4 General Recognition

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

6.11 Data and Information that are Inconsistent with the GRAS Conclusion

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

6.12 Information that is Exempt from Disclosure under FOIA

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

The signatures in the cover letter (agent of the notifier), and in Part 1 of this notice (notifier), are personal privacy information. This personal privacy information has no bearing on the safety of Gyusa.g™.

Part 7: Supporting Data and Information

Literature searches for the safety assessment of guayusa described in Part 6 of this GRAS notice were conducted through November 2018.

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

1. Kapp RW, Jr., Mendes O, et al. General and Genetic Toxicology of Guayusa Concentrate (Ilex guayusa). *Int J Toxicol.* 2016
2. IARC and WHO. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 51. Coffee, Tea, Mate, Methylxanthines and Methylglyoxal. 1991. 1-523.
3. Lewis WH, Kennelly EJ, et al. Ritualistic use of the holly Ilex guayusa by Amazonian Jivaro Indians. *J Ethnopharmacol.* 1991;33(1-2):25-30
4. Dueñas-Serrano J, Jarrett C, et al. A historical and ethno-botanical overview of Ilex guayusa Loes. (Aquifoliaceae) with notes on its distribution. 2014:1-15
5. Alikaridis F. Natural constituents of Ilex species. *J Ethnopharmacol.* 1987;20(2):121-44
6. Dueñas J, Jarrett C, et al. Amazonian Guayusa (Ilex guayusa Loes.): A Historical and Ethnobotanical Overview. *Economic Botany.* 2016:1-7
7. Yi F, Zhao X, et al. Genus llex L.: Phytochemistry, Ethnopharmacology, and Pharmacology. *Chinese Herbal Medicines.* 2016;8(3):209-230
8. Hao D, Gu X, et al. Research progress in the phytochemistry and biology of Ilex pharmaceutical resources. *Acta Pharmaceutica Sinica B.* 2013;3(1):8-19
9. Garcia-Ruiz A, Baenas N, et al. Guayusa (Ilex guayusa L.) new tea: phenolic and carotenoid composition and antioxidant capacity. *J Sci Food Agric.* 2017
10. Villacis-Chiriboga J, Garcia-Ruiz A, et al. Changes in phytochemical composition, bioactivity and in vitro digestibility of guayusa leaves (Ilex guayusa Loes.) in different ripening stages. *J Sci Food Agric.* 2018;98(5):1927-1934
11. Pardau MD, Pereira ASP, et al. Antioxidant and anti-inflammatory properties of Ilex guayusa tea preparations: a comparison to Camellia sinensis teas. *Food Funct.* 2017;8(12):4601-4610
12. Food Chemicals Codex. FCC 10. Caffeine: 2016. 208-209.
13. Mitchell DC, Knight CA, et al. Beverage caffeine intakes in the U.S. *Food Chem Toxicol.* 2014;63:136-42
14. Ahluwalia N and Herrick K. Caffeine intake from food and beverage sources and trends among children and adolescents in the United States: review of national quantitative studies from 1999 to 2011. *Adv Nutr.* 2015;6(1):102-11

15. Tran NL, Barraj LM, et al. Trends and patterns of caffeine consumption among US teenagers and young adults, NHANES 2003-2012. *Food Chem Toxicol*. 2016;94:227-42
16. Drewnowski A and Rehm CD. Sources of Caffeine in Diets of US Children and Adults: Trends by Beverage Type and Purchase Location. *Nutrients*. 2016;8(3):154
17. Mayo Clinic. Caffeine content for coffee, tea, soda and more. [October 1, 2011; Retrieved January 16, 2014, 2014] from http://www.mayoclinic.org/caffeine/ART-20049372?p=1.
18. Rosenfeld LS, Mihalov JJ, et al. Regulatory status of caffeine in the United States. *Nutr Rev*. 2014;72 Suppl 1:23-33
19. Crozier TW, Stalmach A, et al. Espresso coffees, caffeine and chlorogenic acid intake: potential health implications. *Food Funct*. 2012;3(1):30-3
20. Ludwig IA, Mena P, et al. Variations in caffeine and chlorogenic acid contents of coffees: what are we drinking? *Food Funct*. 2014;5(8):1718-26
21. National Center for Biotechnology Information. PubChem Compound Database; CID=2519. Caffeine. [Retrieved March 4, 2016] from https://pubchem.ncbi.nlm.nih.gov/compound/2519.
22. Wu X, Beecher GR, et al. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem*. 2004;52(12):4026-37
23. Perez-Jimenez J, Neveu V, et al. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *Eur J Clin Nutr*. 2010;64 Suppl 3:S112-20
24. Martini D, Del Bo C, et al. Coffee Consumption and Oxidative Stress: A Review of Human Intervention Studies. *Molecules*. 2016;21(8)
25. Graf B, Milbury P, et al. Flavonols, flavones, flavanones, and human health: epidemiological evidence. *J Med Food*. 2005;8(3):281-290
26. Pandey KB and Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev*. 2009;2(5):270-8
27. Santana-Galvez J, Cisneros-Zevallos L, et al. Chlorogenic Acid: Recent Advances on Its Dual Role as a Food Additive and a Nutraceutical against Metabolic Syndrome. *Molecules*. 2017;22(3)
28. Mattila P, Hellstrom J, et al. Phenolic acids in berries, fruits, and beverages. *J Agric Food Chem*. 2006;54(19):7193-9
29. Manach C, Scalbert A, et al. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*. 2004;79(5):727-47
30. Clifford M. Chlorogenic acids and other cinnamates - nature, occurrence and dietary burden. *J Sci Food Agr*. 1999;79:362-372
31. Crozier A, Jaganath IB, et al. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat Prod Rep*. 2009;26(8):1001-43
32. Farah A. Chapter 2. Coffee constituents. In: Emerging health effects and disease prevention. Y-F Chu: Wiley & Sons - Blackwell: 2012. 21-58.
33. Farah A and Donangelo C. Phenolic compounds in coffee. *Braz J Plant Physiol*. 2006;18:23-36
34. Farah A. Coffee as a functional beverage. *AGRO FOOD INDUSTRY HI TECH*. 2009;20(6):36-39

35. Abranko L and Clifford MN. An Unambiguous Nomenclature for the Acyl-quinic Acids Commonly Known as Chlorogenic Acids. *J Agric Food Chem.* 2017;65(18):3602-3608
36. Clifford M and Abranko L. Some Notes on the Chlorogenic Acids. 1. Numbering and Nomenclature. ResearchGate: https://tinyurl.com/ya8xppt2. 2017.
37. Kremr D, Bajer T, et al. Unremitting problems with chlorogenic acid nomenclature: A review. *Química Nova.* 2016;39(4):530-533
38. Lafay S, Gil-Izquierdo A, et al. Chlorogenic acid is absorbed in its intact form in the stomach of rats. *J Nutr.* 2006;136(5):1192-7
39. Mills CE, Oruna-Concha MJ, et al. The effect of processing on chlorogenic acid content of commercially available coffee. *Food Chem.* 2013;141(4):3335-40
40. Jaiswal R, Patras MA, et al. Profile and characterization of the chlorogenic acids in green Robusta coffee beans by LC-MS(n): identification of seven new classes of compounds. *J Agric Food Chem.* 2010;58(15):8722-37
41. Del Rio D, Stalmach A, et al. Bioavailability of coffee chlorogenic acids and green tea flavan-3-ols. *Nutrients.* 2010;2(8):820-33
42. Stavric B. Methylxanthines: toxicity to humans. 2. Caffeine. *Food Chem Toxicol.* 1988;26(7):645-62
43. Stavric B and Gilbert S. Caffeine metabolism: a problem in extrapolating results from animal studies to humans. *Acta Pharm Jugosl.* 1990;40(3):475-489
44. Drugs.com. Theophylline Dosage. [2017; Retrieved February 21, 2017] from https://www.drugs.com/dosage/theophylline.html.
45. Stavric B. Methylxanthines: toxicity to humans. 1. Theophylline. *Food Chem Toxicol.* 1988;26(6):541-65
46. Jara A, Rodriguez Y, et al. Antioxidant activity and total phenolics of plants used in traditional medicine in Ecuador. Presented at the 17th International Electronic Conference on Synthetic Organic Chemistry. www.sciforum.net; 2013.
47. Sigma-Aldrich. Product Information. Caffeine (Anhydrous). 1999.
48. Somogyi L. Caffeine intake by the U.S. population. Prepared for the Food and Drug Administration. (Includes FDA comments - December 2012). 2010. 1-85.
49. IARC and WHO. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 56. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. Caffeic Acid. 1993. 115-134.
50. Chou T. Wake up and smell the coffee. Caffeine, coffee, and the medical consequences. *West J Med.* 1992;157(5):544-53
51. Higdon JV and Frei B. Coffee and health: a review of recent human research. *Crit Rev Food Sci Nutr.* 2006;46(2):101-23
52. Davies E. Chemistry in every cup. *Chemistry World* Chemistry in every cup. May 2011. 36-39

53. Alcohol and Tobacco Tax and Trade Bureau (TTB) and U.S. Department of the Treasury. Kombucha. [2017] from https://www.ttb.gov/kombucha/index.shtml.
54. Gebara KS, Gasparotto-Junior A, et al. Daily Intake of Chlorogenic Acids from Consumption of Mate (Ilex paraguariensis A.St.-Hil.) Traditional Beverages. *J Agric Food Chem*. 2017;65(46):10093-10100
55. Ahluwalia N, Herrick K, et al. Caffeine intake in children in the United States and 10-y trends: 2001-2010. *Am J Clin Nutr*. 2014;100(4):1124-32
56. Mitchell DC, Hockenberry J, et al. Assessing dietary exposure to caffeine from beverages in the U.S. population using brand-specific versus category-specific caffeine values. *Food Chem Toxicol*. 2015;80:247-52
57. Fulgoni VL, 3rd, Keast DR, et al. Trends in intake and sources of caffeine in the diets of US adults: 2001-2010. *Am J Clin Nutr*. 2015;101(5):1081-7
58. Verster JC and Koenig J. Caffeine intake and its sources: A review of national representative studies. *Crit Rev Food Sci Nutr*. 2018;58(8):1250-1259
59. Wright JD, Wang CY, et al. Dietary intake of ten key nutrients for public health, United States: 1999-2000. *Adv Data*. 2003(334):1-4
60. Klein R, Proctor S, et al. Healthy People 2010 criteria for data suppression. *Statistical Notes*. 2002;24
61. Nusser S, Carriquiry A, et al. A semiparametric transformation approach to estimating usual daily intake distributions. *Journal of the American Statistical Association*. 1996;91(436):1440-1449
62. de Smet PA. A multidisciplinary overview of intoxicating snuff rituals in the western hemisphere. *J Ethnopharmacol*. 1985;13(1):3-49
63. Innerhofer S and Bernhardt K. Ethnobotanic garden design in the Ecuadorian Amazon. *Biodiversity and Conservation*. 2011;20(2):429-439
64. Jamieson RW. The essence of commodification: caffeine dependencies in the early modern world. *J Soc Hist*. 2001;35(2):269-94
65. Schultes R. Ilex guayusa from 500 A.D. to the present. *Etnologiska Studier*. 1972;32:115-138
66. Gan RY, Zhang D, et al. Health Benefits of Bioactive Compounds from the Genus Ilex, a Source of Traditional Caffeinated Beverages. *Nutrients*. 2018;10(11)
67. Power F and Chesnut V. Ilex vomitoria as a native source of caffeine. *Journal of the American Chemical Society*. 1919;41(8):1307-1312
68. Palumbo MJ, Putz FE, et al. Nitrogen fertilizer and gender effects on the secondary metabolism of yaupon, a caffeine-containing North American holly. *Oecologia*. 2007;151(1):1-9
69. Zhu F, Cai YZ, et al. Comparison of major phenolic constituents and in vitro antioxidant activity of diverse Kudingcha genotypes from Ilex kudingcha, Ilex cornuta, and Ligustrum robustum. *J Agric Food Chem*. 2009;57(14):6082-9
70. Li L, Xu LJ, et al. The large-leaved Kudingcha (Ilex latifolia Thunb and Ilex kudingcha C.J. Tseng): a traditional Chinese tea with plentiful secondary metabolites and potential biological activities. *J Nat Med*. 2013;67(3):425-37
71. Nawrot P, Jordan S, et al. Effects of caffeine on human health. *Food Additives and Contaminants*. 2003;20(1):1-30

72. Wikoff D, Welsh BT, et al. Systematic review of the potential adverse effects of caffeine consumption in healthy adults, pregnant women, adolescents, and children. *Food Chem Toxicol*. 2017;109(Pt 1):585-648
73. Knight CA, Knight I, et al. Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. *Food Chem Toxicol*. 2004;42(12):1923-30
74. Branum AM, Rossen LM, et al. Trends in caffeine intake among U.S. children and adolescents. *Pediatrics*. 2014;133(3):386-93
75. Bailey RL, Saldanha LG, et al. Estimating caffeine intake from energy drinks and dietary supplements in the United States. *Nutr Rev*. 2014;72 Suppl 1:9-13
76. Chen L, Bell EM, et al. Exploring maternal patterns of dietary caffeine consumption before conception and during pregnancy. *Matern Child Health J*. 2014;18(10):2446-55
77. USDA and DHHS. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. Advisory Report to the Secretary of Health and Human Services and the Secretary of Agriculture. 2015.
78. Duarte G, Pereira A, et al. Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. *Food Chem*. 2010;118(2010):851-855
79. Thom E. The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. *J Int Med Res*. 2007;35(6):900-8
80. Olthof MR, Hollman PC, et al. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am J Clin Nutr*. 2001;73(3):532-8
81. Ludwig IA, Clifford MN, et al. Coffee: biochemistry and potential impact on health. *Food Funct*. 2014;5(8):1695-717
82. Roberts WG and Gordon MH. Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *J Agric Food Chem*. 2003;51(5):1486-93
83. Kim SS, Son YO, et al. Antioxidant property of an active component purified from the leaves of paraquat-tolerant Rehmannia glutinosa. *Redox Report*. 2005;10(6):311-318
84. Mattila P and Hellstrom J. Phenolic acids in potatoes, vegetables, and some of their products. *J Food Compos Anal*. 2007;20:152-160
85. Rodriguez-Mateos A, Cifuentes-Gomez T, et al. Procyanidin, anthocyanin, and chlorogenic Acid contents of highbush and lowbush blueberries. *J Agric Food Chem*. 2012;60(23):5772-8
86. Cui T, Nakamura K, et al. Analyses of arbutin and chlorogenic acid, the major phenolic constituents in Oriental pear. *J Agric Food Chem*. 2005;53(10):3882-7
87. Clifford M. Chlorogenic acids and other cinnamates–nature, occurrence, dietary burden, absorption and metabolism. *J Sci Food Agr*. 2000;80(7):1033-1043
88. Plazas M, Andujar I, et al. Breeding for chlorogenic acid content in eggplant: interest and prospects. *Not Bot Horti Agrobo*. 2013;41(1):26-35

89. Kandil A, Li J, et al. Phenolic acids in some cereal grains and their inhibitory effect on starch liquefaction and saccharification. *J Agric Food Chem.* 2012;60(34):8444-9
90. Perez-Jimenez J, Fezeu L, et al. Dietary intake of 337 polyphenols in French adults. *Am J Clin Nutr.* 2011;93(6):1220-8
91. Grosso G, Stepaniak U, et al. Estimated dietary intake and major food sources of polyphenols in the Polish arm of the HAPIEE study. *Nutrition.* 2014;30(11-12):1398-403
92. Witkowska AM, Zujko ME, et al. Comparison of Various Databases for Estimation of Dietary Polyphenol Intake in the Population of Polish Adults. *Nutrients.* 2015;7(11):9299-308
93. Ovaskainen ML, Torronen R, et al. Dietary intake and major food sources of polyphenols in Finnish adults. *J Nutr.* 2008;138(3):562-6
94. Nascimento-Souza MA, de Paiva PG, et al. Estimated dietary intake and major food sources of polyphenols in elderly of Vicosa, Brazil: a population-based study. *Eur J Nutr.* 2018;57(2):617-627
95. Miranda AM, Steluti J, et al. Dietary intake and food contributors of polyphenols in adults and elderly adults of Sao Paulo: a population-based study. *Br J Nutr.* 2016;115(6):1061-70
96. Tresserra-Rimbau A, Medina-Remon A, et al. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. *Nutr Metab Cardiovasc Dis.* 2013;23(10):953-9
97. FDA and DHHS. Guidance for industry: estimating dietary intake of substances in food. 2006. 1-21.
98. Krieger DR, Kalman DS, et al. The Safety, Pharmacokinetics, and Nervous System Effects of Two Natural Sources of Caffeine in Healthy Adult Males. *Clin Transl Sci.* 2016
99. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. 2011. 1-25.
100. Redbook F. Toxicological principles for the safety assessment of food ingredients US (FDA). *Center for Food Safety and Nutrition (CFSAN).* 2000
101. FDA. Redbook 2000. Toxicological Principles for the Safety Assessment of Food Ingredients. IV.C.1.a. Bacterial Reverse Mutation Test; 2003: 59-67.
102. OECD. Test No. 473: In Vitro Mammalian Chromosomal Aberration Test, OECD Guidelines for the Testing of Chemicals. 1997.
103. OECD. Test No. 425: Acute Oral Toxicity - Up and Down Procedure (UDP), OECD Guidelines for the Testing of Chemicals. 2008.
104. OECD SIDS. Cafeine. CAS: 58-08-2. 2002. 376.
105. OECD. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. 2008.
106. FDA. Redbook 2000. Toxicological principles for the safety assessment of food ingredients. IV.C.4.a. Subchronic toxicity studies with rodents; 2003.
107. OECD. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. 1998.

108. Milanez S and Toxicology and Hazard Assessment Group. Adverse health effects of caffeine: review and analysis of recent human and animal research. Office of Nutrition, Labeling and Dietary Supplements, Office of Food Safety and Applied Nutrition, CFSAN, FDA. 2011. 392.
109. Gans JH. Comparative toxicities of dietary caffeine and theobromine in the rat. *Food Chem Toxicol*. 1984;22(5):365-9
110. Sinha RA, Farah BL, et al. Caffeine stimulates hepatic lipid metabolism by the autophagy-lysosomal pathway in mice. *Hepatology*. 2014;59(4):1366-80
111. Wilcox AR. The effects of caffeine and exercise on body weight, fat-pad weight, and fat-cell size. *Med Sci Sports Exerc*. 1982;14(4):317-21
112. Zheng G, Sayama K, et al. Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice. *In Vivo*. 2004;18(1):55-62
113. FAO/WHO. Food additives. Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives. 2000.
114. Temple JL and Ziegler AM. Gender Differences in Subjective and Physiological Responses to Caffeine and the Role of Steroid Hormones. *J Caffeine Res*. 2011;1(1):41-48
115. Temple JL, Dewey AM, et al. Effects of acute caffeine administration on adolescents. *Exp Clin Psychopharmacol*. 2010;18(6):510-20
116. Herz RS and Beninger RJ. Comparison of the ability of (+)-amphetamine and caffeine to produce environment-specific conditioning. *Psychopharmacology (Berl)*. 1987;92(3):365-70
117. Zimmerberg B, Carr KL, et al. The effects of postnatal caffeine exposure on growth, activity and learning in rats. *Pharmacol Biochem Behav*. 1991;39(4):883-8
118. Westwood FR, Iswaran TJ, et al. Long-term effects of an inotropic phosphodiesterase inhibitor (ICI 153,110) on the rat salivary gland, harderian gland, and intestinal mucosa. *Toxicol Pathol*. 1991;19(3):214-23
119. Chou TM and Benowitz NL. Caffeine and coffee: effects on health and cardiovascular disease. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol*. 1994;109(2):173-89
120. Wells D and Humphreys-Beher M. Analysis of protein synthesis in rat salivary glands after chronic treatment with β-receptor agonists and phosphodiesterase inhibitors. *Biochem Phamacol*. 1985;34(24):4229-4237
121. Inoue K, Morikawa T, et al. Adaptive parotid gland hypertrophy induced by dietary treatment of GSE in rats. *Toxicol Pathol*. 2014;42(6):1016-23
122. Everds NE, Snyder PW, et al. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. *Toxicol Pathol*. 2013;41(4):560-614
123. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Scientific Opinion on the safety of caffeine. *The EFSA Journal*. 2015;13(5):4102
124. Kot M and Daniel WA. Effect of cytochrome P450 (CYP) inducers on caffeine metabolism in the rat. *Pharmacol Rep*. 2007;59(3):296-305
125. Crisan M, Munteanu C, et al. Red bull induces biochemical changes in Wistar rat liver. *Annals of the Romanian Society for Cell Biology*. 2013;18(2):118

126. Khayyat L, Sorour J, et al. Histological, ultrastructural and physiological studies on the effect of different kinds of energy drinks on the liver of Wistar albino rat. *Int J Res Sci.* 2015;1(2):15-22
127. Akande I and Banjoko O. Assessment of biochemical effect of Power Horse energy drink on hepatic, renal and histological functions in Sprague Dawley rats. *Annual Review and Research in Biology.* 2011;1(3):45-56
128. Bukhar H, ElSawy N, et al. Biological effect of high energy drink on normal and hyperglycemic rats. *Pak J Nutr.* 2012;11(4):301
129. Onuegbu AJ, Olisekodiaka JM, et al. Coffee consumption could affect the activity of some liver enzymes and other biochemical parameters in healthy drinkers. *Med Princ Pract.* 2011;20(6):514-8
130. Modi AA, Feld JJ, et al. Increased caffeine consumption is associated with reduced hepatic fibrosis. *Hepatology.* 2010;51(1):201-9
131. Ruhl CE and Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology.* 2005;128(1):24-32
132. Ruhl CE and Everhart JE. Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. *Gastroenterology.* 2005;129(6):1928-36
133. Cadden IS, Partovi N, et al. Review article: possible beneficial effects of coffee on liver disease and function. *Aliment Pharmacol Ther.* 2007;26(1):1-8
134. Dickson JC, Liese AD, et al. Associations of coffee consumption with markers of liver injury in the insulin resistance atherosclerosis study. *BMC Gastroenterol.* 2015;15:88
135. Tanaka K, Tokunaga S, et al. Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. *Int J Epidemiol.* 1998;27(3):438-43
136. Honjo S, Kono S, et al. Coffee consumption and serum aminotransferases in middle-aged Japanese men. *J Clin Epidemiol.* 2001;54(8):823-829
137. Klatsky AL, Morton C, et al. Coffee, cirrhosis, and transaminase enzymes. *Arch Intern Med.* 2006;166(11):1190-5
138. D'Avanzo B, Santoro L, et al. Coffee consumption and serum cholesterol. GISSI-EFRIM Study Group. *Prev Med.* 1993;22(2):219-24
139. Hongu N and Sachan DS. Caffeine, carnitine and choline supplementation of rats decreases body fat and serum leptin concentration as does exercise. *J Nutr.* 2000;130(2):152-7
140. Sugiura C, Nishimatsu S, et al. Catechins and Caffeine Inhibit Fat Accumulation in Mice through the Improvement of Hepatic Lipid Metabolism. *J Obes.* 2012;2012:520510
141. Hasegawa N and Mori M. Effect of powdered green tea and its caffeine content on lipogenesis and lipolysis in 3T3-L1 cell. *J Health Sci.* 2000;46(2):153-155
142. Arciero PJ, Gardner AW, et al. Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *Am J Physiol.* 1995;268(6 Pt 1):E1192-8
143. Jung RT, Shetty PS, et al. Caffeine: its effect on catecholamines and metabolism in lean and obese humans. *Clin Sci (Lond).* 1981;60(5):527-35

144. Hursel R and Westerterp-Plantenga MS. Thermogenic ingredients and body weight regulation. *Int J Obes (Lond)*. 2010;34(4):659-69
145. Benowitz NL, Jacob P, 3rd, et al. Sympathomimetic effects of paraxanthine and caffeine in humans. *Clin Pharmacol Ther*. 1995;58(6):684-91
146. Brent RL, Christian MS, et al. Evaluation of the reproductive and developmental risks of caffeine. *Birth Defects Res B Dev Reprod Toxicol*. 2011;92(2):152-87
147. Swanston-Flatt SK, Day C, et al. Glycaemic effects of traditional European plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetes Res*. 1989;10(2):69-73
148. Tajik N, Tajik M, et al. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *Eur J Nutr*. 2017;56(7):2215-2244
149. Taylor L and Antonio J. Chapter 24: Coffee as a functional beverage. Handbook of nutraceuticals and functional foods. R Wildman. Boca Raton, FL: CRC Press: 2007. 453-465.
150. Xu JG, Hu QP, et al. Antioxidant and DNA-protective activities of chlorogenic acid isomers. *J Agric Food Chem*. 2012;60(46):11625-30
151. Liang N and Kitts DD. Role of Chlorogenic Acids in Controlling Oxidative and Inflammatory Stress Conditions. *Nutrients*. 2015;8(1)
152. Deshpande SN and Aguilar AA. Effects of roasting temperatures and gamma irradiation on the content of chlorogenic acid, caffeic acid and soluble carbohydrates of coffee. *Int J Appl Radiat Isot*. 1975;26(11):656-61
153. Jaiswal R, Matei MF, et al. Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food Funct*. 2012;3(9):976-84
154. Moon JK, Yoo HS, et al. Role of roasting conditions in the level of chlorogenic acid content in coffee beans: correlation with coffee acidity. *J Agric Food Chem*. 2009;57(12):5365-9
155. Perrone D, Farah A, et al. Influence of coffee roasting on the incorporation of phenolic compounds into melanoidins and their relationship with antioxidant activity of the brew. *J Agric Food Chem*. 2012;60(17):4265-75
156. Wei F, Furihata K, et al. Roasting process of coffee beans as studied by nuclear magnetic resonance: time course of changes in composition. *J Agric Food Chem*. 2012;60(4):1005-12
157. National Coffee Association. How to brew coffee: the NCA Guide to brewing essentials. [Retrieved March 26, 2018] from http://www.ncausa.org/About-Coffee/How-to-Brew-Coffee.
158. CoffeeFAQ.com. Just how much ground coffee do I need for X amount of coffee? [Retrieved March 26, 2108] from https://coffeefaq.com/just-how-much-ground-coffee-do-i-need-for-x-amount-of-coffee/.
159. Anderson JW, Baird P, et al. Health benefits of dietary fiber. *Nutr Rev*. 2009;67(4):188-205
160. Farah A, de Paulis T, et al. Effect of roasting on the formation of chlorogenic acid lactones in coffee. *J Agric Food Chem*. 2005;53(5):1505-13

161. Committee on Military Nutrition Research, Food and Nutrition Board, et al. Caffeine for the sustainment of mental task performance: formulations for military operations. Washington DC, National Academy Press; 2001: 172.
162. Drapeau C, Hamel-Hebert I, et al. Challenging sleep in aging: the effects of 200 mg of caffeine during the evening in young and middle-aged moderate caffeine consumers. *J Sleep Res*. 2006;15(2):133-41
163. Hursel R, Viechtbauer W, et al. The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: a meta-analysis. *Obes Rev*. 2011;12(7):e573-81
164. Beaudoin MS and Graham TE. Methylxanthines and human health: epidemiological and experimental evidence. *Handb Exp Pharmacol*. 2011(200):509-48
165. Arnaud M. Caffeine. In: Encyclopedia of Human Nutrition. Second Edition. Nestle S.A. Vevey, Switzerland: Elsevier: 2005. 247-252.
166. Nathanson JA. Caffeine and related methylxanthines: possible naturally occurring pesticides. *Science*. 1984;226(4671):184-7
167. Lee Y, Moon SJ, et al. Multiple gustatory receptors required for the caffeine response in Drosophila. *Proc Natl Acad Sci U S A*. 2009;106(11):4495-500
168. Wright GA, Baker DD, et al. Caffeine in floral nectar enhances a pollinator's memory of reward. *Science*. 2013;339(6124):1202-4
169. Health Canada. Caffeine in food. [2012; Retrieved June 19, 2014] from http://www.hc-sc.gc.ca/fn-an/securit/addit/caf/food-caf-aliments-eng.php.
170. Millen BE, Abrams S, et al. The 2015 Dietary Guidelines Advisory Committee Scientific Report: Development and Major Conclusions. *Adv Nutr*. 2016;7(3):438-44
171. Loomis D, Guyton KZ, et al. Carcinogenicity of drinking coffee, mate, and very hot beverages. *Lancet Oncol*. 2016;17(7):877-878
172. IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 116. Drinking coffee, mate, and very hot beverages. 2018. 501.
173. La Vecchia C and Tavani A. Coffee and cancer risk: an update. *Eur J Cancer Prev*. 2007;16(5):385-9
174. Bull S, Brown T, et al. External scientific report. Extensive literature search as preparatory work for the safety assessment for caffeine. EFSA, Ricardo-AEA. 2015. 1-98.
175. IARC and WHO. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.
176. Cano-Marquina A, Tarin JJ, et al. The impact of coffee on health. *Maturitas*. 2013;75(1):7-21
177. Pourshahidi L, Navarini L, et al. A Comprehensive Overview of the Risks and Benefits of Coffee Consumption. *Comprehensive Reviews in Food Science and Food Safety*. 2016;15(4):671-684
178. Grosso G, Godos J, et al. Coffee, Caffeine, and Health Outcomes: An Umbrella Review. *Annu Rev Nutr*. 2017;37:131-156
179. Zhang L, Kujawinski DM, et al. Caffeine in your drink: natural or synthetic? *Anal Chem*. 2012;84(6):2805-10

180. Morton K, Knight K, et al. A Prospective Randomized, Double-Blind, Two-Period Crossover Pharmacokinetic Trial Comparing Green Coffee Bean Extract-A Botanically Sourced Caffeine-With a Synthetic USP Control. *Clin Pharmacol Drug Dev.* 2018
181. White JR, Jr., Padowski JM, et al. Pharmacokinetic analysis and comparison of caffeine administered rapidly or slowly in coffee chilled or hot versus chilled energy drink in healthy young adults. *Clin Toxicol (Phila).* 2016;54(4):308-12
182. Kot M and Daniel WA. Caffeine as a marker substrate for testing cytochrome P450 activity in human and rat. *Pharmacol Rep.* 2008;60(6):789-97
183. Kot M and Daniel WA. The relative contribution of human cytochrome P450 isoforms to the four caffeine oxidation pathways: an in vitro comparative study with cDNA-expressed P450s including CYP2C isoforms. *Biochem Pharmacol.* 2008;76(4):543-51
184. Rybak ME, Sternberg MR, et al. Urine excretion of caffeine and select caffeine metabolites is common in the U.S. population and associated with caffeine intake. *J Nutr.* 2015;145(4):766-74
185. Nehlig A. Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption. *Pharmacol Rev.* 2018;70(2):384-411
186. Yu T, Campbell SC, et al. Pregnancy-induced changes in the pharmacokinetics of caffeine and its metabolites. *J Clin Pharmacol.* 2016;56(5):590-6
187. Gajewska A, Blumenthal TD, et al. Effects of ADORA2A gene variation and caffeine on prepulse inhibition: a multi-level risk model of anxiety. *Prog Neuropsychopharmacol Biol Psychiatry.* 2013;40:115-21
188. Cornelis MC, Monda KL, et al. Genome-wide meta-analysis identifies regions on 7p21 (AHR) and 15q24 (CYP1A2) as determinants of habitual caffeine consumption. *PLoS Genet.* 2011;7(4):e1002033
189. Amin N, Byrne E, et al. Genome-wide association analysis of coffee drinking suggests association with CYP1A1/CYP1A2 and NRCAM. *Mol Psychiatry.* 2012;17(11):1116-29
190. McMahon G, Taylor AE, et al. Phenotype refinement strengthens the association of AHR and CYP1A1 genotype with caffeine consumption. *PLoS One.* 2014;9(7):e103448
191. Robertson TM, Clifford MN, et al. Postprandial glycaemic and lipaemic responses to chronic coffee consumption may be modulated by CYP1A2 polymorphisms. *Br J Nutr.* 2018;119(7):792-800
192. Gierach GL, Freedman ND, et al. Coffee intake and breast cancer risk in the NIH-AARP diet and health study cohort. *Int J Cancer.* 2012;131(2):452-60
193. Gardener H, Rundek T, et al. Coffee and tea consumption are inversely associated with mortality in a multiethnic urban population. *J Nutr.* 2013;143(8):1299-308
194. Sugiyama K, Kuriyama S, et al. Coffee consumption and mortality due to all causes, cardiovascular disease, and cancer in Japanese women. *J Nutr.* 2010;140(5):1007-13

195. Zhang W, Lopez-Garcia E, et al. Coffee consumption and risk of cardiovascular diseases and all-cause mortality among men with type 2 diabetes. *Diabetes Care*. 2009;32(6):1043-5
196. Happonen P, Laara E, et al. Coffee consumption and mortality in a 14-year follow-up of an elderly northern Finnish population. *Br J Nutr*. 2008;99(6):1354-61
197. Lopez-Garcia E, van Dam RM, et al. The relationship of coffee consumption with mortality. *Ann Intern Med*. 2008;148(12):904-14
198. Lopez-Garcia E, Rodriguez-Artalejo F, et al. Coffee consumption and mortality in women with cardiovascular disease. *Am J Clin Nutr*. 2011;94(1):218-24
199. Je Y and Giovannucci E. Coffee consumption and total mortality: a meta-analysis of twenty prospective cohort studies. *Br J Nutr*. 2014;111(7):1162-73
200. Crippa A, Discacciati A, et al. Coffee consumption and mortality from all causes, cardiovascular disease, and cancer: a dose-response meta-analysis. *Am J Epidemiol*. 2014;180(8):763-75
201. Poole R, Kennedy OJ, et al. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. *BMJ*. 2017;359:j5024
202. Liu JJ, Crous-Bou M, et al. Coffee Consumption Is Positively Associated with Longer Leukocyte Telomere Length in the Nurses' Health Study. *J Nutr*. 2016;146(7):1373-8
203. Arab L. Epidemiologic evidence on coffee and cancer. *Nutr Cancer*. 2010;62(3):271-83
204. Bohn SK, Blomhoff R, et al. Coffee and cancer risk, epidemiological evidence, and molecular mechanisms. *Mol Nutr Food Res*. 2014;58(5):915-30
205. Floegel A, Pischon T, et al. Coffee consumption and risk of chronic disease in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study. *Am J Clin Nutr*. 2012;95(4):901-8
206. Yu X, Bao Z, et al. Coffee consumption and risk of cancers: a meta-analysis of cohort studies. *BMC Cancer*. 2011;11:96
207. Baker JA, Boakye K, et al. Consumption of black tea or coffee and risk of ovarian cancer. *Int J Gynecol Cancer*. 2007;17(1):50-4
208. Goodman MT, Tung KH, et al. Association of caffeine intake and CYP1A2 genotype with ovarian cancer. *Nutr Cancer*. 2003;46(1):23-9
209. Larsson SC and Wolk A. Coffee consumption is not associated with ovarian cancer incidence. *Cancer Epidemiol Biomarkers Prev*. 2005;14(9):2273-4
210. Riman T, Dickman PW, et al. Some life-style factors and the risk of invasive epithelial ovarian cancer in Swedish women. *Eur J Epidemiol*. 2004;19(11):1011-9
211. Song YJ, Kristal AR, et al. Coffee, tea, colas, and risk of epithelial ovarian cancer. *Cancer Epidemiol Biomarkers Prev*. 2008;17(3):712-6
212. Gosvig CF, Kjaer SK, et al. Coffee, tea, and caffeine consumption and risk of epithelial ovarian cancer and borderline ovarian tumors: Results from a Danish case-control study. *Acta Oncol*. 2015;54(8):1144-51

213. Lueth NA, Anderson KE, et al. Coffee and caffeine intake and the risk of ovarian cancer: the Iowa Women's Health Study. *Cancer Causes Control.* 2008;19(10):1365-72
214. Silvera SA, Jain M, et al. Intake of coffee and tea and risk of ovarian cancer: a prospective cohort study. *Nutr Cancer.* 2007;58(1):22-7
215. Steevens J, Schouten LJ, et al. Tea and coffee drinking and ovarian cancer risk: results from the Netherlands Cohort Study and a meta-analysis. *Br J Cancer.* 2007;97(9):1291-4
216. Tworoger SS, Gertig DM, et al. Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer. *Cancer.* 2008;112(5):1169-77
217. Villanueva CM, Silverman DT, et al. Coffee consumption, genetic susceptibility and bladder cancer risk. *Cancer Causes Control.* 2009;20(1):121-7
218. Kurahashi N, Inoue M, et al. Coffee, green tea, and caffeine consumption and subsequent risk of bladder cancer in relation to smoking status: a prospective study in Japan. *Cancer Sci.* 2009;100(2):294-91
219. Boggs DA, Palmer JR, et al. Tea and coffee intake in relation to risk of breast cancer in the Black Women's Health Study. *Cancer Causes Control.* 2010;21(11):1941-8
220. Fagherazzi G, Touillaud MS, et al. No association between coffee, tea or caffeine consumption and breast cancer risk in a prospective cohort study. *Public Health Nutr.* 2011;14(7):1315-20
221. Ganmaa D, Willett WC, et al. Coffee, tea, caffeine and risk of breast cancer: a 22-year follow-up. *Int J Cancer.* 2008;122(9):2071-6
222. Harris HR, Bergkvist L, et al. Coffee and black tea consumption and breast cancer mortality in a cohort of Swedish women. *Br J Cancer.* 2012;107(5):874-8
223. Ishitani K, Lin J, et al. Caffeine consumption and the risk of breast cancer in a large prospective cohort of women. *Arch Intern Med.* 2008;168(18):2022-31
224. Kotsopoulos J, Ghadirian P, et al. The CYP1A2 genotype modifies the association between coffee consumption and breast cancer risk among BRCA1 mutation carriers. *Cancer Epidemiol Biomarkers Prev.* 2007;16(5):912-6
225. Lehrer S, Green S, et al. Coffee Consumption Associated with Increased Mortality of Women with Breast Cancer. *J Caffeine Res.* 2013;3(1):38-40
226. Lowcock EC, Cotterchio M, et al. High coffee intake, but not caffeine, is associated with reduced estrogen receptor negative and postmenopausal breast cancer risk with no effect modification by CYP1A2 genotype. *Nutr Cancer.* 2013;65(3):398-409
227. Jiang W, Wu Y, et al. Coffee and caffeine intake and breast cancer risk: an updated dose-response meta-analysis of 37 published studies. *Gynecol Oncol.* 2013;129(3):620-9
228. Salomone F, Galvano F, et al. Molecular Bases Underlying the Hepatoprotective Effects of Coffee. *Nutrients.* 2017;9(1)
229. Bamia C, Lagiou P, et al. Coffee, tea and decaffeinated coffee in relation to hepatocellular carcinoma in a European population: multicentre, prospective cohort study. *Int J Cancer.* 2015;136(8):1899-908

230. Godos J, Micek A, et al. Coffee Consumption and Risk of Biliary Tract Cancers and Liver Cancer: A Dose-Response Meta-Analysis of Prospective Cohort Studies. *Nutrients*. 2017;9(9)
231. Bai K, Cai Q, et al. Coffee consumption and risk of hepatocellular carcinoma: a meta-analysis of eleven epidemiological studies. *Onco Targets Ther*. 2016;9:4369-75
232. Kerzendorfer C and O'Driscoll M. UVB and caffeine: inhibiting the DNA damage response to protect against the adverse effects of UVB. *J Invest Dermatol*. 2009;129(7):1611-3
233. Caini S, Cattaruzza S, et al. Coffee, tea and caffeine intake and the risk of non-melanoma skin cancer: a review of the literature and meta-analysis. *Eur J Nutr*. 2017;56(1):1-12
234. Wang J, Li X, et al. Coffee consumption and the risk of cutaneous melanoma: a meta-analysis. *Eur J Nutr*. 2016;55(4):1317-29
235. Yew YW, Lai YC, et al. Coffee Consumption and Melanoma: A Systematic Review and Meta-Analysis of Observational Studies. *Am J Clin Dermatol*. 2016;17(2):113-23
236. Ferrucci LM, Cartmel B, et al. Tea, coffee, and caffeine and early-onset basal cell carcinoma in a case-control study. *Eur J Cancer Prev*. 2014;23(4):296-302
237. Song F, Qureshi AA, et al. Increased caffeine intake is associated with reduced risk of basal cell carcinoma of the skin. *Cancer Res*. 2012;72(13):3282-9
238. Caini S, Masala G, et al. Coffee, tea and melanoma risk: findings from the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*. 2017;140(10):2246-2255
239. World Cancer Research Fund and American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Endometrial Cancer. 2013.
240. Uccella S, Mariani A, et al. Intake of coffee, caffeine and other methylxanthines and risk of Type I vs Type II endometrial cancer. *Br J Cancer*. 2013;109(7):1908-13
241. Giri A, Sturgeon SR, et al. Caffeinated coffee, decaffeinated coffee and endometrial cancer risk: a prospective cohort study among US postmenopausal women. *Nutrients*. 2011;3(11):937-50
242. Gunter MJ, Schaub JA, et al. A prospective investigation of coffee drinking and endometrial cancer incidence. *Int J Cancer*. 2012;131(4):E530-6
243. Hashibe M, Galeone C, et al. Coffee, tea, caffeine intake, and the risk of cancer in the PLCO cohort. *Br J Cancer*. 2015;113(5):809-16
244. Zhou Q, Luo ML, et al. Coffee consumption and risk of endometrial cancer: a dose-response meta-analysis of prospective cohort studies. *Sci Rep*. 2015;5:13410
245. Dik VK, Bueno-de-Mesquita HB, et al. Coffee and tea consumption, genotype-based CYP1A2 and NAT2 activity and colorectal cancer risk-results from the EPIC cohort study. *Int J Cancer*. 2014;135(2):401-12
246. Michels KB, Willett WC, et al. Coffee, tea, and caffeine consumption and incidence of colon and rectal cancer. *J Natl Cancer Inst*. 2005;97(4):282-92

247. Dominianni C, Huang WY, et al. Prospective study of the relationship between coffee and tea with colorectal cancer risk: the PLCO Cancer Screening Trial. *Br J Cancer*. 2013;109(5):1352-9
248. Bonaventure A, Rudant J, et al. Childhood acute leukemia, maternal beverage intake during pregnancy, and metabolic polymorphisms. *Cancer Causes Control*. 2013;24(4):783-93
249. Milne E, Royle JA, et al. Maternal consumption of coffee and tea during pregnancy and risk of childhood ALL: results from an Australian case-control study. *Cancer Causes Control*. 2011;22(2):207-18
250. Cheng J, Su H, et al. Maternal coffee consumption during pregnancy and risk of childhood acute leukemia: a metaanalysis. *Am J Obstet Gynecol*. 2014;210(2):151 e1-151 e10
251. Thomopoulos TP, Ntouvelis E, et al. Maternal and childhood consumption of coffee, tea and cola beverages in association with childhood leukemia: a meta-analysis. *Cancer Epidemiol*. 2015;39(6):1047-59
252. Hart AR, Kennedy H, et al. Pancreatic cancer: a review of the evidence on causation. *Clin Gastroenterol Hepatol*. 2008;6(3):275-82
253. Ghadirian P, Lynch HT, et al. Epidemiology of pancreatic cancer: an overview. *Cancer Detect Prev*. 2003;27(2):87-93
254. Michaud DS, Gallo V, et al. Coffee and tea intake and risk of brain tumors in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort study. *Am J Clin Nutr*. 2010;92(5):1145-50
255. Wijarnpreecha K, Thongprayoon C, et al. Association between coffee consumption and risk of renal cell carcinoma: A Meta-analysis. *Intern Med J*. 2017
256. Sajja KC, El-Serag HB, et al. Coffee or Tea, Hot or Cold, Are Not Associated With Risk of Barrett's Esophagus. *Clin Gastroenterol Hepatol*. 2016;14(5):769-72
257. Bonita JS, Mandarano M, et al. Coffee and cardiovascular disease: in vitro, cellular, animal, and human studies. *Pharmacol Res*. 2007;55(3):187-98
258. O'Keefe JH, Bhatti SK, et al. Effects of habitual coffee consumption on cardiometabolic disease, cardiovascular health, and all-cause mortality. *J Am Coll Cardiol*. 2013;62(12):1043-51
259. You DC, Kim YS, et al. Possible health effects of caffeinated coffee consumption on Alzheimer's disease and cardiovascular disease. *Toxicol Res*. 2011;27(1):7-10
260. Wilson PW and Bloom HL. Caffeine Consumption and Cardiovascular Risks: Little Cause for Concern. *J Am Heart Assoc*. 2016;5(1)
261. Ding M, Bhupathiraju SN, et al. Long-term coffee consumption and risk of cardiovascular disease: a systematic review and a dose-response meta-analysis of prospective cohort studies. *Circulation*. 2014;129(6):643-59
262. Cornelis MC, El-Sohemy A, et al. Coffee, CYP1A2 genotype, and risk of myocardial infarction. *JAMA*. 2006;295(10):1135-41
263. Dixit S, Stein PK, et al. Consumption of Caffeinated Products and Cardiac Ectopy. *J Am Heart Assoc*. 2016;5(1)

264. Frost L and Vestergaard P. Caffeine and risk of atrial fibrillation or flutter: the Danish Diet, Cancer, and Health Study. *Am J Clin Nutr*. 2005;81(3):578-82
265. Shen J, Johnson VM, et al. Dietary factors and incident atrial fibrillation: the Framingham Heart Study. *Am J Clin Nutr*. 2011;93(2):261-6
266. Cheng M, Hu Z, et al. Caffeine intake and atrial fibrillation incidence: dose response meta-analysis of prospective cohort studies. *Can J Cardiol*. 2014;30(4):448-54
267. Larsson SC, Drca N, et al. Coffee consumption is not associated with increased risk of atrial fibrillation: results from two prospective cohorts and a meta-analysis. *BMC Med*. 2015;13:207
268. Pelchovitz DJ and Goldberger JJ. Caffeine and cardiac arrhythmias: a review of the evidence. *Am J Med*. 2011;124(4):284-9
269. Zuchinali P, Ribeiro PA, et al. Effect of caffeine on ventricular arrhythmia: a systematic review and meta-analysis of experimental and clinical studies. *Europace*. 2016;18(2):257-66
270. Geleijnse JM. Habitual coffee consumption and blood pressure: an epidemiological perspective. *Vasc Health Risk Manag*. 2008;4(5):963-70
271. Mesas AE, Leon-Munoz LM, et al. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. *Am J Clin Nutr*. 2011;94(4):1113-26
272. Riksen NP, Rongen GA, et al. Acute and long-term cardiovascular effects of coffee: implications for coronary heart disease. *Pharmacol Ther*. 2009;121(2):185-91
273. Palatini P, Ceolotto G, et al. CYP1A2 genotype modifies the association between coffee intake and the risk of hypertension. *J Hypertens*. 2009;27(8):1594-601
274. Mineharu Y, Koizumi A, et al. Coffee, green tea, black tea and oolong tea consumption and risk of mortality from cardiovascular disease in Japanese men and women. *J Epidemiol Community Health*. 2011;65(3):230-40
275. Winkelmayer WC, Stampfer MJ, et al. Habitual caffeine intake and the risk of hypertension in women. *JAMA*. 2005;294(18):2330-5
276. Kim B, Nam Y, et al. Coffee Consumption and Stroke Risk: A Meta-analysis of Epidemiologic Studies. *Korean J Fam Med*. 2012;33(6):356-65
277. Lopez-Garcia E, van Dam RM, et al. Coffee consumption and coronary heart disease in men and women: a prospective cohort study. *Circulation*. 2006;113(17):2045-53
278. Heckman MA, Weil J, et al. Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. *J Food Sci*. 2010;75(3):R77-87
279. Akash MS, Rehman K, et al. Effects of coffee on type 2 diabetes mellitus. *Nutrition*. 2014;30(7-8):755-63
280. Krebs JD, Parry-Strong A, et al. A cross-over study of the acute effects of espresso coffee on glucose tolerance and insulin sensitivity in people with type 2 diabetes mellitus. *Metabolism*. 2012;61(9):1231-7
281. Greenberg JA, Owen DR, et al. Decaffeinated coffee and glucose metabolism in young men. *Diabetes Care*. 2010;33(2):278-80

282. Sartorelli DS, Fagherazzi G, et al. Differential effects of coffee on the risk of type 2 diabetes according to meal consumption in a French cohort of women: the E3N/EPIC cohort study. *Am J Clin Nutr.* 2010;91(4):1002-12
283. Bhupathiraju SN, Pan A, et al. Changes in coffee intake and subsequent risk of type 2 diabetes: three large cohorts of US men and women. *Diabetologia.* 2014;57(7):1346-54
284. Jiang X, Zhang D, et al. Coffee and caffeine intake and incidence of type 2 diabetes mellitus: a meta-analysis of prospective studies. *Eur J Nutr.* 2014;53(1):25-38
285. Shi X, Xue W, et al. Acute caffeine ingestion reduces insulin sensitivity in healthy subjects: a systematic review and meta-analysis. *Nutr J.* 2016;15(1):103
286. Whitehead N and White H. Systematic review of randomised controlled trials of the effects of caffeine or caffeinated drinks on blood glucose concentrations and insulin sensitivity in people with diabetes mellitus. *J Hum Nutr Diet.* 2013;26(2):111-25
287. Robertson TM, Clifford MN, et al. A single serving of caffeinated coffee impairs postprandial glucose metabolism in overweight men. *Br J Nutr.* 2015;114(8):1218-25
288. Jahanfar S and Jaafar SH. Effects of restricted caffeine intake by mother on fetal, neonatal and pregnancy outcome. *Cochrane Database Syst Rev.* 2013;2:CD006965
289. Chen LW, Wu Y, et al. Maternal caffeine intake during pregnancy is associated with risk of low birth weight: a systematic review and dose inverted question markresponse meta-analysis. *BMC Med.* 2014;12(1):174
290. Greenwood DC, Thatcher NJ, et al. Caffeine intake during pregnancy and adverse birth outcomes: a systematic review and dose-response meta-analysis. *Eur J Epidemiol.* 2014;29(10):725-34
291. Peck JD, Leviton A, et al. A review of the epidemiologic evidence concerning the reproductive health effects of caffeine consumption: a 2000-2009 update. *Food Chem Toxicol.* 2010;48(10):2549-76
292. ACOG Committee. ACOG Committee Opinion No. 462: Moderate caffeine consumption during pregnancy. *Obstet Gynecol.* 2010;116(2 Pt 1):467-8
293. Santos IS, Matijasevich A, et al. Maternal caffeine consumption and infant nighttime waking: prospective cohort study. *Pediatrics.* 2012;129(5):860-8
294. Choi E, Choi KH, et al. The Benefit of Bone Health by Drinking Coffee among Korean Postmenopausal Women: A Cross-Sectional Analysis of the Fourth & Fifth Korea National Health and Nutrition Examination Surveys. *PLoS One.* 2016;11(1):e0147762
295. Hallstrom H, Byberg L, et al. Long-term coffee consumption in relation to fracture risk and bone mineral density in women. *Am J Epidemiol.* 2013;178(6):898-909
296. Harter DL, Busnello FM, et al. Association between low bone mass and calcium and caffeine intake among perimenopausal women in Southern Brazil: cross-sectional study. *Sao Paulo Med J.* 2013;131(5):315-22

297. McLellan TM, Caldwell JA, et al. A review of caffeine's effects on cognitive, physical and occupational performance. *Neurosci Biobehav Rev.* 2016;71:294-312
298. Nehlig A. Effects of coffee/caffeine on brain health and disease: What should I tell my patients? *Pract Neurol.* 2016;16(2):89-95
299. Turnbull D, Rodricks JV, et al. Neurobehavioral hazard identification and characterization for caffeine. *Regul Toxicol Pharmacol.* 2016;74:81-92
300. Wang L, Shen X, et al. Coffee and caffeine consumption and depression: A meta-analysis of observational studies. *Aust N Z J Psychiatry.* 2016;50(3):228-42
301. Grosso G, Micek A, et al. Coffee, tea, caffeine and risk of depression: A systematic review and dose-response meta-analysis of observational studies. *Mol Nutr Food Res.* 2016;60(1):223-34
302. Lucas M, O'Reilly EJ, et al. Coffee, caffeine, and risk of completed suicide: results from three prospective cohorts of American adults. *World J Biol Psychiatry.* 2014;15(5):377-86
303. Hall S, Desbrow B, et al. A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. *Food Res Int.* 2015;76(Pt 3):626-636
304. Flaten V, Laurent C, et al. From epidemiology to pathophysiology: what about caffeine in Alzheimer's disease? *Biochem Soc Trans.* 2014;42(2):587-92
305. Wierzejska R. Can coffee consumption lower the risk of Alzheimer's disease and Parkinson's disease? A literature review. *Arch Med Sci.* 2017;13(3):507-514
306. Cao C, Loewenstein DA, et al. High Blood Caffeine Levels in MCI Linked to Lack of Progression to Dementia. *J Alzheimers Dis.* 2012;30(3):559-72
307. Onatibia-Astibia A, Franco R, et al. Health benefits of methylxanthines in neurodegenerative diseases. *Mol Nutr Food Res.* 2017;61(6)
308. Kolahdouzan M and Hamadeh MJ. The neuroprotective effects of caffeine in neurodegenerative diseases. *CNS Neurosci Ther.* 2017;23(4):272-290
309. Beydoun MA, Beydoun HA, et al. Epidemiologic studies of modifiable factors associated with cognition and dementia: systematic review and meta-analysis. *BMC Public Health.* 2014;14:643
310. Kim YS, Kwak SM, et al. Caffeine intake from coffee or tea and cognitive disorders: a meta-analysis of observational studies. *Neuroepidemiology.* 2015;44(1):51-63
311. Arab L, Khan F, et al. Epidemiologic evidence of a relationship between tea, coffee, or caffeine consumption and cognitive decline. *Adv Nutr.* 2013;4(1):115-22
312. Roshan MH, Tambo A, et al. Potential Role of Caffeine in the Treatment of Parkinson's Disease. *Open Neurol J.* 2016;10:42-58
313. Qi H and Li S. Dose-response meta-analysis on coffee, tea and caffeine consumption with risk of Parkinson's disease. *Geriatr Gerontol Int.* 2014;14(2):430-9
314. Gaba A. Recent studies on nutrition and Parkinson's disease prevention: a systematic review. *Open Journal of Preventive Medicine.* 2015;5(05):197

315. Maughan RJ and Griffin J. Caffeine ingestion and fluid balance: a review. *J Hum Nutr Diet*. 2003;16(6):411-20
316. Zhang Y, Coca A, et al. Caffeine and diuresis during rest and exercise: A meta-analysis. *J Sci Med Sport*. 2015;18(5):569-74
317. Goldstein ER, Ziegenfuss T, et al. International society of sports nutrition position stand: caffeine and performance. *J Int Soc Sports Nutr*. 2010;7(1):5
318. Killer SC, Blannin AK, et al. No evidence of dehydration with moderate daily coffee intake: a counterbalanced cross-over study in a free-living population. *PLoS One*. 2014;9(1):e84154
319. Silva AM, Judice PB, et al. Total body water and its compartments are not affected by ingesting a moderate dose of caffeine in healthy young adult males. *Appl Physiol Nutr Metab*. 2013;38(6):626-32
320. Ruxton CH and Hart VA. Black tea is not significantly different from water in the maintenance of normal hydration in human subjects: results from a randomised controlled trial. *Br J Nutr*. 2011;106(4):588-95
321. Grandjean AC, Reimers KJ, et al. The effect of caffeinated, non-caffeinated, caloric and non-caloric beverages on hydration. *J Am Coll Nutr*. 2000;19(5):591-600
322. Armstrong LE, Pumerantz AC, et al. Fluid, electrolyte, and renal indices of hydration during 11 days of controlled caffeine consumption. *Int J Sport Nutr Exerc Metab*. 2005;15(3):252-65
323. FDA. Letter from Acting Associate Commissioner for Legislation, Michele Mital, to Senator Richard J. Durbin. Department of Health and Human Services; 2012.
324. FDA. Consumer health information: FDA to investigate added caffeine. 2013. 1-2
325. IOM (Institute of Medicine). Caffeine in food and dietary supplements: examining safety. Workshop summary. 2014.
326. NIH and Office of Dietary Supplements. News and Events. "The Use and Biology of Energy Drinks Meeting" Summary: Current Knowledge and Critical Gaps. [2013; Retrieved March 25, 2018] from https://ods.od.nih.gov/News/EnergyDrinksWorkshopSummary2013.aspx.
327. Sorkin BC, Camp KM, et al. Executive summary of NIH workshop on the Use and Biology of Energy Drinks: Current Knowledge and Critical Gaps. *Nutr Rev*. 2014;72 Suppl 1:1-8
328. Taylor M and FDA. FDA Voice. Defining boundaries for caffeine in today's marketplace. [2013; Retrieved March 25, 2018] from https://blogs.fda.gov/fdavoice/index.php/2013/08/defining-boundaries-for-caffeine-in-todays-marketplace/.
329. Scientific Committee on Food. Opinion on caffeine, taurine, and d-glucurono-g-lactone as constituents of so-called "energy" drinks (expressed 21 January 1999). European Commission. 1999. 1-14.
330. Scientific Committee on Food. Opinion of the Scientific Committee on Food on additional information on "energy" drinks. European Commission,. 2003. 1-26.

331. EFSA. Scientific opinion: The use of taurine and d-glucurono-g-lactone as constituents of the so-called "energy" drinks. *The EFSA Journal*. 2009;935:1-31
332. Sved DW, Godsey JL, et al. Absorption, tissue distribution, metabolism and elimination of taurine given orally to rats. *Amino Acids*. 2007;32(4):459-66
333. Riesenhuber A, Boehm M, et al. Diuretic potential of energy drinks. *Amino Acids*. 2006;31(1):81-3
334. Health Canada and Food Directorate - Health Products and Food Branch. Category specific guidance for temporary marketing authorization. Caffeinated energy drinks. 2012. 1-28.
335. Rotstein J, Barber J, et al. Energy drinks: an assessment of the potential health risks in the Canadian context. *Int Food Risk Anal*. 2013;3(5):1-29
336. FDA and DHHS. Warning Letter. Hard Eight Nutrition LLC; 2015.
337. FDA and DHHS. Warning Letter. Bridge City LLC; 2015.
338. FDA and DHHS. Warning Letter. Kreativ Health Inc Dba Natural Food Supplements; 2015.
339. FDA and DHHS. Warning Letter. Smartpowders; 2015.
340. FDA and DHHS. Warning Letter: PureBulk, Inc.; 2015.
341. DHHS and FDA. Highly concentrated caffeine in dietary supplements; guidance for industry; availability. Federal Register Vol. 83, No. 73 (April 16, 2018) / Notices.
342. FDA. Pure and highly concentrated caffeine. [2018; Retrieved June 13, 2018] from https://www.fda.gov/Food/DietarySupplements/ProductsIngredients/ucm460095.htm.
343. Ferruzzi MG. The influence of beverage composition on delivery of phenolic compounds from coffee and tea. *Physiol Behav*. 2010;100(1):33-41
344. Lang R, Dieminger N, et al. Bioappearance and pharmacokinetics of bioactives upon coffee consumption. *Anal Bioanal Chem*. 2013;405(26):8487-503
345. Borges G, Lean ME, et al. Bioavailability of dietary (poly)phenols: a study with ileostomists to discriminate between absorption in small and large intestine. *Food Funct*. 2013;4(5):754-62
346. Crozier A, Del Rio D, et al. Bioavailability of dietary flavonoids and phenolic compounds. *Mol Aspects Med*. 2010;31(6):446-67
347. Nardini M, Cirillo E, et al. Absorption of phenolic acids in humans after coffee consumption. *J Agric Food Chem*. 2002;50(20):5735-41
348. Williamson G, Dionisi F, et al. Flavanols from green tea and phenolic acids from coffee: critical quantitative evaluation of the pharmacokinetic data in humans after consumption of single doses of beverages. *Mol Nutr Food Res*. 2011;55(6):864-73
349. Konishi Y and Kobayashi S. Transepithelial transport of chlorogenic acid, caffeic acid, and their colonic metabolites in intestinal caco-2 cell monolayers. *J Agric Food Chem*. 2004;52(9):2518-26
350. Stalmach A, Mullen W, et al. Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans:

identification of biomarkers of coffee consumption. *Drug Metab Dispos.* 2009;37(8):1749-58

351. Olthof MR, Hollman PC, et al. Chlorogenic acid and caffeic acid are absorbed in humans. *J Nutr.* 2001;131(1):66-71
352. Farah A, Monteiro M, et al. Chlorogenic acids from green coffee extract are highly bioavailable in humans. *J Nutr.* 2008;138(12):2309-15
353. Erk T, Hauser J, et al. Structure- and dose-absorption relationships of coffee polyphenols. *Biofactors.* 2014;40(1):103-12
354. Erk T, Williamson G, et al. Dose-dependent absorption of chlorogenic acids in the small intestine assessed by coffee consumption in ileostomists. *Mol Nutr Food Res.* 2012;56(10):1488-500
355. Farrell TL, Dew TP, et al. Absorption and metabolism of chlorogenic acids in cultured gastric epithelial monolayers. *Drug Metab Dispos.* 2011;39(12):2338-46
356. Matsui Y, Nakamura S, et al. Liquid chromatography-electrospray ionization-tandem mass spectrometry for simultaneous analysis of chlorogenic acids and their metabolites in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007;858(1-2):96-105
357. Stalmach A, Williamson G, et al. Impact of dose on the bioavailability of coffee chlorogenic acids in humans. *Food Funct.* 2014;5(8):1727-37
358. Stalmach A, Steiling H, et al. Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy. *Arch Biochem Biophys.* 2010;501(1):98-105
359. Azuma K, Ippoushi K, et al. Absorption of chlorogenic acid and caffeic acid in rats after oral administration. *J Agric Food Chem.* 2000;48(11):5496-500
360. Gonthier MP, Verny MA, et al. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J Nutr.* 2003;133(6):1853-9
361. Camarasa J, Escubedo E, et al. Pharmacokinetics of caffeic acid in rats by a high-performance liquid chromatography method. *J Pharm Biomed Anal.* 1988;6(5):503-10
362. Heimbach JT, Marone PA, et al. Safety studies on products from whole coffee fruit. *Food Chem Toxicol.* 2010;48(8-9):2517-2525
363. Mullen W, Nemzer B, et al. The antioxidant and chlorogenic acid profiles of whole coffee fruits are influenced by the extraction procedures. *J Agric Food Chem.* 2011;59(8):3754-62
364. Tice R, Integrated Laboratory Systems, et al. Chlorogenic Acid [327-97-9] and Caffeic Acid [331-39-5]. Review of Toxicological Literature. 1998. 112.
365. Eklund A. Effect of chlorogenic acid in a casein diet for rats. Nutritional and pathological observations. *Nutr Metab.* 1975;18(5-6):258-64
366. Proctor DM, Gatto NM, et al. Mode-of-action framework for evaluating the relevance of rodent forestomach tumors in cancer risk assessment. *Toxicol Sci.* 2007;98(2):313-26
367. Chaube S and Swinyard CA. Teratological and toxicological studies of alkaloidal and phenolic compounds from Solanum tuberosum L. *Toxicol Appl Pharmacol.* 1976;36(2):227-37

368. Fung VA, Cameron TP, et al. Mutagenic activity of some coffee flavor ingredients. *Mutat Res.* 1988;204(2):219-28
369. Seifried HE, Seifried RM, et al. A compilation of two decades of mutagenicity test results with the Ames Salmonella typhimurium and L5178Y mouse lymphoma cell mutation assays. *Chem Res Toxicol.* 2006;19(5):627-44
370. Stich HF, Rosin MP, et al. A comparative genotoxicity study of chlorogenic acid (3-0-caffeoylquinic acid). *Mutat Res.* 1981;90(3):201-12
371. Suzuki A, Kagawa D, et al. Green coffee bean extract and its metabolites have a hypotensive effect in spontaneously hypertensive rats. *Hypertens Res.* 2002;25(1):99-107
372. Liu B, Cao L, et al. Preparation, Phytochemical Investigation, and Safety Evaluation of Chlorogenic Acid Products from Eupatorium adenophorum. *Molecules.* 2016;22(1)
373. Nolen GA. A reproduction/teratology study of brewed and instant decaffeinated coffees. *J Toxicol Environ Health.* 1982;10(4-5):769-83
374. Collins T, Welsh J, et al. A study of the teratogenic potential of caffeine given by oral intubation to rats. *Regul Toxicol Pharmacol.* 1981;1(3):335-378
375. Collins TF, Welsh JJ, et al. A study of the teratogenic potential of caffeine ingested in drinking-water. *Food Chem Toxicol.* 1983;21(6):763-77
376. Collins TF, Welsh JJ, et al. Potential reversibility of skeletal effects in rats exposed in utero to caffeine. *Food Chem Toxicol.* 1987;25(9):647-62
377. Eskenazi B, Stapleton AL, et al. Associations between maternal decaffeinated and caffeinated coffee consumption and fetal growth and gestational duration. *Epidemiology.* 1999;10(3):242-9
378. Johnston KL, Clifford MN, et al. Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine. *Am J Clin Nutr.* 2003;78(4):728-33
379. van Dijk AE, Olthof MR, et al. Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. *Diabetes Care.* 2009;32(6):1023-5
380. Mubarak A, Bondonno CP, et al. Acute effects of chlorogenic acid on nitric oxide status, endothelial function, and blood pressure in healthy volunteers: a randomized trial. *J Agric Food Chem.* 2012;60(36):9130-6
381. Iwai K, Narita Y, et al. Study on the postprandial glucose responses to a chlorogenic acid-rich extract of decaffeinated green coffee beans in rats and healthy human subjects. *Food Sci Technol Res.* 2012;18(6):849-860
382. Ward NC, Hodgson JM, et al. Acute effects of chlorogenic acids on endothelial function and blood pressure in healthy men and women. *Food Funct.* 2016;7(5):2197-203
383. Camfield DA, Silber BY, et al. A randomised placebo-controlled trial to differentiate the acute cognitive and mood effects of chlorogenic acid from decaffeinated coffee. *PLoS One.* 2013;8(12):e82897
384. Cropley V, Croft R, et al. Does coffee enriched with chlorogenic acids improve mood and cognition after acute administration in healthy elderly? A pilot study. *Psychopharmacology (Berl).* 2012;219(3):737-49

385. Olthof MR, van Dijk AE, et al. Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on incretin hormones. *Nutr Metab (Lond)*. 2011;8:10
386. Park I, Ochiai R, et al. Effects of subacute ingestion of chlorogenic acids on sleep architecture and energy metabolism through activity of the autonomic nervous system: a randomised, placebo-controlled, double-blinded cross-over trial. *Br J Nutr*. 2017;117(7):979-984
387. Henry-Vitrac C, Ibarra A, et al. Contribution of chlorogenic acids to the inhibition of human hepatic glucose-6-phosphatase activity in vitro by Svetol, a standardized decaffeinated green coffee extract. *J Agric Food Chem*. 2010;58(7):4141-4
388. Thom E. The effect of chlorogenic acid (Svetol™) and chlorogenic enriched coffee (CoffeeSLENDER®) on the glucose profile and bodyweight in healthy volunteers. Parexel. 1-5.
389. Dellalibera S, Lemaire B, et al. Svetol, green coffee extract, induces weight loss and increases the lean to fat mass ratio in volunteers with overweight problem. *Phytotherapie*. 2006;4(4):194-197
390. Bakuradze T, Boehm N, et al. Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: Results from an intervention study. *Mol Nutr Food Res*. 2011;55(5):793-797
391. Soga S, Ota N, et al. Stimulation of postprandial fat utilization in healthy humans by daily consumption of chlorogenic acids. *Biosci Biotechnol Biochem*. 2013;77(8):1633-6
392. Ochiai R, Jokura H, et al. Green coffee bean extract improves human vasoreactivity. *Hypertension Research*. 2004;27(10):731-737
393. Watanabe T, Arai Y, et al. The blood pressure-lowering effect and safety of chlorogenic acid from green coffee bean extract in essential hypertension. *Clin Exp Hypertens*. 2006;28(5):439-49
394. Yamaguchi T, Chikama A, et al. Hydroxyhydroquinone-free coffee: a double-blind, randomized controlled dose-response study of blood pressure. *Nutr Metab Cardiovasc Dis*. 2008;18(6):408-14
395. Chikama A, Yamaguchi T, et al. Effects of hydroxyhydroquinone-reduced coffee on blood pressure in high-normotensives and mild hypertensives. *J Health Sci*. 2008;54(2):162-173
396. Ochiai R, Chikama A, et al. Effects of hydroxyhydroquinone-reduced coffee on vasoreactivity and blood pressure. *Hypertens Res*. 2009;32(11):969-74
397. Watanabe K, Yamaguchi T, et al. Consumer health benefits of habitual consumption of chlorogenic acid-enriched coffee: a large single-arm study. *Nutrafoods*. 2014;13(3):103-111
398. Kozuma K, Tsuchiya S, et al. Antihypertensive effect of green coffee bean extract on mildly hypertensive subjects. *Hypertens Res*. 2005;28(9):711-8
399. Onakpoya I, Terry R, et al. The use of green coffee extract as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. *Gastroenterol Res Pract*. 2011;2011
400. Thompson GR, Mason MM, et al. Chronic oral toxicity of cannabinoids in rats. *Toxicol Appl Pharmacol*. 1973;25(3):373-90

401. Heitman E and Ingram DK. Cognitive and neuroprotective effects of chlorogenic acid. *Nutr Neurosci.* 2017;20(1):32-39
402. Jin S, Chang C, et al. Chlorogenic acid improves late diabetes through adiponectin receptor signaling pathways in db/db mice. *PLoS One.* 2015;10(4):e0120842
403. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of health claims related to coffee, including chlorogenic acids from coffee, and protection of DNA, proteins and lipids from oxidative damage (ID 1099, 3152, 4301), maintenance of normal blood glucose concentrations (ID 1100, 1962), and contribution to the maintenance or achievement of a normal body weight (ID 2031, 4326) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal.* 2011;9(4)
404. Loader TB, Taylor CG, et al. Chlorogenic acid from coffee beans: evaluating the evidence for a blood pressure-regulating health claim. *Nutr Rev.* 2017;75(2):114-133
405. Meng S, Cao J, et al. Roles of chlorogenic Acid on regulating glucose and lipids metabolism: a review. *Evid Based Complement Alternat Med.* 2013;2013:801457
406. Bassoli BK, Cassolla P, et al. Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. *Cell Biochem Funct.* 2008;26(3):320-8
407. Ong KW, Hsu A, et al. Chlorogenic acid stimulates glucose transport in skeletal muscle via AMPK activation: a contributor to the beneficial effects of coffee on diabetes. *PLoS One.* 2012;7(3):e32718
408. Hemmerle H, Burger HJ, et al. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J Med Chem.* 1997;40(2):137-45
409. Arion WJ, Canfield WK, et al. Chlorogenic acid and hydroxynitrobenzaldehyde: new inhibitors of hepatic glucose 6-phosphatase. *Arch Biochem Biophys.* 1997;339(2):315-22
410. Williamson G. Possible effects of dietary polyphenols on sugar absorption and digestion. *Mol Nutr Food Res.* 2013;57(1):48-57
411. Oboh G, Agunloye OM, et al. Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study. *J Basic Clin Physiol Pharmacol.* 2015;26(2):165-70
412. Flanagan J, Bily A, et al. Lipolytic activity of Svetol®, a decaffeinated green coffee bean extract. *Phytother Res.* 2014;28(6):946-8
413. 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
414. Fuentes E and Palomo I. Mechanisms of endothelial cell protection by hydroxycinnamic acids. *Vascul Pharmacol.* 2014;63(3):155-61
415. Onakpoya IJ, Spencer EA, et al. The effect of chlorogenic acid on blood pressure: a systematic review and meta-analysis of randomized clinical trials. *J Hum Hypertens.* 2015;29(2):77-81
416. Zhao Y, Wang J, et al. Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertens Res.* 2012;35(4):370-4

417. Buscemi S, Verga S, et al. Dose-dependent effects of decaffeinated coffee on endothelial function in healthy subjects. *Eur J Clin Nutr.* 2009;63(10):1200-5
418. Jokura H, Watanabe I, et al. Coffee polyphenol consumption improves postprandial hyperglycemia associated with impaired vascular endothelial function in healthy male adults. *Nutr Res.* 2015;35(10):873-881
419. Ochiai R, Sugiura Y, et al. Coffee bean polyphenols ameliorate postprandial endothelial dysfunction in healthy male adults. *Int J Food Sci Nutr.* 2015;66(3):350-4
420. Yamagata K. Do Coffee Polyphenols Have a Preventive Action on Metabolic Syndrome Associated Endothelial Dysfunctions? An Assessment of the Current Evidence. *Antioxidants (Basel).* 2018;7(2)
421. Godos J, Pluchinotta FR, et al. Coffee components and cardiovascular risk: beneficial and detrimental effects. *Int J Food Sci Nutr.* 2014;65(8):925-36
422. Agudelo-Ochoa GM, Pulgarin-Zapata IC, et al. Coffee Consumption Increases the Antioxidant Capacity of Plasma and Has No Effect on the Lipid Profile or Vascular Function in Healthy Adults in a Randomized Controlled Trial. *J Nutr.* 2016;146(3):524-31
423. Boon EAJ, Croft KD, et al. The acute effect of coffee on endothelial function and glucose metabolism following a glucose load in healthy human volunteers. *Food Funct.* 2017;8(9):3366-3373
424. Buscemi S, Verga S, et al. Acute effects of coffee on endothelial function in healthy subjects. *Eur J Clin Nutr.* 2010;64(5):483-9
425. Buscemi S, Batsis JA, et al. Coffee and endothelial function: a battle between caffeine and antioxidants? *Eur J Clin Nutr.* 2010;64(10):1242-3
426. Noguchi K, Matsuzaki T, et al. Effect of caffeine contained in a cup of coffee on microvascular function in healthy subjects. *J Pharmacol Sci.* 2015;127(2):217-22
427. Papamichael CM, Aznaouridis KA, et al. Effect of coffee on endothelial function in healthy subjects: the role of caffeine. *Clin Sci (Lond).* 2005;109(1):55-60
428. Buscemi S, Mattina A, et al. Acute effects of coffee on QT interval in healthy subjects. *Nutr J.* 2011;10:15
429. Kajikawa M, Maruhashi T, et al. Coffee with a high content of chlorogenic acids and low content of hydroxyhydroquinone improves postprandial endothelial dysfunction in patients with borderline and stage 1 hypertension. *Eur J Nutr.* 2018
430. Van Rymenant E, Van Camp J, et al. Ferulic acid-4-O-sulfate rather than ferulic acid relaxes arteries and lowers blood pressure in mice. *J Nutr Biochem.* 2017;44:44-51
431. Zheng Z, Sheng Y, et al. The therapeutic detoxification of chlorogenic acid against acetaminophen-induced liver injury by ameliorating hepatic inflammation. *Chem Biol Interact.* 2015;238:93-101
432. Ali N, Rashid S, et al. Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: An experimental approach. *Chem Biol Interact.* 2017;272:80-91

433. Xu D, Hu L, et al. Tetrachlorobenzoquinone induces acute liver injury, up-regulates HO-1 and NQO1 expression in mice model: the protective role of chlorogenic acid. *Environ Toxicol Pharmacol*. 2014;37(3):1212-20
434. Shi H, Dong L, et al. Chlorogenic acid against carbon tetrachloride-induced liver fibrosis in rats. *Eur J Pharmacol*. 2009;623(1-3):119-24
435. Xu Y, Chen J, et al. Protective effects of chlorogenic acid on acute hepatotoxicity induced by lipopolysaccharide in mice. *Inflamm Res*. 2010;59(10):871-7
436. Yun N, Kang JW, et al. Protective effects of chlorogenic acid against ischemia/reperfusion injury in rat liver: molecular evidence of its antioxidant and anti-inflammatory properties. *J Nutr Biochem*. 2012;23(10):1249-55
437. Mikami Y and Yamazawa T. Chlorogenic acid, a polyphenol in coffee, protects neurons against glutamate neurotoxicity. *Life Sci*. 2015;139:69-74
438. Jiang R, Hodgson JM, et al. Chlorogenic acid improves ex vivo vessel function and protects endothelial cells against HOCl-induced oxidative damage, via increased production of nitric oxide and induction of Hmox-1. *J Nutr Biochem*. 2016;27:53-60
439. Akila P, Asaikumar L, et al. Chlorogenic acid ameliorates isoproterenol-induced myocardial injury in rats by stabilizing mitochondrial and lysosomal enzymes. *Biomed Pharmacother*. 2017;85:582-591
440. Abraham SK, Sarma L, et al. Protective effects of chlorogenic acid, curcumin and beta-carotene against gamma-radiation-induced in vivo chromosomal damage. *Mutat Res*. 1993;303(3):109-12
441. Wang GF, Shi LP, et al. Anti-hepatitis B virus activity of chlorogenic acid, quinic acid and caffeic acid in vivo and in vitro. *Antiviral Res*. 2009;83(2):186-90
442. Matsunaga K, Katayama M, et al. Inhibitory Effects of Chlorogenic Acid on Azoxymethane-induced Colon Carcinogenesis in Male F344 Rats. *Asian Pac J Cancer Prev*. 2002;3(2):163-166
443. Mori H, Tanaka T, et al. Inhibitory effect of chlorogenic acid on methylazoxymethanol acetate-induced carcinogenesis in large intestine and liver of hamsters. *Cancer Lett*. 1986;30(1):49-54
444. Morishita Y, Yoshimi N, et al. Regressive effects of various chemopreventive agents on azoxymethane-induced aberrant crypt foci in the rat colon. *Jpn J Cancer Res*. 1997;88(9):815-20
445. Shimizu M, Yoshimi N, et al. Suppressive effects of chlorogenic acid on N-methyl-N-nitrosourea-induced glandular stomach carcinogenesis in male F344 rats. *J Toxicol Sci*. 1999;24(5):433-9
446. Brune M, Rossander L, et al. Iron absorption and phenolic compounds: importance of different phenolic structures. *Eur J Clin Nutr*. 1989;43(8):547-57
447. Gutnisky A, Rizzo N, et al. The inhibitory action of chlorogenic acid on the intestinal iron absorption in rats. *Acta Physiol Pharmacol Ther Latinoam*. 1992;42(3):139-46
448. Hurrell RF, Reddy M, et al. Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *Br J Nutr*. 1999;81(4):289-95

449. Miranda L, Deusser H, et al. The impact of in vitro digestion on bioaccessibility of polyphenols from potatoes and sweet potatoes and their influence on iron absorption by human intestinal cells. *Food Funct.* 2013;4(11):1595-601
450. Coudray C, Bousset C, et al. Short-term ingestion of chlorogenic or caffeic acids decreases zinc but not copper absorption in rats, utilization of stable isotopes and inductively-coupled plasma mass spectrometry technique. *Br J Nutr.* 1998;80(6):575-84
451. WHO. Thiamine deficiency and its prevention and control in major emergencies. 1999. 52.
452. Somogyi JC and Bonicke R. Connection between chemical structure and antithiamine activity of various phenol derivatives. *Int Z Vitaminforsch.* 1969;39(1):65-73
453. Davis JS and Somogyi JC. Reaction mechanism of the inactivation of thiamine by 3,4-dihydroxycinnamic acid. (Preliminary publication). *Int Z Vitaminforsch.* 1969;39(4):401-6
454. Panijpan B and Ratanaubolchai K. Kinetics of thiamine-polyphenol interactions and mechanism of thiamine disulphide formation. *Int J Vitam Nutr Res.* 1980;50(3):247-53
455. Somogyi JC and Nageli U. Antithiamine effect of coffee. *Int J Vitam Nutr Res.* 1976;46(2):149-53
456. Hilker DM and Somogyi JC. Antithiamins of plant origin: their chemical nature and mode of action. *Ann N Y Acad Sci.* 1982;378:137-45
457. Kono Y, Kashine S, et al. Iron chelation by chlorogenic acid as a natural antioxidant. *Biosci Biotechnol Biochem.* 1998;62(1):22-7
458. Braunstein E. Iron deficiency anemia (anemia of chronic blood loss; chlorosis). Merck Manual (Professional Version). 2016.
459. Johnson L. Zinc. Merck Manual (Professional Version). 2017.
460. de Andrade F, de Albuquerque CA, et al. Safety assessment of yerba mate (Ilex paraguariensis) dried extract: results of acute and 90 days subchronic toxicity studies in rats and rabbits. *Food Chem Toxicol.* 2012;50(2):328-34
461. Bracesco N, Sanchez AG, et al. Recent advances on Ilex paraguariensis research: minireview. *J Ethnopharmacol.* 2011;136(3):378-84
462. Heck CI and de Mejia EG. Yerba Mate Tea (Ilex paraguariensis): a comprehensive review on chemistry, health implications, and technological considerations. *J Food Sci.* 2007;72(9):R138-51
463. Dasanayake AP, Silverman AJ, et al. Mate drinking and oral and oro-pharyngeal cancer: a systematic review and meta-analysis. *Oral Oncol.* 2010;46(2):82-6
464. Loria D, Barrios E, et al. Cancer and yerba mate consumption: a review of possible associations. *Rev Panam Salud Publica.* 2009;25(6):530-9
465. Abnet CC. Carcinogenic food contaminants. *Cancer Invest.* 2007;25(3):189-96
466. Gonzalez de Mejia E, Song YS, et al. Effect of yerba mate (Ilex paraguariensis) tea on topoisomerase inhibition and oral carcinoma cell proliferation. *J Agric Food Chem.* 2005;53(6):1966-73
467. Marques V and Farah A. Chlorogenic acids and related compounds in medicinal plants and infusions. *Food Chem.* 2009;113(4):1370-1376

468. Miranda DD, Arcari DP, et al. Protective effects of mate tea (Ilex paraguariensis) on H2O2-induced DNA damage and DNA repair in mice. *Mutagenesis*. 2008;23(4):261-5
469. de Morais EC, Stefanuto A, et al. Consumption of yerba mate (Ilex paraguariensis) improves serum lipid parameters in healthy dyslipidemic subjects and provides an additional LDL-cholesterol reduction in individuals on statin therapy. *J Agric Food Chem*. 2009;57(18):8316-24
470. Boaventura BC, Di Pietro PF, et al. Association of mate tea (Ilex paraguariensis) intake and dietary intervention and effects on oxidative stress biomarkers of dyslipidemic subjects. *Nutrition*. 2012;28(6):657-64
471. Klein GA, Stefanuto A, et al. Mate tea (Ilex paraguariensis) improves glycemic and lipid profiles of type 2 diabetes and pre-diabetes individuals: a pilot study. *J Am Coll Nutr*. 2011;30(5):320-32
472. Panza VP, Diefenthaeler F, et al. Effects of mate tea consumption on muscle strength and oxidative stress markers after eccentric exercise. *Br J Nutr*. 2016;115(8):1370-8
473. Petrilli AA, Souza SJ, et al. Effect of Chocolate and Yerba Mate Phenolic Compounds on Inflammatory and Oxidative Biomarkers in HIV/AIDS Individuals. *Nutrients*. 2016;8(5)
474. Yu S, Yue S, et al. Yerba mate (Ilex paraguariensis) improves microcirculation of volunteers with high blood viscosity: a randomized, double-blind, placebo-controlled trial. *Exp Gerontol*. 2015;62:14-22
475. Santos IS, Matijasevich A, et al. Mate drinking during pregnancy and risk of preterm and small for gestational age birth. *J Nutr*. 2005;135(5):1120-3
476. Food Safety Authority of Ireland. Substantial equivalence opinion. Ilex guayusa. 2017. 2.
477. The European Commission. Commission implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. The European Parliament and the Council on Novel Foods. Official Journal of the European Union; 2017: 351/72-351/201.

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October 21, 2019

Renata Kolanos, PhD
Regulatory Review Scientist/Chemistry Reviewer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Food Ingredients

Re: Responses to GRN 869 questions

Dear Dr. Kolanos,
Please find our responses to your questions for GRN 869 below. The original FDA questions are in blue, and responses to the questions are in black.

- (QUESTION #1) We noted contradictory statements in the notice. The following are examples only:

  a. On page 73 of the notice, the notifier states, "3 mg/kg bw/day could potentially serve as a no concern level"; on page 80 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 (80) 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 88 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 89, 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 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 88, 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

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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 #1): 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 869, 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

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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 869: The conservative caffeine exposure estimates (Part 3 of the notice), which take into account background caffeine consumption plus caffeine consumption from the Gyusa.g™ intended uses, resulted in an estimated 90th percentile exposure of 2.09 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 Gyusa.g™ 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 cited in GRN 869 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 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 869, 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 Gyausa.g™ intended uses, resulted in an estimated 90th percentile exposure of less than 400 mg/kg bw/day in adults.**

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Thus Gyusa.g™, 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 106 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 (≤ 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/mg. 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 869, the conservative caffeine exposure estimates (part 3 of the GRN), which take into account background caffeine consumption as well as caffeine consumption from the Gyausa.g™ 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 exposure estimates were between 200 and 300 mg per day from *both* background caffeine exposure alone, and background plus Gyusa.g™ intended uses. As discussed on page 38 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 these estimates (from background caffeine intake alone *and* background plus Gyusa.g™ intake) are higher than the 200 mg limit suggested by some EFSA, the Knight et al. data suggests that the GRN 869 exposure estimates during pregnancy would be well under 200 mg/day.

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- 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 99 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 Higon 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 in adults aged 50+ was 375.7 mg/day (4.8 mg/kg bw/day) just from background caffeine consumption. The estimate was actually slightly decreased to 358 mg/kg bw/day (4.5 mg/kg bw/day) when the Gyusa.g™ intended uses were assessed with background. Thus, while both estimates fall between 300 and 400 mg/day, adding the intended uses for the ingredient slightly decreased the exposure level in this age group.
- On page 108, 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 Gyusa.g™ intended use products are much lower than 250 mg (they range from 60–125 mg per serving), 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.91, 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 #2): We noted that on pages 64-65 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

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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 #2): 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 #3): 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

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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 #3): 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 #4): On page 139, 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 #4): The study was reference #413 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 #5): 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/fpsyt.2017.00080.

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(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:

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 #5): 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 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 is 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

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which consumption of Gyusa.g™ 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 #6): Address the adverse effects of caffeine in normal adults in conditions of overdose in no more than 1-2 pages in your own words. State the safe levels of oral caffeine consumption for healthy adults, pregnant women, adolescents, and children. This information should be discussed 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 477 references in the current notice.

- (RESPONSE #6): 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 Gyusa.g™ 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 myoblobin and creatine kinase levels. An electrocardiogram may show results of tachycardia, ST segment depressions, or T wave inversions.(a)(b)

  Wikoff et al., (2017) found that 400 mg/day for healthy adults, 300 mg/day for pregnant women, and 2.5 mg/kg bw/day for adolescents and children are supported as safe for consumption, using a robust systematic review of the literature related to five specific outcomes (calcium and bone status, cardiovascular effects, behavioral effects, reproductive and developmental toxicity, and acute

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toxicity). Note that Wikoff used comparator levels determined safe in the Nawrot et al., (2003) publication to assess if those levels were still safe. While Nawrot et al. did not suggest a safe use level for adolescents, Wikoff et al. utilized the level for children (2.5 mg/kg bw/day) as their comparator, and determined that it was an acceptably protective dose limit.(c) Temple et al., (2017) pointed out that there are organizations (e.g. EFSA) that suggest a more conservative 200 mg/day safe limit for pregnant women, and that a higher safe level has been suggested by EFSA for children and adolescents (3 mg/kg bw/day).(b) **The conservative exposure estimates in part 3 of the GRN, which take into account background caffeine consumption plus caffeine consumption from the Gyusa.g™ intended uses, resulted in an estimated 90th percentile exposure of < 400 mg/day for adults, < 300 mg/day for women of childbearing age (14–44), and < 2.5 mg/kg bw/day in children and < 3 mg/kg bw/day for adolescents.**

Regarding the differences in determination of safe levels of use in pregnancy (200 mg/day versus 300 mg/day), additional discussion of estimated exposures during pregnancy versus in non-pregnant women of child-bearing age (the former is likely <200 mg/day even though the latter is 200–300 mg/day) can be found in a previous response above. With regard to adolescent safe levels; safe levels have been concluded at 2.5 mg/kg bw/day (by Wikoff et al. (2017) and estimated at 3.0 mg/kg bw/day (by EFSA, 2015). In GRN 869, the adolescent exposure estimate for background plus intended use caffeine was determined for adolescents aged 13–18, and the result was 2.76 mg/kg bw/day (compared to 1.6 mg/kg bw/day from background caffeine consumption). For the purposes of addressing FDA's questions for this GRN, because the conservative exposure estimates for adolescents aged 13–18 fell between the 2.5 and 3.0 mg/kg bw/day safe levels, **we also assessed exposure utilizing the age range for adolescents as defined by EFSA (ages 10–17). The resulting 90th percentile lifetime caffeine exposure from background diet plus the new intended uses for this new age range was lower at 2.3 mg/kg bw/day, which falls below the lower estimate of caffeine safety of 2.5 mg/kg bw/day**.

With regard to the results shown for adolescents in the GRN notice (ages 13–18), in order to see estimated increases or decreases in caffeine exposure in adolescents from individual intended use categories as compared to background, we have broken them down in the table below. The data follows a similar pattern to that shown in Table 16 of the GRN for children.

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New Table: Comparison of Exposure to Caffeine in *Adolescents* from Background Sources to that from Background Plus Gyusa.g™'s Proposed Use Categories Using NHANES 2013–14 data

Intended Use Food Category	90th Percentile Daily Average Consumption as mg/day (mg/kg bw/day)	
	Background Caffeine Exposure	Estimated New Caffeine Exposure from Background Plus Intended Use Categories
Bars* / Energy Bars	1.6 (0.2)	69.5 (1.1)
Energy Drinks	198.5 (2.6)	209.7 (2.9)
RTD Tea	109.6 (1.7)	211.8 (3.1)
Carbonated Soft-drinks	90.1 (1.3)	148.6 (2.2)
Coffee-like Beverages	206.5 (3.2)	132.4 (1.9)
Enhanced Water	0 (0)	124.6 (1.9)

Creme runs #204 and #312
*Note that NHANES surrogate food codes for the energy bar Gyusa.g™ application included non-caffeinated nutrition bars

As discussed below Table 16 in the GRN, products containing Gyusa.g™ will be clearly labeled with caffeine content. The large jump in exposure in adolescents compared to background from bars, carbonated soft-drinks, teas, and enhanced waters is expectedly due to the fact that many of the food codes contained in these categories do not normally contain caffeine, hence the lower level of caffeine exposures in the background column. During our assessment, suddenly *100% of these food codes* were assigned to contain the *maximum* intended addition levels of Gyusa.g™/caffeine, leading to the rise and likely large over-estimation of caffeine consumption. In reality, caffeine containing products are not expected to be substituted for non-caffeine containing nutrition type bars and non-caffeinated soft-drinks, teas or waters (note that the intended addition level for carbonated soft drinks is that allowed by FDA). Instead, these surrogate food codes were utilized to show conservative estimates for general food groups, despite not all surrogate categories being a perfect match. In reality, Gyusa.g™ beverages products are expected to represent a relatively small segment of the total marketplace, and exposure may realistically look more like the background exposures shown in the GRN (i.e. 1.6 mg/kg bw/day for adolescents). **Importantly, as mentioned a number of times in the GRN, while these exposure estimates are intended to be very conservative (representing maximum addition levels in 100% of the categories and products on the market), recent data from a number of publications**

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suggests that caffeine consumption has remained stable in the U.S. population over the last decade, including by children and adolescents, despite various new caffeine products being added to the market (references cited in the GRN include citation numbers 14–16 and 55–58).

- (QUESTION #7): 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 #7): 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 869, 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)

  Such 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 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

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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 #8): 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 #8): 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 above 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 869, 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 Gyusa.g™ is expected to be labelled with regard to caffeine content, individuals who are sensitive are expected to avoid or limit consumption of Gyusa.g™/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 Gyusa.g™ intended uses are compared to estimated exposures from background caffeine consumption in part 3 of the GRN. While increases in caffeine consumption are shown compared to background, such estimates are likely overly conservative, since in reality, studies show that caffeine consumption in adults, adolescents and children has remained stable over the last decade despite new caffeine products being added to the marketplace. Regardless, the potentially overly-conservative estimates still fall below daily intake levels considered safe by various scientific bodies (see additional discussions with respect to pregnant women and adolescents above). Levels of caffeine per serving in each of the intended use categories are considered reasonable and moderate overall, 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, Gyusa.g™'s intended uses are expected to be safe for humans.

- (QUESTION #9): 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.

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- (RESPONSE #9): The review by Wise et al. was published online August 1st, 2019, after GRN 869 was submitted 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 869, 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 Gyusa.g™.

  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 869 (Gyusa.g™) 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 Gyusa.g™ 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 Gyusa.g™ 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

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raw leaf material utilized in every batch of the Gyusa.g™ manufacturing process undergoes pesticide evaluation, and batches would be rejected if they were to ever exceed the specified tolerances.

As in GRN 869, 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 869 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 Gyusa.g™ 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,

Amy Clewell, ND, DABT
VP Scientific and Regulatory Affairs

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