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#868

June 7, 2019

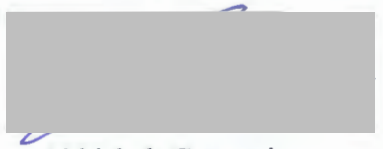
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

**Re: Generally Recognized as Safe (GRAS) Notice for Coffeeberry® Coffee Fruit Extract**

Dear Sir/Madam:

Pursuant to 21 C.F.R. part 170, subpart E, VDF FutureCeuticals, Inc. hereby submits the enclosed notice, that use of its Coffeeberry® Coffee Fruit Extract at levels of up to 300 mg/serving in conventional foods is excluded from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because the notifier has determined that such use is generally recognized as safe (GRAS).

Sincerely,



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Encl.

# EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF

# COFFEEBERRY® COFFEE FRUIT EXTRACT

## AS A FOOD INGREDIENT

Prepared for:
**VDF FutureCeuticals, Inc.**
2692 N. St. Rt. 1-17
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&

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## Panel Members

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May, 2019

# EVALUATION OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF COFFEEBERRY® COFFEE FRUIT EXTRACT AS A FOOD INGREDIENT

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## Part I- SIGNED STATEMENT AND CERTIFICATION

### 1.1. Basis of Conclusion

This GRAS conclusion for use of Coffeeberry® Coffee Fruit Extract as a food ingredient has been reached in accordance with requirements as defined in 21 CFR 170.220.

### 1.2. Name and address of organization:

**VDF FutureCeuticals, Inc.**
2692 N. St. Rt. 1-17
Momence, IL 60954
USA

Phone: 1-815-507-1470
Fax: 1-815-472-3529
Email: RWexler@futureceuticals.com

### 1.3. Name of substance:

The name of the substance of this GRAS assessment is Coffeeberry® Coffee Fruit Extract.

### 1.4. Intended conditions of use of Coffeeberry® Coffee Fruit Extract:

Coffeeberry® Coffee Fruit Extract is intended to be used as a food ingredient and as an antioxidant in selected conventional food products, such as Flavored Water/Energy Drink; Coffee/Tea; RTM Beverages; Milk Products (pre-work out); Clusters/Bars; Fruit Juices; Vegetable Juices/Blends; Chocolate; Candy; and Chewing gum, at use levels of up to 300 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). It is recognized that there are Standard of Identity requirements for some of the specified foods and these foods will not be referred to by their commonly recognized names.

### 1.5. Statutory Basis for GRAS conclusion:

This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and 170.30(b).

### 1.6. Exemption from Premarket approval requirements:

VDF FutureCeuticals, Inc. (FutureCeuticals) has concluded that Coffeeberry® Coffee Fruit Extract is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on our conclusion that Coffeeberry® Coffee Fruit Extract, meeting the specifications cited herein, and when used as a food ingredient and as an antioxidant, is GRAS and is therefore exempt from the premarket approval requirements.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Coffeeberry® Coffee Fruit Extract, when used as described in this dossier, is GRAS based on scientific procedures.

### 1.7. Availability of data and information:

The data and information that are the basis for this GRAS conclusion will be made available to FDA upon request by contacting Mr. Wexler at the below address. The data and information will be made available to FDA in a form in accordance with that requested under 21 CFR 170.225(c)(7)(ii)(A) or 21 CFR 170.225(c)(7)(ii)(B).

Mr. J. Randal Wexler
Vice President and General Counsel
VDF FutureCeuticals, Inc.
2692 N. St. Rt. 1-17
Momence, IL 60954
USA

Phone: 1-815-507-1439
Email: RWexler@futureceuticals.com

### 1.8. Data exempt from Disclosure:

Part I through Part VII of this GRAS assessment does not contain any privileged or confidential information such as trade secrets and/or commercial or financial information and can be made publicly available.

### 1.9. Certification:

FutureCeuticals certifies that, to the best of its knowledge, this GRAS conclusion is based on a complete, representative, and balanced dossier that includes all relevant information, available and obtainable by FutureCeuticals, including any favorable or unfavorable information, and pertinent to the evaluation of the safety and GRAS status of the use of Coffeeberry® Coffee Fruit Extract. FutureCeuticals accepts responsibility for the GRAS conclusion that has been made for Coffeeberry® Coffee Fruit Extract as described in this dossier.

### 1.10. Name, position/title of responsible person who signs dossier and signature:

Mr. J. Randal Wexler
Vice President and General Counsel
VDF FutureCeuticals, Inc.
2692 N. St. Rt. 1-17
Momence, IL 60954
USA

Signature: 

### 1.11. FSIS/USDA – Use in Meat and/or Poultry:

FutureCeuticals does not intend to add Coffeeberry® Coffee Fruit Extract to any meat and/or poultry products that come under USDA jurisdiction. Therefore, 21 CFR 170.270 does not apply.

## Part II- IDENTITY AND TECHNICAL INFORMATION

### 2.1. Description

The subject of this GRAS assessment, Coffeeberry® Coffee Fruit Extract is a standardized powder derived from coffee fruit of the plant *Coffea arabica*. The extract is a tan-brown colored powder with characteristic odor and taste. It is prepared by water/ethanol extraction of whole ground coffee fruit (including the coffee bean). General descriptive characteristics of Coffeeberry® Coffee Fruit Extract are summarized in Table 1. The active constituents of the extract are phenolic acids.

**Table 1. General Descriptive Characteristics of Coffeeberry® Coffee Fruit Extract**

| Parameter | Description (FutureCeuticals, 2017)* |
|---|---|
| Plant Source | *Coffea Arabica* |
| Part used | Fruit |
| Starting material | Ground and dried whole fruit |
| Active constituents | Phenolic acids |
| Synonyms | Coffee cherry extract |
| Appearance | Dried powder |
| Color | Tan brown |
| Odor | Characteristic |
| Taste | Characteristic |
| Storage | Cool, Dry |
| Shelf life | 2 years |

*Based on information provided by FututreCeuticals (2017)

### 2.2. Botanical identification

The hierarchical classification of the source material, *Coffea arabica* is presented in Table 2. The plant is famous for its seeds or "beans," which are the source for a commonly consumed drink around the world, coffee. The genus Coffea is taxonomically placed within the flowering plants of the family Rubiaceae and is considered to comprise three subgenera: Subgenus Coffea (having about 90 spp.) with significant commercial relevance, and subgenera Psilanthopsis and Baracoffea that have only minor commercial relevance (Chevalier, 1942; 1947; Leroy, 1961). The Coffea plant, an evergreen shrub or small tree having dark green, glossy leaves, is thought to have originated in southern Asia or in Africa. It is now cultivated as an agricultural crop in various parts of the world, mostly at latitudes less than 30 degrees north or south of the equator, and most commonly at elevations of 1000-2000 meters (Wrigley, 1988). The fruit is a small green cherry that turns bright red when ripe.

**Table 2. Taxonomical Classification of *Coffea arabica***

| Rank | Scientific Name and Common Name |
|---|---|
| Kingdom | Plantae – Plants |
| Subkingdom | Tracheobionta – Vascular plants |
| Superdivision | Spermatophyta – Seed plants |
| Division | Magnoliophyta – Flowering plants |
| Class | Magnolipsoda- Dicotyledons |
| Subclass | Asteridae |
| Order | Rubiales |
| Family | Rubiaceae – Madder family |
| Genus | *Coffee* L. – coffee |
| Species | *Coffea arabica* L. – Arabian coffee |

USDA, Natural Resources Conservation Service[1]

## 2.3. Specifications

Food grade specifications of Coffeeberry® Coffee Fruit Extract by FututreCeuticals are summarized in Table 3. The phenolic acid content of Coffeeberry® Coffee Fruit Extract is 40% or greater, while the caffeine content is approximately 1-2%. Analytical results from four non-consecutive lots (Table 4) demonstrate that Coffeeberry® Coffee Fruit Extract is produced consistently and meets the food grade specifications. Coffeeberry® Coffee Fruit Extract is completely soluble in water. The residual solvent levels for ethanol used in the extraction are below the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and/or Code of Federal Regulations (CFR) limits for these solvents in the manufacturing of other food ingredients. The composition and nutritional profile of Coffeeberry® Coffee Fruit Extract is presented in Table 5.

**Table 3. Specifications of Coffeeberry® Coffee Fruit Extract***

| Parameter | Characteristics | Method |
|---|---|---|
| Particle size | ≥98% passing through #40 sieve | FCCM P.2.1 |
| Moisture (%) | ≤10 | FCCM P.1.1 |
| Color | Tan/brown | Visual |
| Solubility in water | Soluble | In house |
| Phenolic acids (%) | ≥40 | FCCM C.2.5 |
| Caffeine (%) | 1-2 (approximately) | USP |
| Residual solvent (ethanol) levels (mg/kg) | 1000 | USP |
| Identity | Characteristic | FTIR |
| **Contaminants** | | |
| Pesticides | Meets EPA Limits | AOAC 2007.01 |
| Aflatoxins Sum of B1, B2, G1, G2 | < 4 ppb | AOAC 994.08 |
| Ochratoxin A | <10 ppb | AOAC 994.08 |
| **Heavy metals** | | |
| Arsenic (mg/kg) | ≤1 | AOAC 993.14 |
| Cadmium (mg/kg) | ≤1 | AOAC 993.14 |
| Lead (mg/kg) | ≤1 | AOAC 993.14 |
| Mercury (mg/kg) | ≤0.5 | AOAC 993.14 |
| **Microbiological purity** | | |
| Aerobic plate count (cfu/g) | ≤10,000 | AOAC 990.12 |
| Yeast and mold (cfu/g) | ≤200 | AOAC 997.02 |
| Coliforms (cfu/g) | ≤10 | AOAC 991.14 |
| *E. coli* (cfu/g) | <10 | AOAC 991.14 |
| *Salmonella* spp. (in 375 g) | Negative | FDA BAM, Ch. 5 |
| Coag.+ *Staph.* (in 1 g) | Negative | FDA BAM, Ch. 12 |

*Based on information provided by FututreCeuticals (2017)

**Table 4. Results of Testing of Four Lots of Coffeeberry® Coffee Fruit Extract Powder**

| Parameter | Specifications | Lot Number |
|---|---|---|

[1] Available at: https://plants.usda.gov/core/profile?symbol=COAR2

| | | | | | |
|---|---|---|---|---|---|
| Particle size | ≥98% passing through #40 sieve | 100% | 100% | 100% | 100% |
| Moisture (%) | ≤10 | 1.5 | 1.5 | 3.8 | 1.6 |
| Color | Tan/brown | Complies | Complies | Complies | Complies |
| Solubility | Soluble | Complies | Complies | Complies | Complies |
| ORAC (µmol TE/g) | ≥6,000 | 8,326 | 7,572 | 9,642 | 8,770 |
| Phenolic acids (%) | ≥40 | 43.1 | 42.6 | 45.03 | 50.80 |
| Caffeine (%) | 1-2 (approximately) | 1.0 | 1.0 | 2.7 | 2.2 |
| Residual solvent (ethanol) levels (mg/kg) | 1000 | <200 | <200 | <200 | <200 |
| **Contaminants** | | | | | |
| Pesticides | Meets EPA Limits | Complies | Complies | Complies | Complies |
| Aflatoxins Sum of B1, B2, G1, G2 | <4 ppb | ND | ND | ND | ND |
| Ochratoxin A | <10 ppb | ND | ND | ND | ND |
| **Heavy metals** | | | | | |
| Arsenic (mg/kg) | ≤1 | <0.020 | <0.020 | <0.020 | <0.020 |
| Cadmium (mg/kg) | ≤1 | <0.020 | <0.020 | <0.020 | <0.020 |
| Lead (mg/kg) | ≤1 | 0.105 | 0.121 | 0.035 | 0.031 |
| Mercury (mg/kg) | ≤0.5 | 0.061 | 0.081 | 0.021 | <0.020 |
| **Microbiological** | | | | | |
| APC (cfu/g) | ≤10,000 | <1,000 | <1,000 | <1,000 | 2,000 |
| Yeast/mold (cfu/g) | ≤200 | <10 | <10 | <10 | 10 |
| Coliforms (cfu/g) | ≤10 | <10 | <10 | <10 | <10 |
| E. coli (cfu/g) | ≤10 | <10 | <10 | <10 | <10 |
| C. + Staph. | Negative | Complies | Complies | Complies | Complies |
| Salmonella | Negative | Complies | Complies | Complies | Complies |

Source: FutureCeuticals (2017)

**Table 5. Compositional Analysis of Coffeeberry® Coffee Fruit Extract**

| Parameter | Quantitative composition |
|---|---|
| Moisture (%) | 2.0 |
| Protein (%) | 9.0 |
| Carbohydrates (%) | 11.4 |
| Total fat (%) | 0.2 |
| Saturated fat (%) | 0.1 |
| Monounsaturated fat (%) | <0.1 |
| Polyunsaturated fat (%) | <0.1 |
| Ash (%) | 16.2 |
| Polyphenols (%) | 52.0 |
| Trigonelline (%) | 3.4 |
| Organic Acids (%) | 3.8 |
| Caffeine (%) | 2.0 |

Source: FutureCeuticals (2017)

## 2.4. Manufacturing Process

### 2.4.1. Harvesting of Coffee Fruit

The *Coffea* plant produces clusters of simultaneously blooming white flowers, each of which subsequently develops into an oval cherry-like fruit. Each cherry consists of an exocarp, pulp, mucilage, and generally two central seeds (or "beans"). The fruit usually achieves ripeness in seven to nine months. Immature fruit is green, but the fruit gradually turns bright red as it ripens (Wrigley, 1988; Sivetz and Desrosier, 1979). During conventional coffee production, coffee processers strip off the fruit that surrounds the seed (Rothfos, 1980; Clarke and Macrae, 1987). Only the seeds of the *Coffea* fruit are used to produce the well-known and much-consumed beverage known as coffee, while the entire fruit is used to produce Coffeeberry® Coffee Fruit Extract, the subject of the present GRAS assessment.

During designated times within the coffee harvest season, the whole coffee fruit is harvested by hand at specific growth stages ranging from sub-ripe to maturity. Only unblemished fruit is selected. After harvesting, the fruit is washed and subsequently quick-dried in food-grade stainless-steel coffee-cherry dryers according to a proprietary protocol. This process yields a dried whole coffee fruit that can be ground for use as a food ingredient or further processed by various extraction methods.

The coffee fruit has long been recognized as having inherent nutritional and health-enhancing potential, including antioxidant capacity (Napolitano et al. 2007; Garcia et al. 2008; Serafini and Testa, 2009). However, the cherry is highly perishable (Pittet et al., 1996; Bucheli et al., 2000) and, until a recent discovery (Miljkovic et al., 2004a, 2004b), has been prone to rapidly develop both extensive bacterial contamination and molds that generate undesirable toxic secondary metabolites known as mycotoxins.

Frank et al. (1965) analyzed the bacterial load of decomposing Kona coffee fruits and concluded that it was dominated by Gram negative organisms, especially *Erwinia dissolvens*. Later analyses by Silv et al. (2000) of Brazilian coffee fruits isolated over 44 bacterial genera and several yeast genera in which they found that Gram negative bacteria dominated in wet years while Gram positive bacteria were more prevalent during dry years. The primary risk factor is contamination by pectinolytic yeasts such as *Saccharomyces* and *Aspergillus* species (Agate and Bhat, 1966) and *Aspergillus* species which produce ochratoxin A (Bucheli et al., 2000; Bucheli and Taniwaki, 2002; Viani, 2002; Napolitano et al., 2007). Consequently, the coffee fruit, other than its seed, has traditionally been considered to be waste material unsuitable for food use, and has typically been discarded or used as fertilizer (Pandey et al., 2000).

The new proprietary technology for cultivation, harvesting, and subsequent processing of whole coffee fruit (including the seed) has eliminated the risk of bacterial and fungal contamination and the production of mycotoxins (Miljkovic et al., 2004a, 2004b). The whole coffee fruit can then be used to produce dried whole coffee fruit powders and granules.

In order to characterize the degree of concentration achieved by water/ethanol extraction, total polyphenols and Oxygen Radical Absorbent Capacity (ORAC) levels were measured in 8 lots of whole fruit powder and 6 of water/ethanol extract powder. The mean levels of total polyphenols were 4.32 and 45.10% respectively, indicating a concentration of polyphenols of 10.43 times higher in the water/ethanol extract as compared to the whole fruit powder. Based on mean ORAC levels, concentrations of water/ethanol extract over whole fruit powder was 8.18.

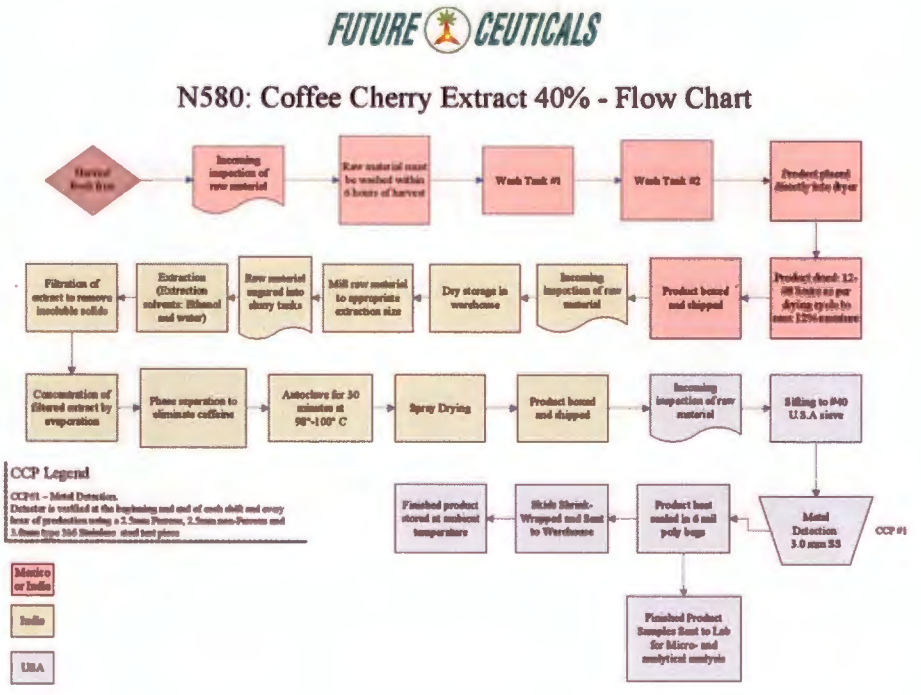
Averaging the degrees of concentration of these two markers—total polyphenols and ORAC levels—it appears that reasonable figures for the overall degree of concentration is 9 for the water/ethanol extract.

### 2.4.2. Production Process

The production process for Coffeeberry® Coffee Fruit Extract is illustrated in Figure 1. Coffeeberry® Coffee Fruit Extract is manufactured according to current good manufacturing practices (GMPs). In brief, the manufacturing starts with harvesting fresh fruits that are subjected to washing with water within six hours of harvest. The washing is repeated one more time and the washed fruits are dried for 12-48 hours to achieve a moisture content of ≤ 12%. The dried fruit is then milled to an appropriate size, augered into slurry tanks, and subjected to extraction with water/ethanol. The extract is then filtered before concentration by evaporation. This is followed by the removal of caffeine via gravimetric phase separation with no added solvents. Next, the material is autoclaved for 30 minutes at 98°- 100° and spray dried. Finally, the material is subjected to sifting, metal detecting, and packaging. The preparation procedure assures a consistent and high-quality product. The extract is a powder that is standardized to 40% or greater phenolic acids and about 1-2% caffeine.

Food-grade ethanol used for extraction of Coffeeberry® Coffee Fruit Extract powder is an unlisted GRAS substance widely used as a solvent in food processing. The ethanol used in the production meets current Food Chemical Codex (FCC) specifications. Except for potable water, no other chemical substances are used in the manufacture of Coffeeberry® Coffee Fruit Extract.

**Figure 1. Manufacturing process of Coffeeberry® Coffee Fruit Extract**



## Part III- DIETARY EXPOSURE

### 3.1. Proposed Use Levels and Food Categories

FutureCeuticals intends to use Coffeeberry® Coffee Fruit Extract in 10 food categories at a maximum proposed use level ranging from 20 to 300 mg per serving. The food serving size to which the extract will be added corresponds to the gram weight or mL volume of food as specified by References Amounts Customarily Consumed (RACCs) for food labeling based on FDA's final rule, effective July 26, 2016, with the compliance date of July 26, 2018 (Federal Register, 2016). Table 6 lists the 10 food categories to which Coffeeberry® Coffee Fruit Extract is proposed for use, descriptions of the types of foods within the category that was included in the assessment, the serving size associated with each food type, and the maximum use level of the extract. The Coffeeberry® Coffee Fruit Extract will not be used in any foods for which food standards would preclude its use. Foods that are intended for infants and toddlers, such as infant formulas or foods formulated for babies or toddlers, and meat and poultry products that come under USDA jurisdiction are excluded from the list of intended food uses of Coffeeberry® Coffee Fruit Extract.

**Table 6. Proposed Uses of Coffeeberry® Coffee Fruit Extract in Foods**

| Proposed Use Category | Description of Foods Selected for Analysis | Serving Size[a] | Maximum Use Level (mg/serving) |
|---|---|---|---|
| Flavored Water/Energy Drink | Sport drinks (i.e., Gatorade, Powerade, etc.), energy drinks (i.e., Monster, Red Bull, etc.), and enhanced/fortified waters (i.e., Propel, Glaceau, etc.) | 360 ml | 300 |
| Coffee/Tea | RTD/bottled/canned coffees and teas. Coffee excludes brewed, instant, and frozen types. Tea excludes hot and brewed tea leaf. Coffee and tea both exclude decaffeinated types. | 360 ml | 100 |
| RTM Beverages | Non-reconstituted protein powders (i.e., Muscle Milk powder) | Amount to make 240 ml | 100 |
| Milk Products (pre-work out) | Ensure, Boost, and RTD high protein nutritional drinks such as Monster Milk | 240 ml | 100 |
| Clusters/Bars | Nutrition bars including Balance, PowerBar, Clif, Zone, etc. | 40 g | 100 |
| Fruit Juices | Fruit juice blends and drinks, carbonated fruit juice drinks, coconut waters | 240 ml | 100 |
| Vegetable Juices/Blends | Carrot juice, tomato juice, celery juice, mixed vegetable juice, fruit and vegetable blend juices | 240 ml | 100 |
| Chocolate | Milk chocolate and dark chocolate with or without caramel, nuts, toffee, and/or dried fruits/seeds inclusions | 30 g | 100 |
| Candy | Gummy candy including Life Savers Gummi Savers and other gummy animals/shapes | 30 g | 20 |
| Chewing gum | Chewing gum, regular and sugar free | 3 g | 20 |

[a] Serving sizes correspond to values in Table 2 – Reference Amounts Customarily Consumed per Eating Occasion: General Food Supply as cited in FR Vol 81, No. 103, Friday, May 27, 2016, pp 34000-47. Available at: https://www.govinfo.gov/content/pkg/FR-2016-05-27/pdf/2016-11865.pdf. RTD = ready-to-drink

### 3.2. Estimated Daily Intake

Intake estimates of Coffeeberry® Coffee Fruit Extract and one of its constituents, caffeine, were based on food consumption records collected in the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) conducted in 2009-2010, 2011-2012, and 2013-2014 (NHANES 2009-2014). The survey data is provided for the total United States (U.S.) population 2 years (y) and older and the following five subpopulation: children 2-12 years, adolescents 13-18 years, adults 19-49 years and 50+ years, and women of childbearing age (WCBA) 14-49 years. The NHANES is a continuous survey that uses a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS, 2013; 2014; 2016). NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection and non-response. The intake analysis was conducted by Exponent, Inc. and the complete report is attached as Appendix I.

The two-day average intake of Coffeeberry® Coffee Fruit Extract from the proposed uses in 10 food categories combined are expressed in units of mg/person/day and mg/kg-bw/day and are provided in Tables 7A and 7B, respectively. Among the U.S. population two years and older, the all users mean and 90th percentile of intake of Coffeeberry® Coffee Fruit Extract is 170 mg/person/day (2.7 mg/kg-bw/day) and 393 mg/person/day (5.9 mg/kg bw/day), respectively. Among the different populations, the highest 90$^{th}$ percentile intake of 459 mg/person/day was noted in adults 19-49 years. On body weight basis the highest intake of 9.6 mg/kg bw/day was noted in children 2 to 12 years of age.

**Table 7A. Summary of the Estimated Daily Intake of Coffeeberry Coffee Berry Extract per Person from Proposed Food-Uses in the United States by Population Group (NHANES 2009-2014 Data)**

| Population Group | Unweighted N[a] | % Users | All-Person Consumption (mg/person/day) | | All-Users Consumption (mg/person/day) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90$^{th}$ Percentile | Mean | 90$^{th}$ Percentile |
| U.S. 2+ years | 12,379 | 55 | 94 | 263 | 170 | 393 |
| Children 2-12 years | 3,386 | 63 | 27 | 188 | 112 | 247 |
| Adolescent 13-18 years | 1,531 | 61 | 126 | 310 | 207 | 432 |
| Adults 19-49 years | 4,264 | 57 | 114 | 338 | 200 | 459 |
| Adults 50+ years | 3,198 | 49 | 72 | 203 | 146 | 335 |
| WCBA 14-49 years | 2,909 | 57 | 87 | 240 | 152 | 340 |

[a] Un-weighted number of users; % users, per capita and per user estimates for NHANES derived using the statistical weights provided by the NCHS. Note: Refer to Table 6 for the proposed uses of the extract.

**Table 7B. Summary of the Estimated Daily Intake of Coffeeberry Coffee Berry Extract on Body Weight Basis from Proposed Food-Uses in the United States by Population Group (NHANES 2009-2014 Data)**

| Population Group | Unwated N[a] | % Users | All-Person Consumption (mg/kg bw/day) | | All-Users Consumption (mg/kg bw/day) | |
|---|---|---|---|---|---|---|
| | | | Mean | 90$^{th}$ Percentile | Mean | 90$^{th}$ Percentile |
| U.S. 2+ years | 12,379 | 55 | 1.5 | 4.2 | 2.7 | 5.9 |
| Children 2-12 years | 3,386 | 63 | 2.7 | 7.4 | 4.3 | 9.6 |
| Adolescent 13-18 years | 1,531 | 61 | 1.9 | 5.0 | 3.1 | 6.4 |
| Adults 19-49 years | 4,264 | 57 | 1.4 | 4.1 | 2.5 | 5.9 |
| Adults 50+ years | 3,198 | 49 | 0.9 | 2.6 | 1.8 | 3.9 |
| WCBA 14-49 years | 2,909 | 57 | 1.2 | 3.4 | 2.2 | 5.0 |

[a] Un-weighted number of users; % users, per capita and per user estimates for NHANES derived using the statistical weights provided by the NCHS. Note: Refer to Table 6 for the proposed uses of the extract.

In addition to the intake of the Coffeeberry® Coffee Fruit Extract the resulting intake of caffeine from the proposed uses as well as background intake of caffeine from other sources was also determined. As regards caffeine intake from the proposed uses, among the U.S. population two years and older, the per user caffeine intake that is associated with the proposed use of Coffeeberry® Coffee Fruit Extract at the per user mean and 90th percentile of intake is estimated as 3.4 mg/person/day (0.05 mg/kg-bw/day) and 7.8 mg/person/day (0.12 mg/kg-bw/day). The caffeine intake from background sources (food and dietary supplement) pre-introduction of Coffeeberry® Coffee Fruit Extract at the per user mean and 90th percentile of intake is estimated as 141 mg/person/day (1.8 mg/kg-bw/day) and 338 mg/person/day (4.2 mg/kg-bw/day), respectively. The cumulative caffeine intake from background sources and the proposed use of Coffeeberry® Coffee Fruit Extract combined at the per user mean and 90th percentile of intake is 137 mg/person/day (1.8 mg/kg-bw/day) and 332 mg/person/day (4.2 mg/kg bw/day), respectively. This intake analysis for caffeine shows that the maximum ($90^{th}$ percentile) additional intake of 7.8 mg caffeine/person/day from the proposed uses of the extract is minor as compared to the existing background intake of caffeine. However, it is interesting to note that the cumulative $90^{th}$ percentile caffeine (background + current proposed uses) intake of 332 mg/person/day is slightly lower than the background caffeine intake of 338 mg/person/day. The possible reason for this is described below.

The cumulative caffeine intake includes consumers of caffeine from background sources and/or proposed uses of Coffeeberry® Coffee Fruit Extract, whereas the background caffeine intake includes consumers of caffeine from the background alone (i.e., pre-introduction of Coffeeberry® Coffee Fruit Extract). Hence, the increase in the user sample size from the background to cumulative analysis is due to the inclusion of NHANES participants who did not consume caffeine but who consume one or more of the foods with the proposed Coffeeberry® Coffee Fruit Extract use coupled with the lower intake of caffeine from these proposed uses that result in slightly lower caffeine intakes for almost all populations in the cumulative intake analysis. In other words, the combined caffeine intake distribution, which includes background and proposed Coffeeberry® Coffee Fruit Extract sources, shifted the mean and 90th percentile of intake lower due to the inclusion of lower caffeine intakes associated with the proposed uses of the extract.

## Part IV- SELF LIMITING LEVELS OF USE

Coffeeberry® Coffee Fruit Extract has a characteristic taste. Excessive amounts of this product are unlikely to be added to food products because of the unpleasant taste at high levels. Additionally, the cost of the product will prohibit excessive use.

## Part V- EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

The statutory basis for the conclusion of the GRAS status of Coffeeberry® Coffee Fruit Extract in this document is not based on common use in food before 1958. The GRAS assessment is based on scientific procedures. Notwithstanding this, the source material of the extract – coffee cherries – has been commonly present in food prior to 1958, as described below.

## Part VI- NARRATIVE

### 6.1. Traditional and Current Uses

Originating in Ethiopia, the coffee plant has been used for centuries in a variety of preparations (Ota, 2018). The popular drink known as coffee – a water extraction made from roasted coffee seeds or "beans" – was likely first consumed in Yemen in the first half of the fifteenth century (Ota, 2018). After spreading from the Middle East and Arabian Peninsula to Europe in the seventeenth century, coffee became widely consumed in global markets by the 1800s, with methods of consumption varying by geographic location (Ota, 2018; Pendergrast, 2010).

In addition to preparations made from roasted coffee beans, the fruit surrounding the coffee bean also has a longstanding history of consumption. "Qishr," a tea made from coffee fruit, has been widely consumed throughout the Middle East for centuries, including in Tihamah, Rayy, and Mecca (Ota, 2018, Thesiger, 1947). In fact, Qishr has traditionally been more popular than coffee in Yemen (Ota, 2018). Coffee fruit has also been used as a source of food. Seventeenth-century reports from Ottoman scholar Katip Celebi detail the consumption of crushed coffee fruit by Yemenis shayks and Islamic mystics. In addition, the Galla tribe in Ethiopia combined fat with crushed whole coffee fruit for food, and natives in equatorial South Sudan and Uganda consumed coffee fruit both raw and after boiling and drying (Ota, 2018).

Today, coffee fruit and its preparations are marketed as ingredients in a wide variety of food applications including ready-to-mix and ready-to drink beverages, chocolate, and other snacks foods. Notable examples include the "Cascara Latte" introduced by Starbucks Corporation in 2017, Kishr's line of "Organic Coffee Fruit" teas, the "Caffé Monster" by Monster Energy Company, and several of the "SuperTea" beverages produced by Bai Brands LLC, all of which contain either coffee fruit or coffee fruit extract.

Furthermore, in 2016, Health Canada evaluated a Novel Food notification from FutureCeuticals and, following a comprehensive assessment, the agency responded that it has no objection to the sale of Coffeeberry® whole coffee fruit derivatives - including the subject of the present GRAS assessment, Coffeeberry® Coffee Fruit Extract - as ingredients for use at levels up to 300 mg/serving in foods and beverages sold in Canada.

This information suggests that coffee fruit and its preparations is consumed as a food without any reported adverse effects.

### 6.2. Data Pertaining to Safety

In a series of well-designed toxicity studies, conducted as per current accepted guidelines, FutureCeuticals investigated the effects of whole coffee fruit preparations (Coffeeberry® products), such as whole coffee fruit powder, dried water extract powder, and dried water/ethanol extract powder (Coffeeberry® Coffee Fruit Extract), in animals and *in vitro* experimental systems. The overall findings from all these studies are published in the journal *Food and Chemical Toxicology* (Heimbach et al., 2010). In the following section, relevant toxicological and other studies on coffee fruit and its preparations are summarized in the order of their importance and in support of the conclusions drawn in this assessment. This information is provided in the following sequence: published pivotal studies, secondary published studies, corroborative unpublished studies and regulatory agency assessments. Efforts have been made to

present both the data supporting the safety as well as any data on the adverse effects of coffee fruit and its preparations.

### 6.2.1. Pivotal Studies of Coffeeberry® Coffee Fruit Extract

#### 6.2.1.1 Fourteen-Day Gavage Study with Coffeeberry® Coffee Fruit Extract

Coffeeberry® Coffee Fruit Extract (the water/ethanol extract; subject of the present GRAS assessment) was tested for its potential toxicity. Based on the initial observations from 14-day feeding studies of whole powder and water-extract powder that revealed palatability issues, for this study, gavage was chosen rather than feeding in the range-finding study of Coffeeberry® Coffee Fruit Extract. The toxicity of Coffeeberry® Coffee Fruit Extract was investigated in Sprague-Dawley (Hsd:SD) rats for a period of 14 days (Heimbach et al., 2010) as per OECD Guideline 407 and US FDA *Redbook 2000*. In this study, groups of 10 rats/sex/dose were orally intubated daily with Coffeeberry® Coffee Fruit Extract at doses of 0 (distilled water vehicle control), 1000, 2000, or 4000 mg/kg bw/day. For this study rats used were approximately 8 weeks of age and males weighed 241±6.05 g, while females weighed 189±7.18 g at the start of treatment. Feed intake and body weight were measured on days 1, 4, 8, 11, and 14. The dose preparations were prepared daily based on the most recent body weights. Fasted rats were placed in metabolism cages one day before scheduled termination to collect urine.

All rats survived until the scheduled termination. During early and intermittently throughout the treatment period, clinical signs in treated animals, such as brown litter staining, facial/ano-genital staining, some nasal/ocular discharge, and piloerection were noted. The study authors considered these signs, which appeared more frequently in test animals than in controls but were not clearly dose-dependent, to be treatment-related but non-adverse. As compared to control, a significant decrease in mean body weights throughout the study (days 1-14) was noted in high-dose rats of both sexes. As compared to control, a significant decrease in mean daily body weight gain in mid- and high-dose males was noted at the start of the study, but became significantly increased from controls on days 4-8 for mid-dose males and days 8-11 for high-dose males. For females, mean daily body weight gain was significantly increased from controls throughout the study at the high dose and during days 11-14 at the lower doses. Generally, feed consumption decreased at the beginning of treatment and increased toward the end of the study in both sexes, particularly at the highest dose level. Feed efficiency followed a similar pattern (Heimbach et al., 2010).

A dose-dependent increase in urine volume collected via metabolism cages was noted that was significantly higher from controls in all treated males (7.4 ml, 18.3 ml, 32.9 ml, and 43.6 ml for control, low-dose, mid-dose, and high-dose, respectively) and high-dose females (7.1 ml, 13.1 ml, 25.8 ml and 42.0 ml for control, low-dose, mid-dose, and high-dose, respectively). Urine volume collected directly from the bladder at necropsy did not differ between groups. Macroscopic examination revealed a proteinaceous white substance of variable size and shape in the urinary bladder of 3/10 control males, 1/10 low-dose males, 3/10 mid-dose males, and 4/10 high-dose males, identified as proteinaceous plugs (Hard et al., 1999). In more than half of treated males, full bladders were noted. Colon/intestinal distention was seen, but not measured, in 1/10 low-dose males, 1/10 mid-dose males, 2/10 high-dose males, and 3/10 high-dose females; it was not observed in controls or in low- or mid-dose females. In one high-dose male and one high-dose female, red nasal discharge and/or facial staining were/was noted. As noted earlier,

this may be attributable to the red color of the test article. Some incidental findings (e.g., small left testis and epididymis and fluid-filled uteri) were reported in both treated and control animals. No other macroscopic observations were reported including any abnormal observations in the lung (Heimbach et al., 2010).

In summary, oral gavage administration of Coffeeberry® Coffee Fruit Extract at dose levels of 0, 1000, 2000, and 4000 mg/kg bw/day for 14 days showed clinical signs in treated animals that were treatment-related but non-adverse and not clearly dose dependent. A significant decrease in mean body weights throughout the study (days 1-14) was noted in high-dose rats of both sexes. Generally, feed consumption decreased at the beginning of treatment and increased toward the end of the study in both sexes, particularly at the highest dose level. Feed efficiency followed a similar pattern. The finding from this study suggest that exposure to high doses of the extract affects feed consumption.

#### 6.2.1.2 Subchronic Toxicity Study of Coffeeberry® Coffee Fruit Extract

In order to mimic the distributed intake patterns that humans would have with the intended use of Coffeeberry® Coffee Fruit Extract (water/ethanol extract; the subject of present GRAS), the dietary route of exposure was preferred. It was also recognized that the short-term palatability issues noted in 14 day feeding studies with whole powder and water extract (described later) is unlikely to compromise the results of a longer study. Given this, the subchronic study of Coffeeberry® Coffee Fruit Extract was performed as a feeding study. The study was conducted in compliance with good laboratory practice (GLP) with the exception of the serology analysis, which was not performed under GLP by an outside laboratory. The toxicity of Coffeeberry® Coffee Fruit Extract was evaluated in Sprague-Dawley (Hsd:SD) rats (Heimbach et al., 2010) following OECD guideline 408, EPA Health Effects Test Guidelines, OPPTS 870.3100: 90-Day Oral Toxicity in Rodents, EPA 712-C-98-199, August 1998, and US FDA *Redbook 2000*, IV.C.4.a. "Subchronic Toxicity Studies with Rodents." For this study, groups of 10 rats/sex/dose were fed a diet containing Coffeeberry® Coffee Fruit Extract at levels of 0 (control), 12,500, 25,000, or 50,000 ppm (Heimbach et al., 2010).

Before the initiation of the treatment, rats (6-7 weeks old) were acclimated for six days. At the start, day 0, males and females weighed 231±4.6 g and 161±5.5 g, respectively. Rats were individually housed in suspended stainless steel cages and were maintained on Purina diet and filtered tap water *ad libitum*. Coffeeberry® Coffee Fruit Extract was thoroughly mixed into the animal feed to provide appropriate concentrations on a weekly basis and refrigerated until use. Diets were analyzed for homogeneity. Ophthalmologic evaluations were conducted prior to the commencement of the study and on day 88. Towards the end of the study period (days 86-87), functional observational battery (FOB) was performed on all rats. Blood samples were taken from the orbital sinus of fasted rats while under isoflurane anesthesia for hematology and clinical chemistry during week 13. Blood samples taken for prothrombin time and partial thromboplastin time were collected via the inferior vena cava under isoflurane anesthesia at termination. Prior to scheduled blood collections during week 13 and at termination, rats were fasted for at least 15 hours and placed in metabolism cages to collect urine. At the end of the study period, animals were euthanized and subjected to full necropsy; selected organs and tissues were preserved in 10% neutral buffered formalin or modified Davidson's fixative; and histopathological examination of preserved organs and tissues from control and high-dose groups and any gross lesions of potential toxicological significance from any test group were conducted (Heimbach et al., 2010).

The mean daily intakes of the extract fed at dietary concentrations of 0, 12,500, 25,000, and 50,000 ppm were 0, 846, 1723, and 3446 mg/kg bw/day, respectively, for males and 0, 965, 2030, and 4087 mg/kg bw/day, respectively, for females. All animals survived to scheduled necropsy. 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, 10 and overall (mid-dose group), and during weeks 4, 8, 10, 12, 13, and overall (high-dose group), suggesting an overall dose-response from days 0-91; and females showed a significant change in feed efficiency during week 1 (increased in low-dose group) and week 6 (decreased in mid-dose group).

Ophthalmoscopic examinations showed eyes to be normal. The FOB results were generally comparable to controls and any changes in quantitative measurements or in incidence of open field measurements were minimal and were not considered to indicate a toxicologically significant behavior change. Motor activity also was comparable to controls. Overall (days 0-91) and weekly mean body weights and mean daily body weight gains 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) (Heimbach et al., 2010).

Hematology, coagulation and clinical chemistry parameters did not reveal adverse changes. The only statistically significant changes reported were increased mean platelet (mid- and high-dose males), decreased eosinophil (low-dose males), decreased sorbitol dehydrogenase (mid-dose males), decreased alkaline phosphatase (high-dose males), decreased triglyceride (high-dose males), increased glucose (low-dose males and females), increased cholesterol (high-dose females), increased sodium (mid-dose females), and increased chloride (mid-dose females). These changes were considered non-adverse and not related to exposure because the magnitude of the change was considered not clinically significant and/or the change was not accompanied by any other corresponding pathological change. 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 (Heimbach et al., 2010).

No treatment related gross abnormalities were noted following macroscopic examination. Some incidental changes such as fluid-filled bladders (mostly males of all groups) and fluid-filled uteri (females of all groups) were noted. There were some statistically significant changes in absolute and relative (to body or brain weight) organ weights but none was accompanied by histopathological changes that would suggest toxicological relevancy to treatment. All reported histopathological changes were considered incidental and related to the orbital sinus bleeds or related to the age and strain of the rat used in the study. These included 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. Based on all the findings, the NOAEL for this study is the highest concentration tested, 50,000 ppm, equivalent to 3446 and 4087 mg/kg bw/day for males and females, respectively (Heimbach et al., 2010).

In summary, the results of 90-day study of Coffeeberry® Coffee Fruit Extract did not reveal consistent statistically-significant dose-dependent treatment-related adverse effects at any tested level. The NOAEL was the highest concentration tested, 5% or 50,000 ppm, equivalent to approximately 3446 and 4087 mg/kg bw/day for male and female rats, respectively (Heimbach et al., 2010). The results of this study suggest that the resulting all user maximum intake of 5.9 mg/kg bw/day from the proposed uses of Coffeeberry® Coffee Fruit Extract, the NOAEL is 584-fold lower. The findings from this study support the safe use of Coffeeberry® Coffee Fruit Extract by humans.

### 6.2.1.3 Mutagenicity Study of Coffeeberry® Coffee Fruit Extract (Ames Assay)

A study on the potential mutagenic effects of Coffeeberry® Coffee Fruit Extract (water/ethanol extract) was conducted in compliance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17 OECD, Paris, 1998), and the Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on June 20, 2002 (BGB1.I Nr. 40 SA. 2090), revised October 31, 2006 (BGB1. I Nr. 50 S. 2407). The extract was tested at concentrations of 31.6, 100, 316, 1000, 2500, and 5000 µg/plate in distilled water for potential mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA in the presence and absence of S9 liver microsomal fraction prepared from phenobarbital/ß-naphtoflavone-induced rats (Heimbach et al., 2010). The study was conducted as per OECD guideline 471, EEC Directive 2000/32, L 136, Annex 4D, B 13/14, "Mutagenicity—Reverse Mutation Test Bacteria", dated May 19, 2000, and EPA Health Effects Test Guidelines, OPPTS 870.5100 "Bacterial Reverse Mutation Assay" EPA 712-C-98-247, August 1998. Both the plate incorporation method and the pre-incubation method were performed (Ames et al., 1973a, 1973b; Maron and Ames, 1983). For each method, two independent experiments were run in triplicate for each test article (Heimbach et al., 2010).

Both negative (solvent and untreated) and positive controls were performed simultaneously. Positive controls for cultures without S9 were 4-nitro-o-phenylene-diamine (TA98 and TA1537), sodium azide (TA1535, TA100), and methyl methane sulfonate (WP2 uvrA). For cultures with S9, 2-aminoanthracene was used for all strains. For the plate incorporation method, at each concentration and bacterial strain, 100 µl test solution, negative control, or positive control was mixed in a test tube with 500 µl S9 or S9 substitution buffer (plates without metabolic activation), 100 µl bacterial suspension, and 2000 µl overlay agar. The mixture was poured over the surface of Vogel-Bonner Medium E agar plates with 2% glucose and allowed to solidify. For the pre-incubation assay, the tester strains (100 µl) were preincubated with 100 µl of test substance preparation and 500 µl of S9 or sterile buffer (plates without metabolic activation) at 37°C. After 60 minutes, 2000 µl overlay agar was added and the mixture was poured onto Vogel-Bonner Medium E agar plates with 2% glucose and allowed to solidify. In both methods, once the plates were solidified, bacteria were incubated in the dark at 37°C for at least 48 hours after which colonies were counted (Heimbach et al., 2010).

In the assay with Coffeeberry® Coffee Fruit Extract, no cytotoxicity was observed in any of the strains tested except in TA1535 at 5000 µg/plate in the second experiment. No precipitation was seen in any of the strains tested. There were no biologically relevant increases in the number of revertant colonies of any of the strains tested at any concentration with or without S9. The positive controls induced a distinct increase in the number of revertant colonies,

indicating the validity of the study. It was concluded that Coffeeberry® Coffee Fruit Extract is not genotoxic under the conditions of the experiments (Heimbach et al., 2010).

### 6.2.2. Secondary Published Studies of Other CoffeeBerry® products

In addition to the studies on Coffeeberry® Coffee Fruit Extract, extensive safety analyses have also been conducted on similar substances derived from coffee fruit, including other products in VDF FutureCeuticals' Coffeeberry® line. These studies primarily focus on coffee fruit water extract and the whole coffee fruit itself, which is the raw material used for Coffeeberry® Coffee Fruit Extract. While these substances are not identical in composition to Coffeeberry® Coffee Fruit Extract, the studies nevertheless provide further support for its safety.

#### 6.2.2.1. Animal Toxicity Studies of Coffeeberry® Whole Coffee Fruit Powder and Coffeeberry® Coffee Fruit Water Extract

The toxicity potentials of CoffeeBerry® products that are similar to the subject of the present GRAS were investigated in short-term repeat-dose toxicity studies that included a 7-day dietary/palatability study and a 14-day gavage range finding study. The physical characteristics and composition of the test articles used in these studies are compared with the subject of this GRAS document in Table 6. These studies were conducted according to 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) and consistent with OECD and Redbook Guidelines. The short-term studies were intended primarily to test palatability, dose levels and methods of administration prior to the repeat-dose long-term (subchronic) study with the subject of present GRAS.

It should be noted, the range of caffeine levels (0.6 to 9.08%) in the water/ethanol extract reported by Heimbach et al. (2008) and presented in Table 6 is considerably wider compared to the range of caffeine levels (1.0 to 2.0%) reported in Tables 3, 4, and 5. This narrower range is not the result of any change in product composition or manufacturing process; rather, since 2008, FutureCeuticals has taken steps to narrow the caffeine range by *optimizing* the manufacturing process. These steps include improved temperature controls, more precise phase separation to eliminate excess caffeine, and testing the caffeine levels of incoming coffee fruit to mitigate natural variation in caffeine content of the bean. As summarized in Table 3, 4 and 5, the caffeine content of Coffeeberry® Coffee Fruit Extract, the subject of the present GRAS assessment, is approximately 1.0-2.0%, not 0.6-9.08%.

**Table 6. Typical Characteristics of Coffeeberry® Products: Whole Powder, Water Extract, and *Water/Ethanol Extract (Coffeeberry® Coffee Fruit Extract), Tested in Toxicity Studies*****

| Characteristics | Whole Powder | Water Extract | Water/Ethanol Extract[c] |
|---|---|---|---|
| Appearance | Tan/brown powder | Brown powder | Brown powder |
| Extraction solvent | None | Water | Water/ethanol |
| Solids | ≥90% | 96% | 90% |
| Solubility in water | Partially soluble | 100% soluble | 100% soluble |
| Total phenolic acids[a] | ≥2% | 5.0% | 35-40% |
| Caffeine | 0.7-1.0% | 1.0% max. | 0.6 - 9.08% |
| ORAC[b] | 800 µmole/g average | 1500 µmole/g | 6,000 µmole/g |

[a]Chlorogenic acid, caffeic acid, quinic acid, ferulic acid; [b]Oxygen radical absorption capacity; [c]Subject of present GRAS- Coffeeberry® Coffee Fruit Extract; *Adapted from Heimbach et al. (2010)

For each repeat-dose toxicity study, animals were observed twice daily for mortality and once daily for any abnormal clinical signs. Every few days (3 times in the 7-day study and 4 times in the 14-day studies), all animals underwent a more detailed clinical examination including changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions, autonomic activity, and changes in behavior. For the 7- and 14-day studies, feed (PMI LabDiet® Purina Certified Rodent Meal #5002) and filtered tap water were provided *ad libitum*. Body weights and feed consumption were recorded regularly throughout the study and mean daily body weight gain, mean feed consumption, feed efficiency, and mean daily intakes of the test substance were calculated. At the end of the study (for studies up to 14 days), animals were euthanized by carbon dioxide asphyxiation and subjected to gross necropsy (i.e., examination of external surface of the body, all orifices, and the thoracic and abdominal cavities and their contents).

#### 6.2.2.1.1. Seven Day Dietary Exposure Study of CoffeeBerry® Whole Coffee Fruit Powder

In a short-term study, CoffeeBerry® whole coffee fruit powder was tested for palatability and toxicity in Sprague-Dawley (Hsd:SD) rats for a period of 7 days (Heimbach et al., 2010) as per OECD Guideline 407 and US FDA *Redbook 2000*, IV.C.3a. For these investigations, groups of 5 rats/sex/dose (8-week-old) were fed the powder at dietary concentrations of 0 (control), 80,000, 100,000, or 120,000 ppm. The test substance, at the appropriate concentrations, was thoroughly mixed into the animal feed at the start of the study and refrigerated until used. The mean daily intakes of whole powder fed, over the study period, at dietary concentrations of 0, 80,000, 100,000, and 120,000 ppm for male and female rats corresponded to 0, 6586, 7904, and 9055 mg/kg bw/day, and 0, 7419, 8758, and 10574 mg/kg bw/day, respectively. No mortality in any of the test groups and no treatment-related abnormal clinical findings were noted. In treated females, body weight gains and final body weights were similar to control values but treated males tended to show a dose-related decrease in body weight gain and final body weight. For example, on day 7, mean male body weights (standard deviation) were 277.6±7.44, 262.0±7.11, 260.0±5.39, and 248.0±9.51 g for 0, 80,000, 100,000, and 120,000 ppm groups, respectively. The trend toward reduced body weight gain in males was seen only in the first two days; by days 3-7, body weight gain recovered to comparable or greater levels.

Feed consumption in treated females was similar to that of controls, while treated males showed decreases in daily feed intake as compared to controls that were most notable on days 0-3. Feed efficiency in both sexes was decreased in an apparent dose-related manner compared to controls, showing greater reductions during days 0-3 but recovering on days 3-7. Gross necropsy showed no abnormal findings other than an incidental finding of red mottled tissue on the thymus of one low-dose female. These findings indicated that rats should tolerate a dietary concentration of CoffeeBerry® whole coffee fruit powder at levels up to 120,000 ppm (approximately 9055 and 10574 mg/kg bw/day for males and females, respectively) in a 14-day study. Based on this finding, it was believed that the 14-day studies of whole powder and water-extract powder at concentrations up to 100,000 ppm could be conducted as feeding rather than gavage studies without encountering palatability problems.

#### 6.2.2.1.2. Fourteen Day Feeding Studies of CoffeeBerry® Whole Coffee Fruit Powder and Coffeeberry® Coffee Fruit Water Extract

Coffeeberry® whole coffee fruit powder and water extract powder were tested for toxicity in Sprague-Dawley (Hsd:SD) rats for a period of 14 days (Heimbach et al., 2010). These

studies were designed as per OECD Guideline 407 and US FDA *Redbook 2000*, IV.C.3a. In these studies groups of 10 rats/sex/dose were fed a diet containing whole powder or water extract powder at levels of 0 (control), 25,000, 50,000, or 100,000 ppm. At the start of the experiments, rats were approximately eight weeks old and males weighed 236±7.07 g, while females weighed 178±7.10 g. Each test substance at the appropriate concentrations was thoroughly mixed into the animal feed at the start of the study and refrigerated until use.

For the whole powder study, over the period of the study, the mean daily intakes of whole powder fed at dietary concentrations of 0, 25,000, 50,000, and 100,000 ppm for male and female rats were calculated to be equivalent to 0, 2188, 4335, and 8309 mg/kg bw/day and 0, 2108, 4458, and 8858 mg/kg bw/day, respectively. No mortality was noted in any of the test groups. During the middle of the study, reduced fecal volume was noted in a couple of treated animals from each dose group. This reduction was resolved by the end of the study. As compared to the male control group, mean weekly body weights of high-dose males were significantly lower on days 3, 7, and 10 but not on day 14. In the female rat groups, body weights were similar to control values at all time points. In the male rat groups, mean daily body weight gain of mid- and high-dose groups was significantly lower as compared to controls. In females, the mid- and high-dose groups showed significant increases in mean daily body weight gain at different intervals during the study. However, these increases were considered incidental. As compared to control, a significant decrease in feed consumption was noted throughout the study (interval days 0-14) in high-dose males. Feed consumption was significantly increased during the days 7-10 and 10-14 intervals in mid- and high-dose males (Heimbach et al., 2010).

At the beginning of the study, in mid- and high-dose males fed whole powder, feed efficiency was significantly decreased, but was significantly increased in these groups in mid-study period. Feed consumption and feed efficiency in females were comparable to controls. Macroscopic examination showed no abnormal findings in rats of either sex from the control and low-dose groups and in female rats from the high-dose group, but some black speckles were noted in the lungs of 2/10 males in both the mid- and high-dose groups. Also, at the high dose, 2/10 males had somewhat reddish lungs and 5/10 males had urinary bladders containing white, semi-solid material (approximately 0.2 x 0.1 cm). The veterinary pathologist determined these changes to be proteinaceous plugs resulting from abnormal ejaculation and secretion from the male accessory sex glands during euthanasia; these plugs represent an agonal change rather than pathological lesions and are considered to be incidental findings of no toxicological significance (Hard et al., 1999). As Coffeeberry® products are bright red when fresh, although they tend to turn brown when dried, the slightly reddish lungs seen in two of the high-dose males may have resulted from inhalation of volatile color from the feed dish (Heimbach et al., 2010).

In the study with water extract powder, the mean daily intakes of the extract fed at dietary levels of 0, 25,000, 50,000, and 100,000 ppm to male and female rats were equivalent to 0, 2179, 4382, and 7889 mg/kg bw/day, and 0, 2234, 4393, and 8861 mg/kg bw/day, respectively. No mortality was noted in any of the test groups. In the majority of treated animals from each dose group, reduced fecal volume was noted during the middle of the study but was resolved by the end of the study. On day 10 of the study, hyperactivity was noted in two high-dose females. As compared to control, mean weekly body weights were significantly decreased in mid-dose males on days 3 and 7 and in high-dose males throughout the study. As compared to control, high-dose females showed a significant decrease in body weights on day 7. The decrease in mean daily body weight gain in mid- and high-dose males was significant throughout the study. All treated

females showed a significant increase in mean daily body weight gain during days 3-7 and high-dose females also showed this increase on days 7-10. These increases were considered compensatory for the decreases noted during the first week. A decrease in feed consumption was significant from controls during days 0-3 in mid-dose males and days 0-14 in high-dose males but feed consumption was significantly increased during days 7-10 and 10-14 in mid-dose males. Females generally showed no differences from controls (Heimbach et al., 2010).

In both male and female rats, feed efficiency showed a similar pattern. At necropsy, no gross abnormalities were seen in the control animals. Macroscopic examination showed black speckles in the lungs of 1/10 low-dose males and slightly reddish lungs in 2/10 mid-dose males and 1/10 high-dose males, again possibly due to the red color of the test article. Urinary bladders containing white, semi-solid material of variable, measurable size were reported in males from all dose levels (1/10 low-dose male, 5/10 mid-dose males, and 6/10 high-dose males). This material was again identified by the veterinary pathologist as proteinaceous plugs (Hard et al., 1999). At the mid-dose, 2/10 males had enlarged bladders, one of which was accompanied by the white, semi-solid material. No other macroscopic findings attributable to treatment were reported (Heimbach et al., 2010).

In summary, the findings from these feeding studies indicate that male rats tolerated less than 25,000 ppm Coffeeberry® whole powder (equivalent to 2188 mg/kg bw/day or water extract powder (equivalent to 2179 mg/kg bw/day), based on reduced feed intake, feed-conversion efficiency, and weight gain, although these effects reflect poor palatability and intolerance rather than toxicity. Female rats tolerated up to 100,000 ppm (8858 and 8861 mg/kg bw/day for whole powder and water extract, respectively).

### 6.2.2.2. Mutagenicity and Genotoxicity Studies of Other CoffeeBerry® products

These studies were conducted in compliance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17 OECD, Paris, 1998), and the Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Appendix 1 to § 19a as amended and promulgated on June 20, 2002 (BGB1.I Nr. 40 SA. 2090), revised October 31, 2006 (BGB1. I Nr. 50 S. 2407). All work undertaken by the testing laboratory was in accordance with the most recent *Guide for the Care and Use of Laboratory Animals, (*DHEW/NIH, 1996), operated under the surveillance of the Regierung von Oberbayern (German regulatory authority) according to AAALAC standards and accreditation.

#### 6.2.2.2.1. Ames Assay

All three Coffeeberry® products—the whole powder, the water extract, and the water/ethanol extract (Coffeeberry® Coffee Fruit Powder)—were tested at concentrations of 31.6, 100, 316, 1000, 2500, and 5000 µg/plate in distilled water for potential mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA in the presence and absence of S9 liver microsomal fraction prepared from phenobarbital/ß-naphtoflavone-induced rats (Heimbach et al., 2010). Additional details related to the assay, including regulatory guidelines followed and the methods are similar to those described in Section section 6.2.3.1.

In the assay with whole powder, no cytotoxicity to *S. typhimurium* (strains TA98, TA100, TA1535, and TA1537) or *E. coli* (strain WP2 uvrA) in the presence or absence of S9 at the concentrations tested was observed except for *S. typhimurium* strain 1537, which showed toxic

effects at a concentration of 5000 µg/plate without S9 in the first experiment and at concentrations of 316 µg/plate and higher without S9 in the second experiment. Precipitation was observed in all strains at concentrations of 100 µg/plate and higher with S9 and 316 µg/plate and higher without S9 in the first experiment and at concentrations of 316 µg/plate and higher with and without S9 in the second experiment. There were no biologically relevant increases in the number of revertant colonies of any of the strains tested at any concentration with or without S9. The positive controls induced a distinct increase in the number of revertant colonies, indicating the validity of the study. It is concluded that the whole powder is not genotoxic under the conditions of the experiment (Heimbach et al., 2010).

In the assay with water extract powder, no cytotoxicity was observed in any of the strains tested. Precipitation was noted in all strains at concentrations of 1000 µg/plate with or without S9 in the first experiment and at concentrations of 316 µg/plate with or without S9 in the second experiment. There were no biologically relevant increases in the number of revertant colonies of any of the strains tested at any concentration with or without S9. The positive controls induced a distinct increase in the number of revertant colonies, indicating the validity of the study. It is concluded that the water extract powder is not genotoxic under the conditions of the experiment (Heimbach et al., 2010).

In the assay with water/ethanol extract, no cytotoxicity was observed in any of the strains tested except in TA1535 at 5000 µg/plate in the second experiment. No precipitation was seen in any of the strains tested. There were no biologically relevant increases in the number of revertant colonies of any of the strains tested at any concentration with or without S9. The positive controls induced a distinct increase in the number of revertant colonies, indicating the validity of the study. It is concluded that the water/ethanol extract (subject of present GRAS assessment) powder is not genotoxic under the conditions of the experiment (Heimbach et al., 2010).

#### 6.2.2.2.2. *In vivo* Micronucleus Test with Coffeeberry® Whole Coffee Fruit Powder

The potential genotoxicity of Coffeeberry® whole coffee fruit powder was investigated in the micronucleus test using peripheral blood cells of NMRI mice following accepted guidelines and recommendations (Heimbach et al., 2010). In this study, male and female NMRL mice aged 7-12 weeks were used. The whole powder was extracted in 0.9% NaCl for 1 hour at 37±1°C in an ultrasonic bath with a mass/volume ratio of 0.2 g/ml and prior to administration the extract was filtered using folded paper filters. The extraction process was used because of technical issues including insolubility and bacterial contamination of the test item. Based on the results of a preliminary toxicity study, the maximum tolerable dose was determined to be 10 ml/kg bw of a 100% extract concentration. Mice (5 mice/sex/dose) were intraperitoneally injected with this dose, while negative (0.9% NaCl) and positive (cyclophosphamide) controls (5/sex/group) were run simultaneously.

Following administration of the samples, blood was collected from the tail vein at 44 and 68 hours and blood cells were immediately fixed in ultracold methanol for at least 16 hours. Prior to analysis, fixed cells were washed in Hank's balanced salt solution, centrifuged at 600 x g for 5 minutes and the supernatant was discarded. Blood cell populations were discriminated using specific antibodies against CD71 (expressed only at the surface of immature erythrocytes) and CD61 (expressed at the surface of platelets) and the DNA content of micronuclei was determined by the use of DNA-specific stain (propidium iodide). A flow cytometer was used to evaluate all samples. Anti-CD71 and anti-CD61 antibodies were labeled with fluorescein

isothiocyanate and phycoerythrin, respectively. Particles were differentiated using forward scatter and side scatter parameters of the flow cytometer. A minimum of 10,000 immature erythrocytes per mouse was examined for the incidence of micronucleated immature erythrocytes and the ratio between immature and mature erythrocytes was determined and expressed as relative PCE (proportion of polychromatic erythrocytes among total erythrocytes). A finding was considered positive if there was a dose-related increase in the number of micronucleated cells and/or a biologically relevant increase in the number of micronucleated cells for at least one dose group.

Four hours following injection of the NaCl extract of whole powder, mice showed reduction of spontaneous activity, cramps, rough fur, and prone position. These signs cleared by 44 hours (time of first blood sampling). There was no dose-related increase in the number of micronucleated cells and all mean values were within the range of the historical control data of the negative control. Statistical analysis ($p<0.05$) verified these results. The study fulfilled the validity criteria. The investigators concluded that under the experimental conditions tested, the whole powder did not induce structural or numerical chromosomal damage in the immature erythrocytes of the mouse, and thus the test article is considered to be non-mutagenic with respect to clastogenicity and aneugenicity.

### 6.2.3. Human Studies

#### 6.2.3.1. Human Studies with Coffeeberry® Coffee Fruit Extract

##### 6.2.3.1.1. Reyes-Izquierdo et al. (2013a)

In a single-dose study, Reyes-Izquierdo et al. (2013a) investigated the effect of Coffeeberry® Coffee Fruit Extract, green coffee caffeine powder, grape seed extract powder and green coffee bean extract powder on blood levels of brain-derived neurotrophic factor (BDNF). In this study, randomly assorted groups of fasted subjects consumed a single, 100 mg dose of each material. Plasma samples were collected at time zero and at 30 min intervals afterwards, up to 120 min. A total of two control groups were included: subjects treated with silica dioxide (as placebo) or with no treatment. The inclusion criteria required participants to be between the ages of 18 and 55 years and have a BMI between 18 and 25 $kg/m^2$. The collected data revealed that treatments with green coffee caffeine powder and grape seed extract powder increased levels of plasma BDNF by about 31% under these experimental conditions, whereas treatment with whole coffee fruit concentrate powder increased it by 143% (n=10), compared with baseline. The investigators suggested that the results of this study indicate that whole coffee fruit concentrate powder could be used for modulation of BDNF-dependent health conditions. However, larger clinical studies are required to support this possibility.

Reyes-Izquierdo et al. (2013a) also performed a single dose, placebo-controlled, within-subject study to confirm and further investigate the effect noted in the above study. This experiment was performed to verify the reproducibility of the effect of whole coffee fruit concentrate powder. In this subsequent study, 20 healthy subjects with ages ranging from 25 to 35 participated. All subjects fasted and resting subjects received placebo on day 1, whole coffee fruit concentrate powder on day 2, and a cup of freshly brewed coffee on day 3. Treatment with whole coffee fruit concentrate powder resulted in a statistically significant increase in plasma BDNF compared to placebo ($p=0.0073$) or coffee ($p=0.0219$) during first 60 minutes. Furthermore, oral whole coffee fruit concentrate powder consumption acutely increased BDNF levels in serum. The

investigators suggested that available results justify further clinical investigation of whole coffee fruit concentrate powder as a tool to manage BDNF-dependent health conditions.

### 6.2.3.2. Human Studies with Other Coffeeberry® Products

#### 6.2.3.2.1. Ostojic et al. (2008)

In a 4-week prospective, randomized, double-blind, placebo-controlled trial, Ostojic et al. (2008) investigated the changes in total antioxidant capacity and aerobic and anaerobic performance induced by supplementation of whole coffee fruit powder in college athletes. In this study, 20 healthy college athletes (14 males and 6 females, mean age = 22.1±2.7 years; mean body weight = 74.1±15.0 kg; mean fat mass = 15.6±7.0; mean basal metabolic rate = 1847±378 kcal) ingested one tablet containing 400 mg of whole coffee fruit powder (n=10) or cellulose placebo (n=10) twice a day for 28 days. All subjects were instructed to consume a standardized diet four weeks prior to whole coffee fruit powder ingestion and seven days prior to baseline testing. Baseline testing of all the parameters studied was conducted. At the end of 28-day exposure period, blood was drawn from an antecubital vein and analyzed for total antioxidant capacity based on chemiluminescence, glucose, triacylglycerol, lipoproteins, and total cholesterol. After the blood draw, the participants completed a warm-up followed by a 60-second vertical jump test and a shuttle-run, at the end of which heart rate and blood lactate levels were measured.

No significant differences between the experimental and control groups in plasma glucose, triacylglycerol, high- or low-density lipoprotein, or total cholesterol was noted. However, antioxidant capacity of the group receiving whole coffee fruit powder was significantly higher at the end of the treatment period than in the pretest as well as significantly higher than that of the controls. Following exercise, the treated group showed significantly quicker heart recovery rate (a reduction during the first minute post-exercise of 38±4 beats/min versus 32±5 beats/min from the maximum rate observed during exercise) and lower levels of blood lactate (5.5±2.6 mmol/l versus 8.5±3.0 mmol/l) as compared to placebo controls. No adverse effects were reported following ingestion of 800 mg/day of whole coffee fruit powder or approximately 10.8 mg/kg bw/day for 28 days. The investigators concluded that ingestion of whole coffee fruit powder did not significantly affect endurance or anaerobic performance indicators of college athletes but improved total antioxidant capacity and "does not induce any acute adverse effects."

### 6.2.4. Other Studies with Coffee Fruit

Besides the above described specific studies of Coffeeberry® products, very few pertinent studies were found in the published literature. In a series of published articles from 1995 to 2001, anticarcinogenic effects of the coffee cherry with the bean removed for coffee production, focusing on spontaneous mammary tumors in a high-mammary-tumor strain of SHN/Mei virgin mice, were investigated. The test article in all these studies (with one exception) was prepared by repeated extraction of dried coffee cherry with hot water. The supernatant from these extractions was pooled, dried *in vacuo*, and dissolved in tap water to provide a final test concentration of 0.5% of extracted coffee cherry which was used as the single test dose. In these studies, control mice received plain tap water. The mice were housed 4-5 animals/cage in Teflon cages with wood shavings, and with feed and water available *ad libitum*. Unless specifically stated, it was not clear in the reported studies whether treated animals received the 0.5% water extract in lieu of untreated tap water (i.e., *ad libitum*) or if a specific volume of the 0.5% water extract was administered to the mice. However, based on the

descriptions provided in the later studies by the same investigators, it was assumed that, for all the studies, the 0.5% water extract of coffee cherry replaced the drinking water in treated groups.

#### 6.2.4.1. Nagasawa et al. (1995)

In this study, Nagasawa et al. (1995) reported that 2-month-old mice (n=24) ingesting the 0.5% water extract of coffee cherry (amount not stated) for a period up to 12 months showed a statistically significant reduction in the development of spontaneous mammary tumors compared with control mice (n=18) receiving untreated tap water. Body weight was recorded and feed and water consumption were estimated (over a 3-day period) at the start of treatment and monthly thereafter for seven months (no explanation was provided as to why body weights were not recorded to the end of the study). Urine was collected at 2 and 5 months and analyzed using a spectrometer. In addition, vaginal smears were taken daily from 5 test mice and 6 controls for 30 days at 1-2 months treatment to assess estrous cycle.

In this study, mice were palpated once a week for mammary tumors until first tumor appearance or until the end of the treatment period. When a tumor was identified, mice were killed by decapitation under light anesthesia. Blood was collected from the trunk and serum was analyzed for free fatty acid and prolactin levels. In surviving mice, blood was collected at eight months for determination of glucose level. At necropsy, the bilateral third thoracic glands were examined for normal and preneoplastic mammary gland growth; the bilateral inguinal glands were removed and prepared to determine the activities of thymidylate synthetase and thymidine kinase; anterior pituitary, adrenals, and ovaries were removed and weighed; adrenals and ovaries were examined histologically; and the unilateral uterine horns were removed and histologically examined for adenomyosis (Nagasawa et al., 1995).

Appearance of tumors were first noted in controls at around four months and in test mice at six months. The cumulative incidence of tumors was significantly lower in test mice as compared to controls. However, the number of tumors per mouse (1-2) was similar in test and control groups. Areas of normal and preneoplastic mammary glands were significantly smaller in test mice as compared to controls. In tumor-bearing mice, serum free fatty acid levels were significantly lower in treated mice than controls. After two months of treatment, body weights were significantly lower in test mice compared to controls, but feed intake did not differ between groups. Water intake was significantly reduced in test mice compared to controls. Some urinary components were significantly higher in test mice as compared to controls: urea, allantoin, and creatinine at 2 and 5 months; taurine and betaine at two months; and citric acid at five months. Endocrine organ weights and histology, estrous cycle, blood glucose level, uterine adenomyosis, activities of thymidylate synthetase and thymidine kinase, and serum prolactin level did not differ between test and control groups. Although data was not provided, visual examination of a graph presented in the published article led to an estimate of water intake of about 4.5 ml/mouse/day, resulting in an estimated intake of coffee cherry extract of about 22.5 mg/mouse/day or 900 mg/kg bw/day assuming a mouse weighs 25 g (Nagasawa et al., 1995).

#### 6.2.4.2. Nagasawa et al. (1996a)

In another study by the same investigators, Nagasawa et al. (1996a) reported that mice that were "retired after the 2nd or 3rd lactation" (this was the only indication of age reported) were palpated once a week for mammary tumors until first tumor appearance. After tumors developed, half of the tumor-bearing mice were assigned to receive the 0.5% water extract of

coffee cherry (n=13; details of amount administered not stated) and the remainder received plain water (n=9). On the 10th day of the treatment, mice were euthanized by decapitation under light anesthesia and the number and size of tumors were recorded. Body weights were recorded at the start and end of treatment. As in the previous study, blood was collected from the trunk and serum was analyzed for free fatty acid levels. At necropsy, the bilateral third thoracic glands were examined for normal and preneoplastic mammary gland growth; the portions of mammary tumors with no necrosis were removed and prepared to determine the activities of thymidylate synthetase and thymidine kinase; anterior pituitary, adrenals, lung, and ovaries were removed and weighed; and adrenals, lung, and ovaries were examined histologically. Both growth in the palpable size of the tumors and the activity of thymidylate synthetase in the tumors were significantly reduced by ingestion of coffee-cherry extract. It was reported that normal and preneoplastic mammary gland growth, serum free fatty acid, change in body weight, and endocrine organ weight and histopathology were not significantly affected. No metastasis to the lung was noted (Nagasawa et al., 1996a).

#### 6.2.4.3. Nagasawa et al. (1996b)

Nagasawa et al. (1996b) also tested a methanol extract of coffee cherry instead of the water extract for potential effects on spontaneous tumorigenesis in SHN mice. The methanol extract was prepared by repeated extraction of dried coffee cherry with 60% methanol, then the supernatants were pooled and dried *in vacuo*. The water-soluble fraction of dry matter was dissolved in tap water to provide a final concentration of 0.25% methanol-soluble extract of coffee cherry. The water-insoluble fraction was prepared as a 2.0% fat emulsion and then diluted to produce a final concentration of 0.25% methanol-insoluble extract of coffee cherry. Two-month-old SHN mice were given 0.25% methanol soluble extract in their drinking water until all controls (mice receiving untreated tap water concurrently) developed mammary tumors. The number of animals in the study was not clearly reported; different numbers of animals ranging from 5 to 20 appeared in the various results tables. Tumor-bearing mice were killed one week after first tumor appearance. According to the results tables, treatment continued for up to five months, although "some" control and treated mice that did not develop tumors were terminated after two months of treatment. Both the soluble and insoluble methanol extracts were given for 10 days to groups of multifarious retired mice (age and number not specified) with palpable mammary tumors. Mice were palpated weekly for mammary tumors and tumor size was recorded on days 0 (the day prior to start of treatment), 3, 6, and 10. Feed and water consumption were estimated over a 3-day period at the start of treatment and monthly thereafter (Nagasawa et al., 1996b).

Urine was collected (schedule not stated; taken only from non-tumor-bearing mice terminated at two months) and analyzed using a spectrometer. In addition, vaginal smears were taken daily from treated and control mice (number not stated) for 30 days after one month of treatment to assess estrous cycle. Prior to necropsy, blood was collected from the trunk and serum was analyzed for free fatty acid and prolactin levels. At necropsy, the bilateral third thoracic glands were examined for normal and preneoplastic mammary gland growth; the bilateral inguinal glands were removed and prepared to determine the activities of thymidylate synthetase and thymidine kinase; anterior pituitary, adrenals, ovaries, kidney, pancreas, liver, thymus, and/or spleen were removed and weighed; adrenals, uterus, and ovaries were examined histologically; and thymus, spleen, peripheral blood, and natural killer cells in the spleen were

prepared to determine surface antigenic markers (only in non-tumor-bearing mice terminated at two months) (Nagasawa et al., 1996b).

In mice given the methanol soluble extract, time-to-tumor was similar to that of controls (four months) but the cumulative incidence of tumors was significantly lower in treated mice compared to controls. Tumor growth was similar between treated and control mice. In mice with established tumors, the activity of thymidine kinase, but not thymidylate synthetase, was significantly lower in treated mice. Water intake was significantly lower in treated mice than controls, which was considered to be due to the bad taste of the extract. The estrous cycle and serum prolactin and free fatty acid levels did not differ between groups. The only organ weight changes noted in mice receiving the methanol soluble extract was a significant increase in ovary weight in non-tumor-bearing animals terminated after two months of treatment compared to corresponding controls and a significant increase in adrenal weight in mice treated for 10 days (Nagasawa et al., 1996b).

Ingestion of the methanol soluble extract showed no effect as assessed by histological examination. Normal and preneoplastic growth was similar between treated and control groups. Treated mice showed a significant increase in urine hippurate, creatinine, and citrate, but not urea, allantoin, creatine, taurine, betaine, oxoglutarate, acetate, or lactate when compared to controls. A significant increase compared to controls was observed in the percentages of thymocytes expressing helper/inducer ($CD4^+8^-$), cytotoxic/suppressor ($CD4^-8^+$) or pre-T ($CD4^-8^-$) phenotypes with a concomitant significant decrease in immature ($CD4^+8^+$) thymocytes. A significant decrease compared to controls was observed in the percentages of splenic lymphocytes expressing helper/inducer ($CD4^+8^-$) or cytotoxic/suppressor ($CD4^-8^+$). Natural killer cell activity in the spleen showed no difference from controls. In mice receiving methanol insoluble extract (testing was limited due to the small amount of the test material), tumor growth, thymidylate synthetase and thymidine kinase activities, normal and preneoplastic mammary gland growth, pituitary gland and ovary weights, and adrenal and ovary histology were similar to those of controls. Adrenal weight was significantly lower than that of controls. Water intake of the mice treated with the soluble extract was estimated (based on inspection of a graph) to be about 4.5 ml/mouse/day, resulting in an estimated intake of coffee cherry extract of about 22.5 mg/mouse/day or 900 mg/kg bw/day, assuming a mouse weighs 25 g (Nagasawa et al., 1996b).

#### 6.2.4.4. Kobayashi et al. (1996)

In an attempt to examine the possible immunomodulating effect of coffee cherry as a part of the mechanism for suppressing mammary tumors in virgin SHN mice, Kobayashi et al. (1996) treated 2-month-old mice (n=12) with 0.5% water extract of coffee cherry (amount not stated). Controls (n=11) received tap water only. After two months of treatment, mice were killed by decapitation under light anesthesia. At necropsy, blood was collected from the trunk. Thymus and spleen were removed and weighed and cell suspensions were prepared. Thymic cells were incubated with phycoerythrin (PE)-conjugated rat anti-mouse CD4 IgG and fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD8a IgG monoclonal antibody. Splenic and peripheral blood lymphocytes were stained with PE-conjugated rat anti-mouse CD25 IgG and FITC-conjugated hamster anti-mouse $CD3_E$ monoclonal antibody. After washing, all treated cells were analyzed by flow cytometer. To assess natural killer activity, splenic cells also were prepared to determine cytotoxicity against YAC-1 lymphoma cells in an enzyme-release assay and to measure released lactate dehydrogenase by a Cyto Tox 96 non-radioactive cytotoxicity assay kit.

As compared to control, the spleen weights were significantly higher in treated mice, although there were no changes in natural killer activity of splenic cells. A significant increase compared to controls was observed in the percentages of cells expressing helper/inducer ($CD4^+8^-$) or pre-T ($CD4^-8^-$) phenotypes with a concomitant significant decrease in immature ($CD4^+8^+$) thymocytes. The increase reported in cytotoxic/suppressor ($CD4^-8^+$) phenotypes did not reach statistical significance. CD25 expression showed no differences between treated and control groups (Kobayashi et al., 1996).

**6.2.4.5. Kobayashi et al. (1997)**

In another study, Kobayashi et al. (1997) investigated the effects of coffee cherry on the activation of splenic lymphocytes in SHN mice. For these studies, two different extracts were prepared: (1) the 0.5% water extract and (2) the 0.25% methanol soluble extract, both previously described (see above). Two-month-old mice (number not reported) received either 0.5% water extract or 0.25% methanol soluble extract as drinking water for three weeks. A group of control mice received plain tap water. At the end of study period, mice were euthanized by decapitation under light anesthesia. At necropsy, the spleen was removed and weighed, and cell suspensions were prepared. Splenic lymphocytes were stained with PE-conjugated rat anti-mouse CD25 IgG and FITC-conjugated rat anti-mouse CD45R/B220 monoclonal antibodies. After washing, all treated cells were analyzed by flow cytometer. To assess mitogenic activity, splenic lymphocyte suspensions were incubated in 96-well plates with concanavalin A (a lymphocyte mitogen) or *E. coli*-lipopolysaccharide for 48 hours at 37°C in 5% $CO_2$. Alamarblue solution was added and the cells were further incubated for four hours after which the optimal density of each well was measured using a micro plate reader (Kobayashi et al., 1997).

As compared to control, no changes in spleen weights were noted in the treated group. The lymphocyte response to mitogen exposure was not affected by either of the coffee cherry extracts, but the lipopolysaccharide response was significantly enhanced with increased percentages of CD45R/B220+ cells in the splenic lymphocytes and CD25+ cells in B-lymphocytes. Water intake was estimated to be 4.5 and 2.4 g/mouse/day for the water and methanol soluble extract, respectively, resulting in an intake of coffee cherry extract of about 22.5 and 6 mg/mouse/day or 900 and 240 g/kg bw/day, respectively, assuming a mouse weighs 25 g (Kobayashi et al., 1997).

**6.2.4.6. Nasagawa et al. (1999)**

In yet another study, Nasagawa et al. (1999) investigated the effects of ingestion of the 0.5% water extract of coffee cherry with or without simultaneous treatment with hydroxyapatite. In this study, two-month-old control mice (n=40) were given feed pellets formulated with 5% calcium carbonate while 2-month-old treated mice (n=30) were given feed pellets formulated with 5% hydroxyapatite. Both groups received untreated tap water for 3 months and then treated mice were switched to the 0.5% water extract of coffee cherry. The feed pellets, plain tap water (controls), and treated water containing 0.5% extract of coffee cherry were provided *ad libitum* to the mice. Mice were palpated weekly and treatment continued until a week after the appearance of mammary tumors or, in the absence of tumor formation, for nine months. To assess tumor growth, "some" tumor-bearing mice were continued on the treatment regime for an additional 12 days after the mean tumor size reached 5-6 mm in diameter. Feed and water intake were estimated over 5 consecutive days (time within study not specified) and body weights were recorded monthly. At necropsy, the bilateral third thoracic glands were examined for normal and

preneoplastic mammary gland growth. The unilateral uterine horns were removed and histologically examined for adenomyosis; and anterior pituitary, adrenals, and ovaries were removed and weighed. At seven months, blood was collected from 10 fasted mice/group at intervals of 30, 60, and 120 minutes following intraperitoneal injection of glucose for determination of glucose level. Also, at seven months, urine was collected and urine components were determined by spectrometer (Nagasawa et al., 1999).

Feed intake in both controls and treated groups decreased for the first six months, then stabilized. After about four months of treatment, water intake was significantly higher in treated mice as compared to controls. Body weight changes were similar to those of control mice for the first five months, but then treated mice showed a significant increase compared to controls until the end of the study. The time-to-tumor and incidence of mammary tumors were significantly lower in treated mice compared to controls at each month from 2-8 months of treatment. Ovarian weights were significantly greater than those of controls. Most of the urinary components (hippurate, allantoin, creatinine, creatine, taurine, betaine, acetate, and lactic acid) showed no difference between treated and control animals. However, urea was significantly higher in treated mice whereas citrate was significantly lower in treated mice. Mammary tumor growth, normal and preneoplastic mammary gland growth, uterine adenomyosis, glucose tolerance, and anterior pituitary and adrenal weights did not differ between treated and control groups. It was noted by the study authors that co-administration of hydroxyapatite with water extract of coffee cherry did not enhance any of the studied effects but actually showed a reduction in any reported effects. Water intake was estimated to be about 7.5 ml/mouse/day (based on graph in the published paper) resulting in an estimated intake of coffee cherry extract of about 37.5 mg/mouse/day or 1340 mg/kg bw/day, assuming an average body weight of 28 g (also estimated by visual inspection of a graph) (Nagasawa et al., 1999).

### 6.2.4.7. Udagawa and Nagasawa (2000)

In another study, Udagawa and Nagasawa (2000) investigated the effects of concurrent treatment with 0.5% water extract of coffee cherry and whole-body hyperthermia on the growth of spontaneous mammary tumors in SHN mice. In this study, three-month-old mice were palpated for mammary tumors twice a week until tumors reached a diameter of 5-7 mm. At this time, tumor-bearing mice were divided into treated (n=21) and control (n=20) groups. The drinking water of treated animals was switched from plain tap water to 0.5% water extract of coffee cherry, while controls were maintained on untreated tap water. Approximately half of the mice from each group (n=11 for treated; n=10 for control) were weighed and then exposed to whole-body hyperthermia (room temperature was maintained at 37-42°C for this session) for 3 hours/day for 5 consecutive days. Mammary tumor growth was measured daily. Five days after the last whole-body hyperthermia treatment, mice were weighed and killed by decapitation under light anesthesia. Prior to necropsy, mice were fasted for 18 hours and blood was collected from the trunk for determination of plasma components. At necropsy, the bilateral third thoracic glands were examined for normal and preneoplastic mammary gland growth; and anterior pituitary, adrenals, and ovaries were removed and weighed.

In mice receiving 0.5% water extract of coffee cherry, mammary tumor growth was significantly lower regardless of exposure to whole-body hyperthermia. Treated mice with or without exposure to whole-body hyperthermia had significantly higher body weights than corresponding controls. Ovarian weight was significantly higher in treated mice exposed to whole-body hyperthermia compared to corresponding controls, while all other organ weights

were similar to controls. Normal and preneoplastic mammary gland growth did not differ between treated and control groups with the exception of a significant increase in the number of hyperplastic alveolar nodules in treated mice exposed to whole-body hyperthermia compared to corresponding controls. Plasma component levels were similar between treated and control groups with the exception of albumin, which was significantly lower in treated mice not exposed to whole-body hyperthermia compared to corresponding controls (Udagawa and Nagasawa, 2000).

**6.2.4.8. Nagasawa et al. (2001)**

In the most recent study from this group that involves measurements of some safety parameters, Nagasawa et al. (2001) administered the 0.5% water extract of coffee cherry or plain tap water to 2-month-old female SHN mice (number of animals not reported) for 60 days. At the start of treatment, just after motor skills testing, and prior to necropsy, body weights were recorded. Feed and water intake were estimated over three consecutive days starting after one week of treatment (details of schedule not mentioned). After 30 and 60 days of treatment, blood and urine were collected for plasma component and urine component levels, and spontaneous motor activity was assessed by a sensor monitor mounted above the cage to detect body heat. Both urinalysis and plasma measures included the following: albumin, alkaline phosphatase, alanine aminotransferase, amylase, aspartate aminotransferase, globulin, total bilirubin, urea nitrogen, calcium, cholesterol, creatinine, glucose, and total protein. At necropsy, the bilateral third thoracic glands were examined for normal and preneoplastic mammary gland growth; and anterior pituitary, adrenals, ovaries, thymus, spleen, heart, lung, pancreas, liver, and kidneys were removed and weighed.

Water intake was estimated to be 5.5-6.5 g/mouse/day resulting in an intake of coffee cherry extract of about 27.5-32.5 mg/mouse/day or 1100-1300 mg/kg bw/day, assuming a mouse weighs 25 g [estimated from body weight data reported in Nagasawa et al. (1995; 1996b; 1999) and Kobayashi et al. (1997)]. At 30 days of treatment, body weight in treated mice was significantly lower than that of controls. However, at day 60, body weight was similar between treated and control groups. Spontaneous motor activity and feed and water intake were similar between treated and control groups. In the plasma of treated mice, when compared to control values, alanine aminotransferase, aspartate, aminotransferase, and blood urea nitrogen levels were significantly lower at 60 days; cholesterol levels were significantly lower at 30 days but significantly higher at 60 days; amylase levels were significantly lower at 30 days but not at 60 days; and creatinine levels were significantly higher at 30 days but not at 60 days. In the urine of treated mice, when compared with control values, alanine aminotransferase, aspartate aminotransferase, and glucose levels were significantly lower at 60 days. All other plasma and urine parameters were similar to those of controls. The only significant difference reported in organ weight was a significant increase in the weight of the pancreas. Normal and preneoplastic mammary gland growth did not differ between treated and control groups with the exception of a significant decrease in the number of hyperplastic alveolar nodules in treated mice (about one-third that of controls).

In summary, none of the above described studies were designed to investigate the toxicity of ingestion of coffee-cherry extracts. However, these studies did not reveal any adverse effects at the tested concentrations of 0.25% and 0.5% of the coffee cherry extracts, equivalent to approximately 900-1340 mg coffee cherry extract/kg bw/day.

### 6.2.5. Safety of Chlorogenic Acids

Among the phenolic acids (≥40%) found in Coffeeberry® Coffee Fruit Extract, chlorogenic acid has been reported as a major component present at levels up to 40%. Chlorogenic acids are a family of esters formed between certain cinnamic acids (such as caffeic, *p*-coumaric, and ferulic acids) and quinic acid. The term "chlorogenic acid" encompasses at least 5 subclasses including caffeoylquinnic acid, dicaffeoylquinic acids, feruloylquinic acids, *p*-coumaroylquinic acid, and caffeoylferuloylquinic acid, with each subclass containing at least 3 isomers (Monteiro et al., 2007).

The available information shows that chlorogenic acids are found naturally in fruits, leaves, and other tissues of many dicotyledonous plant species (NTP, 1998). Standard coffee products typically contain 70 to 350 mg of chlorogenic acids per serving (Clifford, 1999). In addition to its presence in coffee beans, chlorogenic acid is found at high concentrations in a number of fruits and vegetables (Gonthier et al., 2003). The daily intake of chlorogenic acid among coffee drinkers has been reported to vary from 500 to 1000 mg, whereas coffee abstainers consume approximately <100 mg of chlorogenic acid/day (Olthof et al., 2001). Based on the concentration of chlorogenic acid (40%) and the 90$^{th}$ percentile intake of Coffeeberry® Coffee Fruit Extract of 393 mg/person/day, the resulting maximum intake of chlorogenic acid from the proposed uses of Coffeeberry® Coffee Fruit Extract is estimated as 157mg/person/day.

#### 6.2.5.1. Metabolic Fate of Chlorogenic Acids

The absorption, distribution, metabolism and excretion of chlorogenic acids has been extensively studied in animals and human subjects. The available studies in animals suggest that small amounts of ingested chlorogenic acids are absorbed intact from the stomach and small intestines, while the majority is transported intact to the lower gastrointestinal tract (Azuma et al., 2000; Gonthier et al., 2003; Lafay et al., 2006a,b; Ren et al., 2007). Upon reaching the colon, chlorogenic acids are hydrolyzed to caffeic acid and quinic acid that are further metabolized through common metabolic pathways mediated by the intestinal microflora. Quinic acid is converted to benzoic acid, which can be further metabolized in the liver by conjugation reaction with glycine to produce hippuric acid. Gonthier et al. (2003) reported that approximately 60% of an ingested dose of chlorogenic acid is recovered as metabolites in the urine of rats, with hippuric acid being the predominant metabolite accounting for 36.5% of the ingested dose.

The available studies in humans also indicate that approximately 70% of an ingested dose of chlorogenic acid is transported to the colon intact (Olthof et al., 2001; Farah et al., 2008). Only small amounts (less than 2% of an administered dose) of intact chlorogenic acid have been recovered in the urine unchanged following oral ingestion. Olthof et al. (2003) reported that humans have a high metabolic capacity for chlorogenic acid. No evidence of saturation of metabolic pathways was noted at doses up to 2 g/day as indicated by the small amount of intact chlorogenic acid recovered in the urine even at this high dose. The urinary metabolites identified in humans were derivatives of caffeic acid, ferulic acid and quinic acid, as well as their glucuronidated and sulfated forms (Rechner et al., 2001; Olthof et al., 2003, Monteiro et al., 2007; Farah et al., 2008; Stalmach et al., 2009, 2010). Some of these metabolites are likely to be formed through metabolism mediated by the colonic microflora.

### 6.2.5.2. Toxicity Studies of Chlorogenic Acids

Chlorogenic acid is of low acute toxicity. In a study in female Wistar rats, no mortality or morbidity were observed following administration of chlorogenic acid *via* intraperitoneal route at a dose of 2437 mg/kg bw (Chaube and Swinyard, 1976). In short-term oral toxicity studies, chlorogenic acid was without toxicologically relevant adverse effects when supplemented to the diets of rats at 1%, corresponding to approximately 1000 mg/kg bw/day, for 3 weeks (Eklund, 1975). In another study, female BALB/C mice fed diets supplemented with 0.2% chlorogenic acid, corresponding to intakes at approximately 300 mg/kg bw/day, for 10 weeks did not reveal any significant adverse effects on total body weight, as well as liver and intestinal weights (Kitts and Wijewickreme, 1994). There is limited data pertaining to developmental/reproductive toxicity of chlorogenic acids. Intraperitoneal administration of chlorogenic acid at doses up to 500 mg/kg bw/day to rats during gestational days 5 to 12 was without toxicologically relevant effects in the dams or offsprings (Chaube and Swinyard, 1976).

In various genotoxicity/mutagenicity studies, conducted *in vitro*, both positive and negative results have been obtained. However, the positive results occurred under experimental conditions that were deemed not physiologically relevant, and as such, these findings were not considered to be of concern with respect to human health. Findings from an *in vivo* micronucleus test conducted in mice provided further evidence that chlorogenic acid was not genotoxic/mutagenic. Moreover, several *in vitro* and *in vivo* studies have suggested that chlorogenic acid may in fact possess anti-mutagenic and anti-genotoxic properties. Mori et al. (1986) reported that supplementation of chlorogenic acid to the diets of Syrian golden hamsters at doses providing approximately 30 mg/kg bw/day for 24 weeks did not induce any neoplasms or hyperplastic lesions in the liver, small intestines, or large intestines.

Among the metabolites of chlorogenic acid, only caffeic acid is considered to be of toxicological relevance. Hirose et al. (1987) reported evidence of hyperplasia in the forestomach epithelium following supplementation of caffeic acid to the diets of rats at 2% (corresponding to approximately 2000 mg/kg bw/day) for 28 days. It is notable that in this study, chlorogenic acid did not produce forestomach hyperplasia (Hirose et al., 1987). In a long-term study, Hagiwara et al. (1991) reported hyperplasia and squamous-cell papillomas and carcinomas in the forestomach of rats and mice following chronic supplementation of caffeic acid in the diet for durations of up to 96 to 104 weeks. In this same study, some evidence of renal tubular-cell hyperplasia and adenomas were also observed in the rodents (Hagiwara et al., 1991). However, these findings are of limited relevance as the dose of caffeic acid used (1000 to 2000 mg/kg bw/day) are vastly higher than levels that would be expected from the exposure to Coffeeberry® Coffee Fruit Extract. The relevance of forestomach tumors in rodents to human carcinogenesis is also unclear. Furthermore, both chlorogenic acid and caffeic acid have been reported to have anti-carcinogenic effects in animal studies (Lesca, 1983; Huang et al., 1988; Steele et al., 1994; NTP, 1998; Park et al., 2010).

In summary, the available information suggests that chlorogenic acid present in Coffeeberry® Coffee Fruit Extract is unlikely to cause any adverse effects.

### 6.2.6. Corroborative Information

#### 6.2.6.1. Evaluation by Health Canada

In 2016, Health Canada's Food Directorate critically assessed FutureCeuticals' Novel Food notification on Coffeeberry® whole coffee fruit derivatives, including the extract that is the subject of the present GRAS assessment, and the agency responded that it has no objection to the sale of these preparations as ingredients for use at levels up to 300 mg/serving in foods and beverages (Health Canada, 2016). The safety assessment performed by the Food Directorate considered the development of the products, their intended use, the estimated level of consumption by consumers, the incidental intake of heavy metals and mycotoxins, the nutritional and anti-nutrient composition, the microbiological and toxicological information, and the presence of potential allergens.

In its assessment, the Food Directorate also critically reviewed the toxicological studies to support the safety of Coffeeberry® products that included a sub-chronic (90-day) oral toxicity study in rats, an *in vitro* bacterial reverse mutation assay, and an *in vivo* micronucleus assay in mice. These studies were conducted in accordance with accepted international standards. A scientific rationale was provided in the Novel Food notification to support the safe use of the products by pregnant women. Dietary exposure was also calculated for caffeine and chlorogenic acids (CGA), the major active constituents of Coffeeberry® products. The Food Directorate concluded that the information provided was sufficient to assess the safety of the Coffeeberry® whole powder and aqueous ethanolic extract (the subject of the present GRAS assessment – Coffeeberry® Coffee Fruit Extract) in foods.

The Food Directorate noted that the sub-chronic oral toxicity study conducted in rats used Coffeeberry® Coffee Fruit Extract at doses ranging from 0 to 5.0% in the diet. A No Observed Adverse Effect Level (NOAEL) of 5% in the diet was established, which is equivalent to 3446 and 4087 mg/kg bw/day for males and females, respectively. The dietary intake estimate considered that individuals would consume up to 8 servings/day of Coffeeberry® products; 4 servings from beverages and 4 servings from other foods. This is equivalent to doses of 34.3 mg/kg bw/day in adults weighing 70 kg and 68.6 mg/kg bw/day in a 35 kg child. As compared to the NOAEL established in the 90-day study, a margin of exposure (MOE) was determined to be 100 for adults and 50 for children. The lower MOE in children reflects the assumption that children would consume the same amount of Coffeeberry® from food and beverages as an adult. The Food Directorate considered this MOE large enough to be safe for consumption of Coffeeberry®.

Regarding exposure to caffeine, the Coffeeberry® whole coffee fruit powder contains about 1% caffeine while Coffeeberry® Coffee Fruit Extract contains 1-2%. Based on a maximum daily intake of 2400 mg of the extract, the maximum dietary intake of caffeine from consuming 8 servings would be 48 mg/day. The agency noted that this amount would be below the Health Canada guidelines for caffeine in all populations except for young children in whom it would be marginally above their recommended levels (Recommended Maximum Daily Intake of caffeine for 4 to 6-year olds = 45 mg/day) (Health Canada, 2016). Considering the conservative exposure scenario and the fact that the daily exposure to caffeine per day would resemble consuming 1 can of cola (typically containing 36-50 mg/355 mL serving), this dietary intake of caffeine would not be expected to pose a safety concern. The Food Directorate also noted that Coffeeberry® Coffee Fruit Extract consists of < 45.0% chlorogenic acids (CGAs). CGAs are

common constituents of the diet, with the greatest intake being from the consumption of coffee. As CGAs are regularly consumed in the diet with no apparent toxicity, exposure to CGAs from the consumption of 8 servings of Coffeeberry® per day would not be expected to result in any adverse health effects (Health Canada, 2016).

The Food Directorate also noted that no reproductive studies on Coffeeberry® products were submitted. However, FutureCeuticals argued that Coffeeberry® products did not exhibit any genotoxic potential and a 90-day oral toxicity study did not result in any adverse health effects at levels much higher than those expected to be consumed in the diet. Furthermore, exposure to caffeine was within Health Canada's recommended maximum daily intake for pregnant women, and the Food Directorate noted that the level of chlorogenic acids in one serving of Coffeeberry® Coffee Fruit Extract is similar to consuming one cup of coffee or less. Compositional analyses also demonstrated that any component identified by FutureCeuticals in the extract that was not present in the coffee bean (all identified as polyphenols) was a normal constituent of other commonly consumed foods at comparable levels. No new compounds not previously known to be a component of food were identified by FutureCeuticals. Based on this information, the Health Directorate stated that there is no reason to expect that Coffeeberry® would pose a risk to pregnant women or the developing fetus (Health Canada, 2016).

In summary, based on the information presented in support of the use of Coffeeberry® Coffee Fruit Extract as a food ingredient in Canada within the proposed conditions of use, the Food Directorate concluded that there are no food safety concerns for the general population.

It should be noted that for the present GRAS assessment, the intake analysis was performed using the NHANES 2009-2014 data and the resulting maximum ($90^{th}$ percentile) intake of Coffeeberry® Coffee Berry Extract was 393 mg/person/day (5.9 mg/kg bw/day). This value is 6-fold lower than mentioned in Novel Food notification submitted to Health Canada. Furthermore the $90^{th}$ percentile caffeine intake from the proposed uses of 7.8 mg/person/day is minor. In fact as described earlier in Section 3.2. Estimated Daily Intake the cumulative caffeine intake from background and the addition of Coffeeberry® Coffee Berry Extract to the selected food categories is lower than background intake (see Appendix I).

### 6.7. Expert Panel Review, Summary and Discussion

At the request of Amin Talati Upadhye, LLP (AminTalati), USA and its client VDF FutureCeuticals, Inc. (FutureCeuticals), an independent panel of recognized experts (hereinafter referred to as the Expert Panel)[2], qualified by their scientific training and relevant national and international experience to evaluate the safety of food and food ingredients, was convened to evaluate the Generally Recognized As Safe (GRAS) status of Coffeeberry® Coffee Fruit Extract, for use as a food ingredient and as an antioxidant in multiple selected food products, described in this dossier, and at use levels ranging from 20 to 300 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). A comprehensive search of the scientific literature for safety and toxicity information on coffee fruit extract, its constituents such as caffeine, and related preparations was conducted through April 2019 and made available to the Expert Panel. The Expert Panel independently and critically evaluated materials submitted by AminTalati and

[2]Modeled after that described in section 201(s) of the Federal Food, Drug, and Cosmetic Act, As Amended. See also attachments (curriculum vitae) documenting the expertise of the Panel members.

FutureCeuticals and other information deemed appropriate or necessary. Following an independent, critical evaluation, the Expert Panel conferred on May 20, 2019 and unanimously agreed to the decision described herein.

AminTalati and FutureCeuticals ensured that all reasonable efforts were made to identify and select a balanced Expert Panel with expertise in food safety, toxicology, and nutrition. The Expert Panel was selected and convened in accordance with the Food and Drug Administration (FDA)'s guidance for industry on "Best Practices for Convening a GRAS Panel"[3]. Efforts were placed on identifying conflicts of interest or relevant "appearance issues" that could potentially bias the outcome of the deliberations of the Expert Panel and no such conflicts of interest or "appearance issues" were identified. The Expert Panel members received a reasonable honorarium as compensation for their time; the honoraria provided to the Expert Panel members were not contingent upon the outcome of their deliberations.

The *Coffea arabica* plant bears a cherry like fruit that consists of an exocarp, pulp, mucilage, and generally two central seeds (or "beans"). This coffee fruit has long been recognized as having inherent nutritional and health-enhancing potential. Despite its nutritional properties, the fruit historically could not be harvested because it is highly perishable and prone to rapidly develop both extensive bacterial contamination and molds. FutureCeuticals developed a new proprietary technology that has eliminated the risk of bacterial and fungal contamination. This technology allows FutureCeuticals to produce dried whole coffee fruit powders, granules, and extracts (Coffeeberry® products), including the water/ethanol extract that is the subject of this GRAS assessment (Coffeeberry® Coffee Fruit Extract), with high levels of phenolic acids.

FutureCeuticals intends to market the standardized Coffeeberry® Coffee Fruit Extract, produced from whole ground coffee fruits by water/ethanol extraction, as an antioxidant and as a food ingredient in selected conventional food products such as Flavored Water/Energy Drink; Coffee/Tea; RTM Beverages; Milk Products (pre-work out); Clusters/Bars; Fruit Juices; Vegetable Juices/Blends; Chocolate; Candy; and Chewing gum at use levels of ranging from 20 to 300 mg/serving (reference amounts customarily consumed, 21 CFR 101.12). The extract is a tan brown colored powder with characteristic odor and taste. FututreCeuticals has developed the food grade specifications of Coffeeberry® Coffee Fruit Extract. The phenolic acid content of Coffeeberry® Coffee Fruit Extract is greater than 40%, while the caffeine content is approximately 1 to 2%. Coffeeberry® Coffee Fruit Extract is manufactured according to current good manufacturing practices. The intended use of Coffeeberry® Coffee Fruit Extract in the above mentioned conventional foods and beverages is estimated to result in the U.S. population two years and older, the per user mean and 90th percentile of intake of Coffeeberry® Coffee Fruit Extract of 170 mg/person/day (2.7 mg/kg-bw/day) and 393 mg/person/day (5.9 mg/kg bw/day), respectively. Coffeeberry® preparations are also marketed as dietary supplements. Health Canada has permitted the use of Coffeeberry® products, including the subject of the present GRAS assessment, in conventional foods.

In a series of specifically designed studies, the potential toxicity of Coffeeberry® products has been extensively investigated. These studies included 7- and 14-day feeding studies of Coffeeberry® whole powder and water extract powder, a 14-day oral-gavage study of Coffeeberry® Coffee Fruit Extract, and a 90-day feeding study of Coffeeberry® Coffee Fruit Extract (the subject of the present GRAS assessment). Additionally, genotoxicity studies

[3] Available at: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm583856.htm

included Ames assays of all three Coffeeberry® products and a mouse micronucleus test of the whole powder. All of these studies were published in the peer-reviewed scientific literature. In the short-term feeding studies, some issues with palatability and tolerance of the extract were noted, however no toxicity was observed. The no observed adverse effect level (NOAEL) in the 90-day feeding study was the highest level tested, 5% dietary concentration, equivalent to 3446 and 4087 mg/kg bw/day for male and female rats, respectively. No evidence of genotoxicity under the conditions of the experiments was noted with the Coffeeberry® products. As compared to the NOAEL of 3446 mg/kg determined from the subchronic toxicity study (the highest dose tested), the maximum intake of 5.9 mg/kg bw/day (for an individual weighing 60 kg) of Coffeeberry® Coffee Fruit Extract from its proposed food uses at levels up to 300 mg/serving is over 580-fold lower.

As Coffeeberry® Coffee Fruit Extract is derived from whole coffee fruit, it naturally contains caffeine at levels of approximately 1-2%. It was estimated that the addition of Coffeeberry® Coffee Fruit Extract to conventional foods and beverages as proposed will result in 7.8 mg caffeine/person/day (0.12 mg/kg-bw/day) at 90$^{th}$ percentile. This additional intake of caffeine is minor and will have a negligible impact on total daily caffeine intake. The available information suggest that chlorogenic acid present in Coffeeberry® Coffee Fruit Extract is unlikely to cause any adverse effects.

In summary, there is sufficient qualitative and quantitative scientific evidence, including animal data, to assess the safety-in-use for Coffeeberry® Coffee Fruit Extract, the subject of this present GRAS assessment. The safety assessment of Coffeeberry® Coffee Fruit Extract is based on the totality of available evidence, including a variety of specifically designed animal toxicity studies. The totality of available evidence supports the safety of Coffeeberry® Coffee Fruit Extract at the maximum (90$^{th}$ percentile) all users intake of 393 mg/person/day. On the basis of scientific procedures[4], the consumption of Coffeeberry® Coffee Fruit Extract as an added food ingredient is considered safe at use levels up to 300 mg/serving. The intended uses are compatible with current regulations, *i.e.,* Coffeeberry® Coffee Fruit Extract is used in specified foods (described in this document) and is produced according to current good manufacturing practices (cGMP).

[4] 21 CFR §170.3 Definitions. (h) Scientific procedures include those human, animal, analytical, and other scientific studies, whether published or unpublished, appropriate to establish the safety of a substance.

### 6.8. Expert Panel Conclusion

Based on a critical evaluation of the publicly available data, summarized herein, the Expert Panel members whose signatures appear below, have individually and collectively concluded that Coffeeberry® Coffee Fruit Extract, meeting the specifications cited herein, and when used as an antioxidant at use levels ranging from 20 to 300 mg/serving in conventional foods such as Flavored Water/Energy Drink; Coffee/Tea; RTM Beverages; Milk Products (pre-work out); Clusters/Bars; Fruit Juices; Vegetable Juices/Blends; Chocolate; Candy; and Chewing gum (when not otherwise precluded by a Standard of Identity) as described in this monograph, and resulting in the maximum (90$^{th}$ percentile) estimated intake of 393 mg Coffeeberry® Coffee Fruit Extract/person/day, is safe.

It is also our opinion that other qualified and competent scientists reviewing the same publicly available toxicological and safety information would reach the same conclusion. Therefore, we have also concluded that Coffeeberry® Coffee Fruit Extract, when used as described, is Generally Recognized As Safe (GRAS) based on scientific procedures.

### Signatures

[redacted]
Robert L. Martin, Ph.D.

May 21, 2019
Date

[redacted]
John A. Thomas, Ph.D., F.A.T.S., F.A.C.T.

May 23, 2019
Date

[redacted]
Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S.

May 24, 2019
Date

## Part VII- SUPPORTING DOCUMENTS AND REFERENCES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**Appendix I**

**Estimated Daily Intake of Coffeeberry® Coffee Fruit Extract Proposed for Use in Select Foods Among the U.S. Population**

**Report from Exponent- attached separately (pages 1 to 27)**

Exponent®

*Center for Chemical Regulation and Food Safety*

# Estimated Daily Intake of N580 Coffee Fruit Extract Proposed for Use in Select Foods Among the U.S. Population

# Estimated Daily Intake of N580 Coffee Fruit Extract Proposed for Use in Select Foods Among the U.S. Population

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September 21, 2018



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# Contents

# List of Tables

# List of Acronyms

| | |
|---|---|
| bw | Bodyweight |
| CEDI | Cumulative EDI |
| DHHS | U.S. Department of Health and Human Services |
| EDI | Estimated Daily Intake |
| FDA | U.S. Food and Drug Administration |
| FNDDS | Food and Nutrient Database for Dietary Studies |
| FR | Federal Register |
| mg | Miligram |
| NCHS | National Center for Health Statistics |
| NHANES | National Health and Nutrition Examination Survey |
| RACC | References Amounts Customarily Consumed |
| RTD | Ready-to-drink |
| WWEIA | What We Eat In America |
| U.S. | United States |
| USDA | U.S. Department of Agriculture |
| WCBA | Women of childbearing age |

# Introduction

At the request of VDF FutureCeuticals, Inc. (FutureCeuticals), Exponent, Inc. (Exponent) conducted an intake assessment to estimate the total daily intake of N580 Coffee Fruit Extract that is proposed for use in 10 food categories. A subsequent analysis was conducted to estimate caffeine intakes associated with the proposed uses of N580. Specifically, background caffeine intakes from food and dietary supplement sources, proposed uses of N580, and the cumulative caffeine intake from background sources and proposed uses combined was assessed. The intake estimates of N850 and caffeine was based on food consumption data from foods reported consumed in the What We Eat in America (WWEIA) dietary component of the National Health and Nutrition Examination Survey (NHANES) 2009-2014 and provided for the total United States (U.S.) population 2 years (y) and older and the following five subpopulation: children 2-12 y, adolescents 13-18 y, adults 19-49 y and 50+ y, and women of childbearing age (WCBA) 14-49 y. The data and methods used to conduct the intake assessment and results are summarized in this report.

# Proposed Use and Levels

N580 coffee fruit extract is proposed for use in 10 food categories at a maximum proposed use level ranging from 20 to 300 mg per serving. The food serving size to which N580 will be added corresponds to the gram weight or mL volume of food as specified by References Amounts Customarily Consumed (RACCs) for food labeling based on FDA's final rule effective July 26, 2016 with the compliance date of July 26, 2018 (FR 2016). Table 1 lists the 10 food categories to which N580 is proposed for use, descriptions of the types of foods within the category that was included in the assessment, the serving size associated with each food type, and the maximum use level of N580.

**Table 1. Proposed Uses of N580 Coffee Fruit Extract in Foods**

| Proposed Use Category | Description of Foods Selected for Analysis | Serving Size[a] | Maximum N580 Use Level (mg/serving) |
|---|---|---|---|
| Flavored Water/Energy Drink | Sport drinks (i.e., Gatorade, Powerade, etc.), energy drinks (i.e., Monster, Red Bull, etc.), and enhanced/fortified waters (i.e., Propel, Glaceau, etc.) | 360 mL | 300 |
| Coffee/Tea | RTD/bottled/canned coffees and teas. Coffee excludes brewed, instant, and frozen types. Tea excludes hot and brewed tea leaf. Coffee and tea both exclude decaffeinated types. | 360 mL | 100 |
| RTM Beverages | Non-reconstituted protein powders (i.e., Muscle Milk powder) | Amount to make 240 mL | 100 |
| Milk Products (pre-work out) | Ensure, Boost, and RTD high protein nutritional drinks such as Monster Milk | 240 mL | 100 |
| Clusters/Bars | Nutrition bars including Balance, PowerBar, Clif, Zone, etc. | 40 g | 100 |
| Fruit Juices | Fruit juice blends and drinks, carbonated fruit juice drinks, coconut waters | 240 mL | 100 |
| Vegetable Juices/Blends | Carrot juice, tomato juice, celery juice, mixed vegetable juice, fruit and vegetable blend juices | 240 mL | 100 |
| Chocolate | Milk chocolate and dark chocolate with or without caramel, nuts, toffee, and/or dried fruits/seeds inclusions | 30 g | 100 |
| Candy | Gummy candy including Life Savers Gummi Savers and other gummy animals/shapes | 30 g | 20 |
| Chewing Gum | Chewing gum, regular and sugar free | 3 g | 20 |

[a] Serving sizes correspond to values in Table 2 – Reference Amounts Customarily Consumed per Eating Occasion: General Food Supply as cited in FR Vol 81, No. 103, Friday, May 27, 2016, pp 34000-47. Available at: https://www.govinfo.gov/content/pkg/FR-2016-05-27/pdf/2016-11865.pdf.
RTD = ready-to-drink

# Estimated Daily Intake (EDI)

## NHANES Data

Intake estimates of N580 and caffeine were based on food consumption records collected in the WWEIA component of NHANES conducted in 2009-2010, 2011-2012, and 2013-2014 (NHANES 2009-2014). The NHANES is a continuous survey that uses a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS 2013, 2014, 2016). NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection and non-response.

## NHANES 24-hour Dietary Recall

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24 hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the U.S. Department of Agriculture (USDA) and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 23,585 individuals in the survey period 2009-2014 provided 2 complete days of dietary recalls.

### Food and Nutrient Database for Dietary Studies (FNDDS)

For each food reported in NHANES, the USDA Food and Nutrient Database for Dietary Studies (FNDDS) databases provide information on the amount of energy and approximately 60 nutrients or food constituents per 100 g of each food. In addition, the FNDDS translates food as reported consumed into its corresponding ingredients (and gram amounts) or recipes. USDA's

FNDDS 2013-2014 was the main source of nutrient composition data and food recipes in this analysis. FNDDS 2013-2014 was based on nutrient values in the USDA National Nutrient Database for Standard Reference, Release 28 (SR 28) (USDA 2016b), and was used by USDA to process dietary recall data reported in NHANES 2013-2014 (USDA 2016a). When a food was unique to the 2011-2012 period (i.e., not reported in 2013-2014 by participants and thus not available in FNDDS 2013-2014), composition and recipe data were based on earlier releases of the food and nutrient database, FNDDS 2011-2012 or FNDDS version 5.0 (USDA 2014, 2012).

### NHANES 24-hour Dietary Supplement Use

Starting in the NHANES 2007-2008 cycle, NHANES collected supplement use data along with food consumption data as part of the 24-hour dietary recall data collection. The data collection for the 24-hour dietary supplement use is administered by the trained dietary interviewers. During the 24-hour recall, NHANES participants who reported taking supplements in the past 30 days in the household questionnaire were asked if they took these supplements in the previous 24 hours, and if so how much they took. All participants in the 24 hour recall were also asked if they took any other supplements, not reported during the 30-day supplement use household interview, and, if so, they were asked to report how much they took. The use of non-prescription antacids containing calcium and/or magnesium is included in this database. NHANES has preprocessed the supplement 24 hour recall data and derived nutrient intakes from supplements for NHANES 2009-2014.

## Analysis

### NHANES Food Selection

Exponent reviewed all foods reported consumed during NHANES 2009-2014 for foods representative of the proposed uses of N580. The FNDDS recipes were utilized to identify the weight of ingredients in foods which allowed for the estimation of the foods with proposed uses of N580 that can be consumed as is or as a component in a food (i.e., energy drink or lemonade component in alcoholic mixed drinks). A summary of the foods included in the

analysis is provided in Table 1 and the listing of foods identified as representative of the proposed uses and used to complete the analysis is presented in Appendix A.

## Two-day Average Daily Intake

Using the NHANES 2009-2014 consumption data, Exponent estimated the 2-day average daily intake on a "per capita" and "per user" basis. *Per capita* estimates refer to the consumption based on the entire population of interest whereas *per user* estimates refer to those who reported consuming any of the foods of interest on either of the survey days. For each subject with a complete 2-day dietary recall, a 2-day average intake estimate was derived by summing the intake(s) of interest on day 1 and day 2 of the survey and dividing that sum by 2. If a survey participant consumed foods or beverages of interest on only one of the survey days, their intake of interest from that day was divided by two, to obtain their 2-day average intake. The two-day average daily intake was estimated for the U.S. 2+ y, children age 2-12 y, adolescents 13-18 y, adults 19-49 y and 50+y, and women of childbearing age (WCBA) 14-49 y. Estimates were also derived on a bodyweight (bw) basis using each participant's reported body weight.

The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS. Exponent uses the statistically weighted values from the survey in its analyses. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population.

### N580 from Proposed Uses

Intake estimates of N580 from proposed uses was calculated for each survey participant by combining their reported intake of the foods of interest from the 24-hour recall with the corresponding serving size of the food and proposed use level (see Table 1) and the cumulative sum over the two 24-hour recalls was divided by two.

## Caffeine

### i) Intake Associated with the Proposed Uses N580

Based on information provided by FutureCeuticals, the concentration of caffeine in N580 is 2%. Estimates of caffeine intake associated with the proposed use of N580 were derived by combining the consumption amounts reported in NHANES for the proposed foods with the corresponding maximum proposed use level of N580 (see Table 1), the serving size of the proposed food, and the level of caffeine in N580 (2%).

### ii) Background EDI

The estimated background caffeine intake from the total diet was derived using NHANES food consumption and dietary supplement data in combination with the caffeine concentration data as reported in the USDA FNDDS databases. The FNDDS database provided both naturally occurring and added caffeine levels in foods. As such, the existing background intake is the total caffeine intake from food and beverages with "naturally occurring" caffeine and the caffeine intakes from foods and beverages with added caffeine. In addition, each participant's total caffeine intake from dietary supplement sources was added to his or her caffeine intake from food and beverage sources to estimate the total background caffeine intake.

Modification to the caffeine concentration reported in the FNDDS was made for food code 92410330 *soft drink, cola-type, with higher caffeine* with an additional description of *Jolt Cola* and *Josta*. This beverage was assigned a caffeine concentration of 338 ppm (or 160 mg caffeine per 473 mL beverage) instead of the current concentration of 90 ppm (or 9 mg caffeine per 100 g beverage) as reported in FNDDS 2011-2012 which was assumed to be erroneous. The higher caffeine concentration of 338 ppm was based on the online nutrition label of Jolt Cola[1] indicating 160 mg caffeine per 473 mL serving.

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[1] Jolt Cola. Nutrition Label. https://www.joltcola.com/news/nutrition-label. Accessed August 8, 2018.

### iii) Cumulative EDI (CEDI)

To estimate the cumulative EDI for caffeine from background and from the proposed uses of N580, each individual's caffeine intake from background sources was added to their caffeine intake associated with the proposed uses of N580.

# Results

## N580 from Proposed Uses

The two-day average intake of N580 from the proposed uses in 10 food categories combined are expressed in units of mg/day and mg/kg-bw/day and are provided in Tables 2A and 2B, respectively. Among the U.S. population two years and older, the *per user* mean and 90$^{th}$ percentile of intake of N580 is 170 mg/day (2.7 mg/kg-bw/day) and 393 mg/day (5.9 mg/kg-bw/day), respectively.

## Caffeine

Caffeine intakes associated with the proposed use of N580 are summarized in Tables 3A (mg/day) and 3B (mg/kg-bw/day). Background caffeine intake estimates from existing food and dietary supplement sources are provided in Tables 4A (mg/day) and 4B (mg/kg-bw/day) and cumulative caffeine intake which reflects total caffeine intake from background food sources, dietary supplements, and from the proposed uses of N580 are summarized in Tables 5A (mg/day) and 5B (mg/kg-bw/day).

Among the U.S. population two years and older, the *per user* caffeine intake that is associated with the proposed use of N580 at the *per user* mean and 90$^{th}$ percentile of intake is 3.4 mg/day (0.05 mg/kg-bw/day) and 7.8 mg/day (0.12 mg/kg-bw/day) (see Tables 3A and 3B). The caffeine intake from background sources (food and dietary supplement) pre-introduction of N580 at the *per user* mean and 90$^{th}$ percentile of intake is 141 mg/day (1.8 mg/kg-bw/day) and 338 mg/day (4.2 mg/kg-bw/day), respectively (see Tables 4A and 4B). The cumulative caffeine intake from background sources and the proposed use of N580 combined at the *per user* mean and 90$^{th}$ percentile of intake is 137 mg/day (1.8 mg/kg-bw/day) and 332 mg/day (4.2 mg/kg-bw/day), respectively (see tables 5A and 5B).

It should be noted that the cumulative caffeine intake includes consumers of caffeine from background sources *and/or* proposed uses of N580 whereas the background caffeine intake

includes consumers of caffeine from the background alone (i.e., pre-introduction of N580). Hence, the increase in the user sample size from the background to cumulative analysis is due to the inclusion of NHANES participants who did not consume caffeine but who consume one or more of the foods with the proposed N580 use coupled with the lower intake of caffeine from these proposed uses that result in slightly lower caffeine intakes for almost all populations in the cumulative analysis. In other words, the combined caffeine intake distribution, which includes background and proposed N580 sources, shifted the mean and 90$^{th}$ percentile of intake lower due to the inclusion of lower caffeine intakes associated with the proposed uses of N580.

**Table 2A. Two-day average intake of N580 from proposed uses (mg/day); NHANES 2009-2014**

| Population | Un-wtd N[a] | % Users | Per Capita (mg/day) Mean | Per Capita (mg/day) 90th Percentile | Per User (mg/day) Mean | Per User (mg/day) 90th Percentile |
|---|---|---|---|---|---|---|
| U.S. 2+ y | 12,379 | 55 | 94 | 263 | 170 | 393 |
| Children 2-12 y | 3,386 | 63 | 70 | 188 | 112 | 247 |
| Adolescents 13-18 y | 1,531 | 61 | 126 | 310 | 207 | 432 |
| Adults 19-49 y | 4,264 | 57 | 114 | 338 | 200 | 459 |
| Adults 50+ y | 3,198 | 49 | 72 | 203 | 146 | 335 |
| WCBA 14-49 y | 2,909 | 57 | 87 | 240 | 152 | 340 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.
Note: Refer to Table 1 for the proposed uses of N580.

**Table 2B. Two-day average intake of N580 from proposed uses (mg/kg-bw/day); NHANES 2009-2014**

| Population | Un-wtd N[a] | % Users | Per Capita (mg/kg-bw/day) Mean | Per Capita (mg/kg-bw/day) 90th Percentile | Per User (mg/kg-bw/day) Mean | Per User (mg/kg-bw/day) 90th Percentile |
|---|---|---|---|---|---|---|
| U.S. 2+ y | 12,379 | 55 | 1.5 | 4.2 | 2.7 | 5.9 |
| Children 2-12 y | 3,386 | 63 | 2.7 | 7.4 | 4.3 | 9.6 |
| Adolescents 13-18 y | 1,531 | 61 | 1.9 | 5.0 | 3.1 | 6.4 |
| Adults 19-49 y | 4,264 | 57 | 1.4 | 4.1 | 2.5 | 5.7 |
| Adults 50+ y | 3,198 | 49 | 0.9 | 2.6 | 1.8 | 3.9 |
| WCBA 14-49 y | 2,909 | 57 | 1.2 | 3.4 | 2.2 | 5.0 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.
Note: Refer to Table 1 for the proposed uses of N580.

**Table 3A. Two-day average intake of caffeine associated with the proposed uses of N580 (mg/day); NHANES 2009-2014**

| | | | Per Capita (mg/day) | | Per User (mg/day) | |
|---|---|---|---|---|---|---|
| Population | Un-wtd N[a] | % Users | Mean | 90th Percentile | Mean | 90th Percentile |
| U.S. 2+ y | 12,379 | 55 | 1.9 | 5.3 | 3.4 | 7.8 |
| Children 2-12 y | 3,386 | 63 | 1.4 | 3.7 | 2.2 | 4.9 |
| Adolescents 13-18 y | 1,531 | 61 | 2.5 | 6.2 | 4.1 | 8.6 |
| Adults 19-49 y | 4,264 | 57 | 2.3 | 6.8 | 4.0 | 9.2 |
| Adults 50+ y | 3,198 | 49 | 1.4 | 4.1 | 2.9 | 6.7 |
| WCBA 14-49 y | 2,909 | 57 | 1.7 | 4.8 | 3.0 | 6.8 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.
Note: Refer to Table 1 for the proposed uses of N580.

**Table 3B. Two-day average intake of caffeine associated with the proposed uses of N580 (mg/kg-bw/day); NHANES 2009-2014**

| | | | Per Capita (mg/kg-bw/day) | | Per User (mg/kg-bw/day) | |
|---|---|---|---|---|---|---|
| Population | Un-wtd N[a] | % Users | Mean | 90th Percentile | Mean | 90th Percentile |
| U.S. 2+ y | 12,379 | 55 | 0.03 | 0.08 | 0.05 | 0.12 |
| Children 2-12 y | 3,386 | 63 | 0.05 | 0.15 | 0.09 | 0.19 |
| Adolescents 13-18 y | 1,531 | 61 | 0.04 | 0.10 | 0.06 | 0.13 |
| Adults 19-49 y | 4,264 | 57 | 0.03 | 0.08 | 0.05 | 0.11 |
| Adults 50+ y | 3,198 | 49 | 0.02 | 0.05 | 0.04 | 0.08 |
| WCBA 14-49 y | 2,909 | 57 | 0.02 | 0.07 | 0.04 | 0.10 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.
Note: Refer to Table 1 for the proposed uses of N580.

**Table 4A. Two-day average intake of caffeine from background sources (food and dietary supplements) (mg/day); NHANES 2009-2014**

| | | | Per Capita (mg/day) | | Per User (mg/day) | |
|---|---|---|---|---|---|---|
| Population | Un-wtd N[a] | % Users | Mean | 90th Percentile | Mean | 90th Percentile |
| U.S. 2+ y | 19,451 | 91 | 129 | 323 | 141 | 338 |
| Children 2-12 y | 4,198 | 82 | 12 | 35 | 15 | 40 |
| Adolescents 13-18 y | 2,075 | 86 | 51 | 132 | 59 | 143 |
| Adults 19-49 y | 6,754 | 92 | 145 | 338 | 158 | 350 |
| Adults 50+ y | 6,424 | 97 | 181 | 391 | 187 | 396 |
| WCBA 14-49 y | 4,459 | 91 | 118 | 286 | 130 | 301 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.

**Table 4B. Two-day average intake of caffeine from background sources (food and dietary supplements) (mg/kg-bw/day); NHANES 2009-2014**

| | | | Per Capita (mg/kg-bw/day) | | Per User (mg/kg-bw/day) | |
|---|---|---|---|---|---|---|
| Population | Un-wtd N[a] | % Users | Mean | 90th Percentile | Mean | 90th Percentile |
| U.S. 2+ y | 19,451 | 91 | 1.7 | 4.1 | 1.8 | 4.2 |
| Children 2-12 y | 4,198 | 82 | 0.4 | 1.1 | 0.5 | 1.3 |
| Adolescents 13-18 y | 2,075 | 86 | 0.8 | 1.8 | 0.9 | 1.9 |
| Adults 19-49 y | 6,754 | 92 | 1.8 | 4.1 | 2.0 | 4.3 |
| Adults 50+ y | 6,424 | 97 | 2.2 | 4.9 | 2.3 | 5.0 |
| WCBA 14-49 y | 4,459 | 91 | 1.6 | 3.9 | 1.8 | 4.2 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS

**Table 5A. Two-day average cumulative intake of caffeine from background sources (food and dietary supplements) and proposed uses of N580 (mg/day); NHANES 2009-2014**

| | | | Per Capita (mg/day) | | Per User (mg/day) | |
|---|---|---|---|---|---|---|
| Population | Un-wtd N[a] | % Users | Mean | 90th Percentile | Mean | 90th Percentile |
| U.S. 2+ y | 20,763 | 96 | 131 | 324 | 137 | 332 |
| Children 2-12 y | 4,857 | 93 | 14 | 37 | 15 | 39 |
| Adolescents 13-18 y | 2,269 | 93 | 53 | 137 | 57 | 144 |
| Adults 19-49 y | 7,082 | 95 | 147 | 340 | 154 | 347 |
| Adults 50+ y | 6,555 | 98 | 182 | 392 | 186 | 395 |
| WCBA 14-49 y | 4,709 | 95 | 120 | 287 | 126 | 296 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS.

**Table 5B. Two-day average cumulative intake of caffeine from background sources (food and dietary supplements) and proposed uses of N580 (mg/kg-bw/day); NHANES 2009-2014**

| | | | Per Capita (mg/kg-bw/day) | | Per User (mg/kg-bw/day) | |
|---|---|---|---|---|---|---|
| Population | Un-wtd N[a] | % Users | Mean | 90th Percentile | Mean | 90th Percentile |
| U.S. 2+ y | 20,763 | 96 | 1.7 | 4.1 | 1.8 | 4.2 |
| Children 2-12 y | 4,857 | 93 | 0.5 | 1.2 | 0.5 | 1.3 |
| Adolescents 13-18 y | 2,269 | 93 | 0.8 | 1.9 | 0.8 | 1.9 |
| Adults 19-49 y | 7,082 | 95 | 1.9 | 4.2 | 2.0 | 4.2 |
| Adults 50+ y | 6,555 | 98 | 2.3 | 5.0 | 2.3 | 5.0 |
| WCBA 14-49 y | 4,709 | 95 | 1.7 | 4.0 | 1.8 | 4.0 |

[a] Un-weighted number of users; % users, *per capita* and *per user* estimates for NHANES derived using the statistical weights provided by the NCHS

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# Appendix A. Food Codes Representative of the Proposed Uses of N580 that were Included in the Analysis

| Food code | Food Description |
|---|---|
| **Flavored Water/Energy Drink** | |
| 92560000 | Fruit-flavored thirst quencher beverage |
| 92560100 | Gatorade Thirst Quencher sports drink |
| 92560200 | Powerade sports drink |
| 92565000 | Fruit-flavored sports drink or thirst quencher beverage, low calorie |
| 92565100 | Gatorade G2 thirst quencher sports drink, low calorie |
| 92565200 | Powerade Zero sports drink, low calorie |
| 92570100 | Fluid replacement, electrolyte solution |
| 92650000 | Red Bull Energy Drink |
| 92650005 | Red Bull Energy Drink, sugar-free |
| 92650200 | Monster Energy Drink |
| 92650205 | Mountain Dew AMP Energy Drink |
| 92650210 | Mountain Dew AMP Energy Drink, sugar-free |
| 92650700 | Rockstar Energy Drink |
| 92650705 | Rockstar Energy Drink, sugar-free |
| 92650800 | Vault Energy Drink |
| 92650805 | Vault Zero Energy drink |
| 92651000 | Energy drink |
| 93301083 | Jagerbomb* |
| 93301216 | Vodka and energy drink* |
| 94210100 | Propel Water |
| 94210200 | Glaceau Vitamin Water |
| 94210300 | SoBe Life Water |
| 94220100 | Propel Zero Water |
| 94220200 | Glaceau Water, low calorie |
| 94220215 | Glaceau Vitamin Water Zero |
| 94220310 | SoBe Life Water Zero |
| 95310200 | Full Throttle Energy Drink |
| 95310400 | Monster Energy Drink |
| 95310500 | Mountain Dew AMP Energy Drink |
| 95310560 | NOS Energy Drink |
| 95310600 | Red Bull Energy Drink |
| 95310700 | Rockstar Energy Drink |
| 95310750 | SoBe Energize Energy Juice Drink |
| 95310800 | Vault Energy Drink |
| 95311000 | Energy Drink |
| 95312400 | Monster Energy Drink, Lo Carb |
| 95312500 | Mountain Dew AMP Energy Drink, sugar-free |
| 95312550 | No Fear Energy Drink, sugar-free |

| Food code | Food Description |
|---|---|
| 95312600 | Red Bull Energy Drink, sugar-free |
| 95312700 | Rockstar Energy Drink, sugar-free |
| 95312800 | Vault Zero Energy Drink |
| 95312900 | XS Energy Drink |
| 95313200 | Energy drink, sugar free |
| 95320200 | Gatorade G sports drink |
| 95320500 | Powerade sports drink |
| 95321000 | Sports drink, NFS |
| 95322200 | Gatorade G2 sports drink, low calorie |
| 95322500 | Powerade Zero sports drink, low calorie |
| 95323000 | Sports drink, low calorie |
| 95330100 | Fluid replacement, electrolyte solution |
| 95330500 | Fluid replacement, 5% glucose in water |
| 95341000 | FUZE Slenderize fortified low calorie fruit juice beverage |
| **Coffee/Tea** | |
| 11561000 | Cafe con leche |
| 92100000 | Coffee, NS as to type |
| 92101600 | Coffee, Turkish |
| 92101610 | Coffee, espresso |
| 92101800 | Coffee, Cuban |
| 92101820 | Coffee, macchiato, sweetened |
| 92101850 | Coffee, cafe con leche |
| 92101900 | Coffee, Latte |
| 92101901 | Coffee, Latte, nonfat |
| 92101903 | Coffee, Latte, with non-dairy milk |
| 92101904 | Coffee, Latte, flavored |
| 92101905 | Coffee, Latte, nonfat, flavored |
| 92101950 | Coffee, Cafe Mocha |
| 92101955 | Coffee, Cafe Mocha, nonfat |
| 92101960 | Coffee, Cafe Mocha, with non-dairy milk |
| 92102450 | Iced Coffee, pre-lightened and pre-sweetened |
| 92102500 | Coffee, Iced Latte |
| 92102501 | Coffee, Iced Latte, nonfat |
| 92102503 | Coffee, Iced Latte, flavored |
| 92102600 | Coffee, Iced Cafe Mocha |
| 92130000 | Coffee, pre-lightened and pre-sweetened with sugar |
| 92130005 | Coffee, pre-lightened and pre-sweetened with low calorie sweetener |
| 92130010 | Coffee, pre-lightened |
| 92130020 | Coffee, pre-sweetened with sugar |
| 92130030 | Coffee, pre-sweetened with low calorie sweetener |
| 92161000 | Coffee, Cappuccino |
| 92161001 | Coffee, Cappuccino, nonfat |
| 92161002 | Coffee, Cappuccino, with non-dairy milk |
| 92161005 | Cappuccino, sweetened |

| Food code | Food Description |
|---|---|
| 92171000 | Coffee, bottled/canned |
| 92171010 | Coffee, bottled/canned, light |
| 92301000 | Tea, NS as to type, unsweetened |
| 92301060 | Tea, NS as to type, presweetened with sugar |
| 92301080 | Tea, NS as to type, presweetened with low calorie sweetener |
| 92301130 | Tea, NS as to type, presweetened, NS as to sweetener |
| 92302200 | Tea, leaf, presweetened with sugar |
| 92302300 | Tea, leaf, presweetened with low calorie sweetener |
| 92302400 | Tea, leaf, presweetened, NS as to sweetener |
| 92306020 | Tea, herbal, presweetened with sugar |
| 92306030 | Tea, herbal, presweetened with low calorie sweetener |
| 92306040 | Tea, herbal, presweetened, NS as to sweetener |
| 92307500 | Iced Tea / Lemonade juice drink |
| 92307510 | Iced Tea / Lemonade juice drink, light |
| 92307520 | Iced Tea / Lemonade juice drink, diet |
| 92309000 | Tea, iced, bottled, black |
| 92309020 | Tea, iced, bottled, black, diet |
| 92309040 | Tea, iced, bottled, black, unsweetened |
| 92309500 | Tea, iced, bottled, green |
| 92309510 | Tea, iced, bottled, green, diet |
| 92309520 | Tea, iced, bottled, green, unsweetened |
| **RTM Beverages** | |
| 11830900 | Protein supplement, milk-based, powdered, not reconstituted |
| 11831500 | Nutrient supplement, milk-based, high protein, powdered, not reconstituted |
| 11836000 | Protein supplement, milk-based, Muscle Milk, powdered, not reconstituted |
| 11836100 | Protein supplement, milk-based, Muscle Milk Light, powdered, not reconstituted |
| 41430000 | Protein powder, NFS |
| 41430010 | Protein supplement, powdered |
| 41430310 | Protein diet powder with soy and casein |
| 95201200 | EAS Whey Protein Powder |
| 95201500 | Herbalife, nutritional shake mix, high protein, powder |
| 95201600 | Isopure protein powder |
| 95202000 | Muscle Milk, regular, powder |
| 95202010 | Muscle Milk, light, powder |
| 95220010 | Nutritional drink mix or meal replacement, high protein, powder, NFS |
| 95230000 | Protein powder, whey based, NFS |
| 95230010 | Protein powder, soy based, NFS |
| 95230020 | Protein powder, light, NFS |
| 95230030 | Protein powder, NFS |
| **Milk Products (pre-work out)** | |
| 11641000 | Meal supplement or replacement, milk-based, high protein, liquid |
| 11641020 | Meal replacement or supplement, milk based, ready-to-drink |
| 41440010 | Ensure liquid nutrition |
| 41440020 | Ensure with fiber, liquid |

| Food code | Food Description |
|---|---|
| 41440050 | Ensure Plus liquid nutrition |
| 95101000 | Boost, nutritional drink, ready-to-drink |
| 95101010 | Boost Plus, nutritional drink, ready-to-drink |
| 95103000 | Ensure, nutritional shake, ready-to-drink |
| 95103010 | Ensure Plus, nutritional shake, ready-to-drink |
| 95105000 | Kellogg's Special K Protein Shake |
| 95106000 | Muscle Milk, ready-to-drink |
| 95106010 | Muscle Milk, light, ready-to-drink |
| 95120010 | Nutritional drink or meal replacement, high protein, ready-to-drink, NFS |
| 95120020 | Nutritional drink or meal replacement, high protein, light, ready-to-drink, NFS |
| **Clusters/Bars** | |
| 41435110 | High protein bar, candy-like, soy and milk base |
| 41435120 | Zone Perfect Classic Crunch nutrition bar |
| 41435300 | Balance Original Bar |
| 41435500 | Clif Bar |
| 41435700 | South Beach Living High Protein Cereal Bar |
| 41435710 | South Beach Living Meal Replacement Bar |
| 53541200 | Meal replacement bar |
| 53541300 | Slim Fast Original Meal Bar |
| 53544450 | PowerBar (fortified high energy bar) |
| 53720100 | Balance Original Bar |
| 53720200 | Clif Bar |
| 53720210 | Clif Kids Organic Zbar |
| 53720300 | PowerBar |
| 53720400 | Slim Fast Original Meal Bar |
| 53720500 | Snickers Marathon Protein bar |
| 53720600 | South Beach Living Meal Bar |
| 53720610 | South Beach Living High Protein Bar |
| 53720700 | Tiger's Milk bar |
| 53720800 | Zone Perfect Classic Crunch nutrition bar |
| 53729000 | Nutrition bar or meal replacement bar, NFS |
| 91780010 | Snickers Marathon Energy bar |
| 91781010 | Snickers Marathon Protein bar |
| **Fruit Juices** | |
| 42403010 | Coconut water, unsweetened (liquid from coconuts) |
| 42404010 | Coconut water, sweetened |
| 61213800 | Fruit juice blend, citrus, 100% juice |
| 61213900 | Fruit juice blend, citrus, 100% juice, with calcium added |
| 64100100 | Fruit juice, NFS |
| 64100110 | Fruit juice blend, 100% juice |
| 64100200 | Cranberry juice blend, 100% juice |
| 64100220 | Cranberry juice blend, 100% juice, with calcium added |
| 92432000 | Fruit juice drink, citrus, carbonated |
| 92433000 | Fruit juice drink, noncitrus, carbonated |

| Food code | Food Description |
|---|---|
| 92510610 | Fruit juice drink |
| 92510720 | Fruit punch, made with fruit juice and soda |
| 92510730 | Fruit punch, made with soda, fruit juice, and sherbet or ice cream |
| 92510955 | Lemonade, fruit juice drink |
| 92510960 | Lemonade, fruit flavored drink |
| 92511250 | Fruit juice beverage, 40-50% juice, citrus |
| 92530510 | Cranberry juice drink, with high vitamin C |
| 92530610 | Fruit juice drink, with high vitamin C |
| 92530950 | Vegetable and fruit juice drink, with high vitamin C |
| 92531030 | Sunny D |
| 92550030 | Fruit juice drink, with high vitamin C, light |
| 92550035 | Fruit juice drink, light |
| 92550040 | Fruit juice drink, diet |
| 92550110 | Cranberry juice drink, with high vitamin C, light |
| 92550200 | Grape juice drink, light |
| 92550350 | Orange juice beverage, 40-50% juice, light |
| 92550360 | Apple juice beverage, 40-50% juice, light |
| 92550370 | Lemonade, fruit juice drink, light |
| 92550400 | Vegetable and fruit juice drink, with high vitamin C, diet |
| 92550405 | Vegetable and fruit juice drink, with high vitamin C, light |
| 92552020 | Sunny D, reduced sugar |
| 92552030 | Capri Sun, fruit juice drink |
| 92582100 | Fruit juice drink, with high vitamin C, plus added calcium |
| 92582110 | Sunny D, added calcium |
| 93301032 | Cape Cod* |
| 93301111 | Martini, flavored* |
| 93301141 | Seabreeze* |
| 93301213 | Vodka and lemonade* |
| 95342000 | MonaVie acai blend beverage |
| **Vegetable Juices/Blends** | |
| 73105010 | Carrot juice, 100% |
| 74301100 | Tomato juice, 100% |
| 74301150 | Tomato juice, 100%, low sodium |
| 74302000 | Tomato juice cocktail |
| 74303000 | Tomato and vegetable juice, 100% |
| 74303100 | Tomato and vegetable juice, 100%, low sodium |
| 74304000 | Tomato juice with clam or beef juice |
| 75132000 | Mixed vegetable juice (vegetables other than tomato) |
| 75132100 | Celery juice |
| 75200700 | Aloe vera juice drink |
| 78101000 | Vegetable and fruit juice, 100% juice, with high vitamin C |
| 93301030 | Bloody Mary* |
| **Chocolate** | |
| 91705010 | Milk chocolate candy, plain |

| Food code | Food Description |
|---|---|
| 91705040 | Chocolate, milk, with nuts, not almond or peanuts |
| 91705050 | Milk chocolate candy, with fruit and nuts |
| 91705060 | Milk chocolate candy, with almonds |
| 91705070 | Chocolate, milk, with peanuts |
| 91705300 | Chocolate, sweet or dark |
| 91705310 | Chocolate, sweet or dark, with almonds |
| **Candy** | |
| 91700010 | Candy, NFS |
| 91745010 | Gumdrops |
| 91770000 | Dietetic or low calorie candy, NFS |
| **Chewing Gum** | |
| 91800100 | Chewing gum, NFS |
| 91801000 | Chewing gum, regular |
| 91802000 | Chewing gum, sugar free |

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November 1, 2019

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**Re: GRN 868 (Coffeeberry® Coffee Fruit Extract)**

Dear Dr. Hall,

This responds to your email of October 18, 2019, regarding your queries that need to be addressed for Coffeeberry® Coffee Fruit Extract GRAS Notice (GRN 868) submitted on behalf of VDF FutureCeuticals, Inc. We are providing a point-by-point response to all your queries along with some additional relevant clarifications/discussion.

**FDA Query 1:** In Table 3 (pp. 9-10), the notifier provided specifications for the coffee fruit extract (the extract). Table 4 contains the results of the analysis of 4 batches of the extract, which demonstrate conformance with the indicated specifications. Table 5 provides the compositional analysis of the extract. It is not clear why there are 2 different sets of compositional data for the extract in Tables 4 and 5. Please explain the differences in the data that are presented in Tables 4 and 5.

**Response to FDA Query 1:** Please note that Table 4 is only intended to show batch compliance with the specifications. It is not a compositional analysis as several of the primary parameters are not included (*e.g.*, protein and carbohydrates). Table 5 provides the compositional analysis of the extract.

**FDA Query 2:** On p. 8, the notifier stated that the extract was prepared by water/ethanol extraction of the whole ground coffee fruit. However, the strength of the extraction medium is not clear. Please state the percentage (or ratio) of the water/ethanol extraction medium and indicate how the dried powder was standardized to contain ≥ 40% phenolic acids and 1-2 % caffeine.

**Response to FDA Query 2:** The extraction medium is 30% water and 70% ethanol. The standardization occurs via gravimetric phase separation. Following concentration by evaporation, the extract settles for 30 minutes, causing the denser caffeine layer to collect at the bottom of the tank. This layer is removed, leaving behind an extract containing approximately 40% phenolic acids (as chlorogenic acids) and 1-2% residual dissolved caffeine. This standardization is assured by precise temperature controls and further confirmed via HPLC analysis prior to sale.

**FDA Query 3:** The notifier did not discuss the stability of the extract under the proposed use conditions. The stability of the product should be described (e.g., the extract is stable at a certain temperature for a stated period of time) and the notifier should indicate that no significant degradation is expected over the indicated shelf life of the product.

**Response to FDA Query 3:** We have confirmed the stability of the extract over its shelf life under a storage temperature of 72°F and a humidity of 55-60%. This stability study included evaluation of the following parameters: color, moisture, total polyphenols by UV analysis, standard plate count, yeast and mold, coliforms, and *E. coli*. Accordingly, no significant degradation is expected over the indicated shelf life of the product.

**FDA Query 4:** On pp. 13-15 and in Appendix I, the notifier provided exposure estimates for the extract (Tables 7A and 7B) and caffeine (Tables 3A and 3B) for the proposed use. However, the notifier did not provide an exposure to polyphenols, which comprise greater than 40% of the coffee fruit extract. The notifier should include a discussion on the potential dietary exposure to polyphenols (> 40%), which may contribute to the overall antioxidant properties of the extract.

**Response to FDA Query 4:** As indicated in Table 5, the extract contains approximately 52% total polyphenols, including the 40% phenolic acids (as chlorogenic acids) to which the extract is standardized. Given the 90$^{th}$ percentile daily estimated intake of 393 mg/person detailed in the exposure analysis, the resulting daily intake of total polyphenols from the extract will be approximately 204 mg/person. Total ORAC (antioxidant potential) has been tested for the ingredient, which yields a result of approximately ≥6,000 µmol TE/g. This result is likely driven by the polyphenol concentration of the extract.

**FDA Query 5:** Since the product is stated to be a tan brown powder, the notifier should indicate that the extract is not intended to be used as a color additive.

**Response to FDA Query 5:** The extract is not intended to be used as a color additive. It is used solely for purposes other than coloring and any color imparted is "clearly unimportant insofar as the appearance, value, marketability, or consumer acceptability is concerned." 21 CFR 70.3(g).

**FDA Query 6:** The notifier should provide a statement indicating that all analytical methods are validated for their particular purpose.

**Response to FDA Query 6:** Yes, all analytical methods are validated for their respective purposes.

**FDA Query 7:** On page 22 (Section 6.2.1.2), the notifier states the "all user maximum intake of 5.9 mg/kg bw/day from the proposed uses of Coffeeberry® coffee fruit extract, the NOAEL is 584-fold lower." This reads like the NOAEL is 584-fold lower than the proposed intake. Please confirm that what you mean is that the all user maximum intake of 5.9 mg/kg bw/day is 584-fold lower than the NOAEL."

**Response to FDA Query 7:** We apologize for this error. We intended to state that the all user maximum intake of 5.9 mg/kg bw/day is 584-fold lower than the NOAEL.

**FDA Query 8:** On page 23 (Section 6.2.2.1, 2nd paragraph), the notifier references "Heimbach et al. (2008). Please confirm that the correct reference is Heimbach et al. (2010). If not, please provide the full reference for Heimbach et al. (2008).

**Response to FDA Query 8:** We apologize for this error. The correct reference is Heimbach et al. (2010).

**FDA Query 9:** On page 29 (Section 6.2.3.2.1), the notifier states that "whole coffee fruit powder" was administered in the clinical study by Ostojic et al. (2008). According to the article, a "coffeeberry (CB) formulation" was used in this study. Please compare the article of commerce in your GRAS notice and CB formulation used in the study to show that this study is relevant to your GRAS determination.

**Response to FDA Query 9:** The formulation studied in Ostojic et al. (2008) was a powder consisting entirely of dried whole coffee fruit and standardized to 10% total phenolic acids. Accordingly, the 800 mg/day dose provided in the study resulted in a daily exposure to 80 mg of phenolic acids. This exposure resulted in no adverse health effects. The subject of the present GRAS notice contains approximately 40% phenolic acids (as chlorogenic acids). Given the 90$^{th}$ percentile daily intake of 393 mg of this extract, the resulting daily intake of phenolic acids from the extract is 157 mg, or approximately twice what was delivered in the study. While not directly relevant to the safety of the extract, this study was included as an additional data point demonstrating the safety of phenolic acids generally as well as the source material (whole coffee fruit) from which our extract is derived.

**FDA Query 10:** On page 36 (Section 6.2.5), the notifier states that the Coffeeberry coffee fruit extract contains ≥40% of phenolic acids and up to 40% of this fraction is chlorogenic acid. Please state what the other 60% is and briefly discuss their safety.

**Response to FDA Query 10:** The premise of this question is based on a misunderstanding of the extract composition. Please allow us to correct the first sentence of Section 6.2.5. The 40% standardization is for phenolic acids *as chlorogenic acids*. Stated differently, chlorogenic acids constitute 100% of the phenolic acid standardization, not 40% as is referenced above in the comment.

**FDA Query 11:**On page 37 (Section 6.2.5.2), the notifier states that Syrian golden hamsters were administered approximately 30 mg chlorogenic acid/kg bw/day. Please explain how you calculated this dose level based on the information (i.e. the dietary dose level of 0.025%) provided in the article.

**Response to FDA Query 11:** For this calculation, we used the Conversion Table given in Priority-based Assessment of Food Additives (FDA PAFA, 1993). As per this Table for hamsters, 1% concentration of an ingredient in food is equivalent to 1200 mg/kg bw/day. As hamsters were fed a diet containing 0.025% chlorogenic acid, the equivalent dose on body weight basis is 0.025 x 1200/1 = 30 mg/kg bw/day.

**FDA Query 12:** (12) On page 37 (Section 6.2.5.2), the notifier discusses the carcinogenicity study of caffeic acid by Hagiwara et al. (1991). While not clearly described in the GRAS notice, this study showed that caffeic acid exerts carcinogenic activity "for the renal tubular cell in male rats and female mice, and for the alveolar type II cell in male mice." The notifier stated that "However,

these findings are of limited relevance as the dose of caffeic acid used (1000 to 2000 mg/kg bw/day) are vastly higher than the levels that would be expected from the exposure to Coffeeberry® Coffee Fruit Extract." Please state what the expected intake of caffeic acid from the proposed use of Coffeeberry® Coffee Fruit Extract is.

**Response to FDA Query 12:** By HPLC analysis, VDF FutureCeuticals has confirmed that caffeic acid comprises only 0.1% of the Coffeeberry® coffee fruit extract. Given the 90$^{th}$ percentile daily estimated intake of 393 mg/person detailed in the extract exposure analysis, the resulting intake of caffeic acid is .393 mg/person/day. For an individual weighing 60 kg, this calculates to .0065 mg/kg bw/day. Compared to the dose used in the caffeic acid carcinogenicity study of 1000 and 2000 mg/kg bw/day, the exposure to caffeic acid resulting from intake of the extract is approximately 150,000 to 300,000-fold lower.

Out of an abundance of caution, we also evaluated the potential intake of caffeic acid resulting from the metabolism of chlorogenic acid in the extract. It is known that chlorogenic acids are hydrolyzed or metabolized to caffeic acid in low quantities. In a pharmacokinetic profile and apparent bioavailability study of chlorogenic acid in plasma and urine of 10 healthy adults for 8 hours after the consumption of a decaffeinated green coffee extract containing 170 mg of chlorogenic acid, Farah et al. (2008) reported that the amount of caffeic acid detected in plasma was 1.1 µmol/L, or 7.4% of the total amount of chlorogenic acids detected in plasma samples. Critically, the material used in this study was standardized to 40% chlorogenic acid, which is identical to the standardization of chlorogenic acid in Coffeeberry® coffee fruit extract.

Though the complexities of metabolism make it difficult to precisely quantify metabolites such as caffeic acid, we conservatively assume based on the Farah study that approximately 10% of the chlorogenic acid in Coffeeberry® coffee fruit extract is metabolized to caffeic acid. Given the 90$^{th}$ percentile daily intake of 393 mg of the extract and the 40% standardization to chlorogenic acid, the resulting intake of caffeic acid from metabolism of chlorogenic acid is estimated at 15.7 mg/person/day (393 x 0.4 x 0.1). For an individual weighing 60 kg, this calculates to 0.26 mg/kg bw/day of caffeic acid. Factoring in the 0.1% caffeic acid present in the extract, the total estimated intake of caffeic acid from the extract, including via metabolism, is 16.1 mg/person/day. For a 60 kg human, this calculates to 0.27 mg/kg bw/day, which is approximately 3,700 to 7,400-fold lower than the 1000 and 2000 mg/kg bw/day dose used in Hagiwara et al. (1991).

In a recent review article, Farah and Lima (2019) reported that after intake of 5-caffeoylquinic acid (chlorogenic acid) or coffee, only small amounts of caffeic acid, a hydrolysis product of both caffeoylquinic acid and dicaffeoylquinic acids, had been identified and quantified in murine or human plasma and urine. Therefore, it was generally concluded that less than 1% of chlorogenic acid ingested was absorbed in animals and humans and that almost the whole ingested amount was degraded during digestion, metabolized by the intestinal microflora, and/or excreted with feces. These reviewers also reported that on average, it is estimated that a third of the amount of chlorogenic acid consumed is absorbed (including all chlorogenic acid compounds and cinnamic acids in plasma) throughout the digestive tract, with a very large variability among individuals. The unabsorbed portion of chlorogenic acid, as with other polyphenols, is extensively hydrolyzed by gut bacteria.

**FDA Query 13:** On page 37 (Section 6.2.5.2), the notifier states that "In various genotoxicity/mutagenicity studies, conducted in vitro, both positive and negative results have been obtained. However, the positive results occurred under experimental conditions that were deemed not physiologically relevant, and as such, these findings were not considered to be of concern with respect to human health. Findings from in vivo micronucleus test conducted in mice provided further evidence that chlorogenic acid was not genotoxic/mutagenic. Moreover, several in vitro and in vivo studies have suggested that chlorogenic acid may in fact possess anti-mutagenic and anti-genotoxic properties." a. Please provide the full references for these "various" genotoxicity/mutagenic studies, in vivo micronucleus test, and several in vitro and in vivo studies you are referencing. b. Please point out in these articles the information or statements about the experimental conditions employed not being relevant to humans.

**Response to FDA Query 13:** The references for the statement "several *in vitro* and *in vivo* studies suggest that chlorogenic acid may also possess anti-mutagenic and anti-genotoxic properties" mentioned in the GRAS dossier include the following: Stich et al., 1982; Wood et al., 1982; Huang et al., 1985; Chan et al., 1986; San and Chan, 1987; Aeschbacher and Jaccaud, 1990; Abraham et al., 1993; Firozi and Bhattacharya, 1995; Yamada and Tomita, 1996; Hossain et al., 1976. The findings from an *in vivo* micronucleus test performed in rats showed that chlorogenic acid was not genotoxic (Hossain et al., 1976). As such, the exposure to chlorogenic acid from the ingestion of Coffeeberry® coffee fruit extract is not expected to pose concern for genotoxicity/mutagenicity.

As the genotoxicity and mutagenicity studies of chlorogenic acid has been extensively reviewed by NTP and in other reviews, we did not summarize these studies in the GRAS Notice. We are providing a summary of available *in vitro* and *in vivo* mutagenicity and genotoxicity studies of chlorogenic acid in Appendix I (included at the end of this document). As described in the NTP (1998) report, chlorogenic acid was shown to induce DNA strand breaks in acellular assays using isolated and plasmid DNA, particularly in the presence of transition metals (*e.g.*, iron). In *in vitro* standard genotoxicity/mutagenicity assays, both positive and negative results have been obtained. The positive results have been attributed to polyphenolic thermal degradation products of chlorogenic acid that reduce oxygen in the presence of transitional metals (*e.g.*, iron), resulting in the formation of hydrogen peroxide and other oxygen radicals (NTP, 1998; Schilter et al., 2001). The addition of exogenous detoxification systems (*e.g.*, addition of S9 liver fraction) generally abolishes the genotoxicity/ mutagenicity of chlorogenic acid, suggesting formation of hydrogen peroxide is an important contributing factor. The positive results noted in *in vitro* studies with chlorogenic acid are not considered to be of relevance to human health, as the key factors involved in the production of hydrogen peroxide, such as oxygen tension and concentration of iron, are present at much higher levels under *in vitro* experimental conditions as compared to those found endogenously in the body (Schilter et al., 2001). Additionally, dietary phenolic compounds can act either as pro-oxidants or antioxidants under experimental conditions depending on the end-point measured, the mechanism of oxidation, and the concentration range tested (Schilter et al., 2001; Sakihama et al., 2002; Lee and Lee, 2006; Perron and Brumaghim, 2009).

**FDA Query 14:** While the above study by Hagiwara et al. (1991) showed carcinogenicity in the forestomach and the kidneys of rats and mice at a high dose level (i.e., 2%, the only dose level tested), the carcinogenicity of caffeic acid was also tested at lower dose levels (0.4 and 0.08%) by Hirose et al. (1998). The notifier did not discuss this study even though the notifier dismissed the

1991 carcinogenicity study results based on the high dose level used. The study from 1998 showed that the compound is still carcinogenic at a lower dose level, hence, the above argument does not hold. Please discuss the results of the Hirose et al. (1998) study in detail. Please make sure you include the following information:

a. Please state whether a NOEL (i.e. no observed effect level) exist for carcinogenicity for caffeic acid and what that NOEL is. If this carcinogenicity NOEL is different for kidney and forestomach carcinogenicity, please state the NOEL for each. Please discuss what the margins of exposure are between the NOELs for kidney and forestomach carcinogenicity and the expected intake of caffeic acid from the proposed use of Coffeeberry® Coffee Fruit Extract.

b. Please state what the NOEL or NOAEL is for general toxicity (i.e. for noncarcinogenic endpoint(s)) and what non-carcinogenic effects were reported.

c. Please expand your discussion on why the observed carcinogenicity results are not relevant to humans. If they are relevant, please explain why you have no safety concern.

Reference: Hirose, M., Takesada, Y., Tanaka, H., Tamano, S., Kato, T., & Shirai, T. (1998). Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4methoxyphenol and catechol at low doses, either alone or in combination, and modulation of their effects in a rat medium-term multi-organ carcinogenesis model. Carcinogenesis, 19(1), 207-212.

**Response to FDA Query 14:** Thank you for bringing the Hirose et al. study to our attention. In this study by Hirose et al. (1997; although PubMed gives the year 1998, publication year is 1997), carcinogenicity of low dietary levels of the antioxidants butylated hydroxyanisole (BHA), caffeic acid, sesamol, 4-methoxyphenol (4-MP) and catechol, known to target the forestomach or glandular stomach, were examined alone or in combination in a 2-year long-term experiment and their modifying effects assessed in a medium-term multiorgan model. As the study was conducted primarily for the carcinogenicity, the general toxicity points were minimum and limited to body weight, organ weight, and histopathological findings. Accordingly, we find it difficult to establish a NOEL for general toxicity. We have attempted to address your queries related to carcinogenicity parameters as below:

**14a(1). Please state whether a NOEL (i.e. no observed effect level) exists for carcinogenicity for caffeic acid and what that NOEL is.**

Yes, a NOEL does exist. It is 0.08% for the forestomach and 0.4% for the kidney. Please see below.

- Hirose et al Carcinogenesis 19(1): 207-212; 1997
    - 2-yr diet carcinogenicity study: Groups of 6-wk old 30-31 male F344 rats, 0.4% Caffeic acid. Result: slightly increased incidence of forestomach papillomas observed compared to basal diet (14.8% vs 0),
    - Medium-term multi-organ model: Groups of 10-15 male F344 rats given known carcinogens (DEN, MNU, DMH, BBN, DHPN) for 1 month to initiate forestomach or glandular stomach carcinogenic process and then given caffeic acid at 0.08% or 0.4%. Result: increased incidence of forestomach papillomas and/or carcinomas were noted only at high dose 0.4% caffeic acid. Caffeic acid by itself did not increase any tumor burden at low dose 0.08%; however, it is NOT clear whether this low dose has contributed to increased incidence of forestomach papillomas seen in combination with other synthetic phenolic antioxidants used in the study.

- Interestingly, in the high dose combination group, the incidence of colon tumors was significantly decreased. Again, it is NOT clear the proportion of contribution to this beneficial effect by caffeic acid.

Based on these data and the overall weight of evidence (carcinogenic potential vs. anticarcinogenic effect): it is concluded that NOEL for caffeic acid is 0.08% based on forestomach papillomas.

**14a(2). If this carcinogenicity NOEL is different for kidney and forestomach carcinogenicity, please state the NOEL for each.**

In the above study, the NOEL for forestomach is 0.08%, and the NOEL for kidney is 0.4%.

**14a(3). Please discuss what the margins of exposure are between the NOELs for kidney and forestomach carcinogenicity and the expected intake of caffeic acid.**

- Direct Exposure from Intake:
  i. Coffeeberry® coffee fruit extract contains 0.1% caffeic acid, which at the 90th percentile estimated daily intake, amounts to 0.393 mg/person/day (0.006 mg/kg bw/day).
  ii. The caffeic acid NOEL for the forestomach was 0.08%.
    - The study did not state a mg/kg bw/day so we used WHO guidelines[1], in which 0.08% corresponds to 800 mg/kg of feed. This calculates to 64 mg/kg bw/day.
  iii. The caffeic acid NOEL for the kidney was 0.4%
    - The same reference to WHO guidelines was used. 0.4% corresponds to 4000 mg/kg of feed. This calculates to 1600 mg/kg bw/day.
  iv. **Comparison Forestomach: This represents a 9,864-fold safety margin (64/0.0065)**
  v. **Comparison Kidney: This represents a 246,154-fold safety margin (1,600/0.0065)**
- Exposure Including Potential Metabolism of Caffeic Acid:
  i. As mentioned above, we conservatively estimate that possibly 10% of the chlorogenic acid could be converted into caffeic acid, and in an abundance of caution have evaluated the safety margin assuming such metabolism.
  ii. For the Coffeeberry® coffee fruit extract:
    1. It contains 40% chlorogenic acid, and at the 90th percentile estimated daily intake of the extract, the dose of chlorogenic acid is 157.2 mg.
    2. Conservatively assuming 10% metabolism of chlorogenic acid into caffeic acid, the intake of caffeic acid would be 15.72 mg/day.
    3. Adding 15.72 mg to the .393 mg resulting from intake of caffeic acid present in the extract, the total intake is 16.1 mg/day. This results in 0.27 mg/kg bw/day for a 60 kg man.
  vi. For NOAEL, as above, for forestomach the result is 64 mg/kg bw/day.

[1] Available in: Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives, 2000.

vii. For NOAEL, as above, for kidney the result is 1600 mg/kg bw/day.
viii. **Comparison Forestomach: This represents a 237-fold safety margin (64/0.27).**
ix. **Comparison Kidney: This represents a 5,926-fold safety margin (1600/0.27).**

**FDA Query 15:** Please discuss the results of the Chaube and Swinyard (1976) safety studies for caffeic acid and compare NOAELs and LOAELs (only if no NOAEL is available) to the intake levels of caffeic acid from the proposed uses.

**Response to FDA Query 15:** It should be noted that the Chaube and Swinyard (1976) studies were conducted following intraperitoneal injections to rats. The findings from this study are of limited toxicological relevance to the oral intakes of Coffeeberry® coffee fruit extract since the test article was administered at high doses through parenteral routes.

In the acute toxicity study described in Chaube and Swinyard (1975), 24 female Wistar rats (9 week old) were administered caffeic acid (purity not provided) at doses of 400 to 1500 mg/kg bw (2.22-8.326 mmol/kg) intraperitoneally. The rats were observed for 11 days. The 1500 mg/kg (8.326 mmol/kg) intraperitoneal dose of caffeic acid induced death in 5 of 8 rats, but doses lower than 1250 mg/kg (6.938 mmol/kg) were nontoxic.

In the short-term study, female Wistar rats were administered caffeic acid intraperitoneally at doses of 20, 40, 100 and 187.5 mg/kg bw/day daily for 8 days. For this study, 9 female rats were used at two lower doses and six female each at 2 higher doses. The rats were euthanized 9 days post-treatment. No lethality was noted.

In the developmental toxicity study, caffeic acid was administered by intraperitoneal route to pregnant rats on days 5 to 12 of the gestation at dose levels of 40, 60, 100 or 187.5 mg/kg/day. In this study, 5 pregnant rats were used at two lower doses and 6 pregnant rats were used at two higher doses. The rats were euthanized on day 21 of gestation. Treatment with caffeic acid did not induce maternal or fetal mortality. No central nervous system defects were observed. In 4% of the fetuses (12/274) rib defects were noted and growth of one fetus was severely retarded, whereas the control group (0/356) had no such effect. The results of this study show that intraperitoneal administration of caffeic acid (40-187.5 mg/kg bw/day) on days 5 through 12 of gestation induced rib defects in 4% of the fetuses. Fetal central nervous system defects and maternal and fetal mortality were not induced at any dose. Given this, the lack of dose-response in the incidence of skeletal abnormalities would indicate that this is not a treatment-related effect. The NOAEL for developmental toxicity may be considered as 187.5 mg/kg bw/day of caffeic acid, the highest dose tested. As compared to the resulting intake of caffeic acid from its presence in Coffeeberry® coffee fruit extract (0.39 mg/person/day or 0.006 mg/kg bw/day), the NOAEL from the rat study is 31,250-fold higher. As mentioned above, the relevance of these findings to the intended oral administration of Coffeeberry® coffee fruit extract is limited by the intraperitoneal route of administration as only a fraction of caffeic acid from chlorogenic acid appears to be absorbed following oral consumption.

**FDA Query 16:** Please discuss the relevant results of the following toxicology studies and what effect, if any, they have on your GRAS conclusion. Please make sure to include a discussion on

what level of caffeic acid intake can be considered safe based on the information presented in these three studies and the studies by Hagiwara et al. (1991), Hirose et al. (1998), and Chaube and Swinyard (1976) and compare this with the intake level from the proposed uses:

a. Kagawa, M., Hakoi, K., Yamamoto, A., Futakuchi, M., & Hirose, M. (1993). Comparison of reversibility of rat forestomach lesions induced by genotoxic and non-genotoxic carcinogens. Japanese journal of cancer research, 84(11), 1120-1129.

b. Ito, N., Hirose, M., & Takahashi, S. (1993). Cell proliferation and forestomach carcinogenesis. Environmental health perspectives, 101(suppl 5), 107-110.

c. Lutz, U., Lugli, S., Bitsch, A., Schlatter, J., & Lutz, W. K. (1997). Dose response for the stimulation of cell division by caffeic acid in forestomach and kidney of the male F344 rat. Toxicological Sciences, 39(2), 131-137.

**Response to FDA Query 16:** In the study by Ito et al. (1993), the researchers analyzed the role of cell proliferation in the forestomach of rats caused by several phenolic compounds, such as BHA, caffeic acid, sesamol, 4-methoxyphenol and 4-methylcatechol. The researchers state that the study "was performed to analyze early histopathological changes induced by these carcinogens in rat forestomach epithelium, as well as oncogene expression and reversibility of early forestomach lesions to clarify the role of cell proliferation and toxicity in forestomach carcinogenicity induced by phenolic compounds."

Please note, given that the subject of this GRAS Notice is coffee fruit extract, our discussion will focus only on the research and results reported for caffeic acid. In this study, twenty-five 6-week-old F344 male rats were fed a powdered basal diet containing 2% caffeic acid or the basal diet alone. The animals were killed for histological examination after 12 hr., 1, 3, or 7 days. For the reversibility study, F344 male rats were treated with caffeic acid at a dose of 2% in powdered basal diet for 24 weeks, and after cessation of caffeic acid treatment, basal diet alone was supplied for a further 24 weeks. A control group of rats were treated with basal diet alone throughout the experiment. Ten animals were killed at weeks 24 and 48 and the forestomach epithelium examined histopathologically for lesion development.

DNA synthesis in the mid-region of the forestomach epithelium, expressed as the number of labeled cells per 100 basal cells, increased 12 hours after treatment with caffeic acid. Hyperplasia was observed 3 days after treatment with caffeic acid at an incidence of 80%; toxic changes such as erosion or ulceration developed in 60% of the animals treated with caffeic acid (Fig. 3, page 108 of the article).

In the reversibility study, moderate hyperplasia was found in all animals treated with caffeic acid; however, the incidence of moderate hyperplasia had decreased to 20% for caffeic acid after cessation of chemical treatment. Also, atypical hyperplasia, which was not observed at 24 weeks, was found 24 weeks later in 10 of the animals receiving caffeic acid.

It should be noted that humans do not have a forestomach. It is not clear from this report to what extent the control animals were examined or what data was obtained from the control animals. However, the authors state that the experiments clearly show that caffeic acid induces increases in DNA synthesis within 3 days after treatment followed by hyperplasia with evidence of epithelial

damage. The authors conclude that the process of forestomach carcinogenesis by non-genotoxic compounds is complex and further studies are required to clarify the factors that cause these changes.

In the study by Kagawa et al. (1993), the reversibility of rat forestomach lesions induced by caffeic acid and other phenolic compounds (non-genotoxic) was studied and compared to genotoxic carcinogens. While the results of this study are comparable to the results of the Ito et al. (1993) study, the authors conclude that caffeic acid can induce strong cell proliferation as well as cytotoxicity in the forestomach, with subsequent tumor development in both male and female rats. However, the authors note that in the forestomach, simple papillary hyperplasia was reversible. The authors also note that the mechanisms by which caffeic acid ("these phenolic compounds") hyperplasia are not fully understood. Also, the authors did not report any data related to the control animals that could be compared. The authors noted that "…caffeic acid could also cause metal-dependent DNA damage through $H_2O_2$ formation *in vitro*. In addition, mutagenic compounds could be formed in the stomach by interaction of amines and nitrite, or nitrite and phenolic compounds, both of the latter being commonly present in the diet. Thus, it is possible that during strong cell proliferation, small amounts of genotoxic compounds such as hydroquinone metabolites, quinone metabolites, active oxygen species or food-derived mutagens interact with forestomach DNA and result in forestomach cell transformation even with 'so-called' non-genotoxic forestomach carcinogens." Further research is needed in this area to further understand the causative effects of carcinogenicity in the rat forestomach and its relationship to humans.

In the study by Lutz et al. (1997), caffeic acid was investigated in male F344 rats after a 4-week feeding study at different dietary concentrations (0, 0.05, 0.14, 0.40, and 1.64%) and the dose response relationship in the forestomach and kidney was determined. Cells in S-phase of DNA replication were visualized by immunohistochemical analysis of incorporated 5-Bromo-2'-deoxyuridine (BrdU), 2 hours after intraperitoneal injection. In the forestomach, both the total number of epithelial cells per millimeter section length and the unit length labeling index of BrdU-positive cells (ULLI) were increased, about 2.5-fold, at 0.40 and 1.64%. The lowest concentration (0.05%) had no effect. At 0.14%, both variables were decreased by about one-third. In the kidney, the labeling index in proximal tubular cells also indicated a J-shaped (or U-shaped) dose response with a 1.8-fold increase at 1.64%. In the glandular stomach and in the liver, which are target organs, no dose-related effect was seen. The data show a good correlation between the organ specificity for cancer induction and stimulation of cell division. The authors conclude that, with respect to the dose-response relationship and the corresponding extrapolation of the animal tumor data to a human cancer risk, a linear extrapolation appears not to be appropriate. The authors further conclude that, "… on the basis of all data combined [ … ] the cancer risk for humans associated with the dietary intake of caffeic acid might be negligible."

In addition to the above described studies and also studies described in the GRAS dossier, a comprehensive review of the scientific literature pertaining to the toxicology of caffeic acid has been conducted by The National Toxicology Program (NTP, 1998), and the International Agency for Research on Cancer (IARC) (IARC, 1993). Caffeic acid administered intraperitoneally at 1500 mg/kg produced mortality in 5 out of 8 rats, but no lethality or other signs of toxicity were observed at doses of 1250 mg/kg or less (Chaube and Swinyard, 1976). From the available repeated-dose studies, a notable finding was the observation of hyperplasia in the forestomach epithelium in rats orally administered caffeic acid. This was observed after rats fed with a diet

containing 2% caffeic acid, corresponding to exposure levels of approximately 2000 mg/kg bw/day, for 4 weeks (FDA PAFA, 1993) (Hirose et al., 1987). As described above, forestomach hyperplasia was also observed in rats consuming caffeic acid at doses of up to 2% in the diet [equivalent to approximately 2,000 mg/kg bw/day (FDA PAFA, 1993)] for up to 24 weeks (Ito et al., 1993; Kagawa et al., 1993).

Hyperplasia of the forestomach was also seen in rats exposed to caffeic acid at either 1% or 2% in the diet [equivalent to approximately 1000 to 2000 mg/kg bw/day (FDA, 1993)] for durations of up to 2 years (Hirose et al., 1986, 1992, 1997; Hagiwara et al., 1991; Ito et al., 1993; Kagawa et al., 1993). Squamous-cell papillomas and carcinomas of the forestomach were also observed in rats treated with 2% caffeic acid in the diet of one chronic study lasting 104 weeks (Hagiwara et al., 1991). In this study, a higher incidence of renal-cell hyperplasia was also observed in male and female rats treated with caffeic acid (70% and 20%, respectively), and 13% of the male rats developed tubular cell adenoma, while none of the control animals exhibited these findings (Hagiwara et al., 1991). These findings were associated with the development of chronic nephropathy.

In male and female mice, chronic treatment with caffeic acid at 2% in the diet [equivalent to 3000 mg/kg body weight/day (FDA PAFA, 1993)] for 96 weeks also produced a significant increase in the incidence of forestomach hyperplasia, with a higher combined incidence of squamous-cell papillomas and carcinomas of the forestomach in male mice (Hagiwara et al., 1991). Chronic exposure to caffeic acid was found to produce renal tubular-cell hyperplasia in male and female mice, and renal-cell adenomas in female mice, though no chronic nephropathy was observed (Hagiwara et al., 1991). Based on these findings, the IARC Working Group (IARC, 1993) has classified caffeic acid as “possibly carcinogenic to humans (Group 2B)”.

In order to interpret the above findings, several important factors need to be considered. As humans do not have forestomach, the observation of forestomach hyperplasia and carcinomas induced by caffeic acid is of limited relevance. The rodent forestomach is located between the esophagus and glandular stomach, and serves the purpose of holding food prior to digestion in the glandular stomach and passage into the intestines (reviewed in Clayson et al., 1990; Hirose and Ito, 1999). Humans possess histologically similar organs such as the oral cavity, pharynx, esophagus, and glandular stomach. However, there are important functional and anatomical differences from rodent forestomach that may limit the relevance of this animal model (Proctor et al., 2007). Moreover, the dose levels of caffeic acid where carcinomas of the forestomach and kidneys were observed (*i.e.*, 1000 to 3000 mg/kg bw/day) are extremely high compared to the estimated intake of caffeic acid from Coffeeberry® coffee fruit extract (0.006 mg/kg bw/day based on actual amount of caffeic acid in the extract, or 0.27 mg/kg bw/day including estimated quantities of caffeic acid metabolized from chlorogenic acid). Based on this discussion, it can be concluded that caffeic acid in Coffeeberry® coffee fruit extract is safe.

The most common source of caffeic acid in the human diet is from drinking coffee. However, it is also found in vegetables, fruits, and herbs, all of which are routinely consumed by humans. Some examples of foods containing caffeic acid include: coffee, wine, turmeric, basil, thyme, oregano, sage, cabbage, apples, strawberries, cauliflower, radishes, mushrooms, kale, pears, olive oil [Source: http://phenol-explorer.eu/contents/polyphenol/457]. Given the wide range of sources from which caffeic acid is consumed in the diet, it is reasonable to conclude that any possible adverse effects resulting from the consumption of caffeic acid would have been detected by now.

The amount of caffeic acid in Coffeeberry® coffee fruit extract is very small and the resulting intake of caffeic acid from the proposed uses is highly unlikely to cause any adverse effects.

**FDA Query 17:** For future reference, no response needed, please note that you must discuss all safety relevant studies including those reporting adverse effects and you need to properly explain why your article of commerce can still be regarded as GRAS despite of those adverse effects.

**FDA Query 18:** If the Reyes-Izquierdo et al. (2013a) human study or any of the animal efficacy studies the notifier mentions in the notice has safety related data that was not included in the notice, please report this information. For future reference, please note that for human clinical efficacy studies and animal efficacy studies, only safety data should be reported and no efficacy outcomes as these are not considered by FDA during its review. Efficacy studies or results (such as tumor suppressing activity or its lack thereof) without the presence of safety data does not support your GRAS conclusion. This had also been communicated to the agent during the pre-submission meeting for the article of commerce.

**Response to FDA Query 18:** The study by Reyes-Izquierdo et al. (2013a) was included in the interest of thoroughness as the extract was tested on human subjects with no adverse health effects reported. Nevertheless, we acknowledge that the study did not include safety related data and is therefore not directly relevant to the safety of the extract.

* * *

We hope the above information and clarification addresses your queries. If you have any questions or need additional explanation, please let me know.

Thank you for the opportunity to provide this explanation to your questions.

Sincerely,

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**Appendix I. Summary of Genotoxicity Studies of Chlorogenic Acid**

| Reference | Assay | Test System | Concentration | Results |
|---|---|---|---|---|
| ***In Vitro*** | | | | |
| MacGregor and Jurd (1978) | Ames Test | *Salmonella typhimurium* TA98 | 58.8 or 588 µg/plate | Negative (+/- S9) |
| Stich et al. (1981) | Ames Test | *S. typhimurium* TA98, TA100 | 19 or 28 mg/plate | Negative (+/- S9)<br>Positive in both strains in the presence of $Mn^{2+}$ but not $Cu^{2+}$. Assays using transition metals were tested in the absence of S9 |
| San and Chan (1987) | Ames Test | *S. typhimurium* TA98 | The suspension method was used, and concentrations of 1, 3, 6, or 9 mg/mL were tested (3, 9, 20 or 30 mM) | Negative (+S9) |
| Fung et al. (1988) | Ames Test | *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 | Up to 10,000 µg/plate | Negative (+/- S9) |
| Duarte et al. (2000) | Ames Test | *S. typhimurium* TA100 | Up to 50 nmol/plate | Negative in absence of nitrosation<br><br>Positive with nitrosation |
| Stich et al. (1981) | Gene conversion | *Saccharomyces cerevisiae* | Up to 80 mg/mL | Positive (-S9)<br>Negative (+S9)<br>Positive in the presence of $Cu^{+2}$ and $Mn^{+2}$. Assays using transition metals were tested in the absence of S9 |
| Rosin (1984) | Gene conversion | *S. cerevisiae* | 1 mg/mL, tested at pH of 10 | Positive (-S9) |

**Appendix I. Summary of Genotoxicity Studies of Chlorogenic Acid**

| Reference | Assay | Test System | Concentration | Results |
|---|---|---|---|---|
| Fung et al. (1988) | Mammalian cell mutation | Mouse lymphoma L5178Y cells | Up to 10,000 µg/mL | Negative (-S9)<br>Positive (+S9) |
| Wood et al. (1982) | Mammalian cell mutation | Chinese Hamster V79-6 cells | 177 µg/mL | Negative (-S9) |
| Whitehead et al. (1983) | Chromosomal aberration | Chinese Hamster Ovary cells | Up to 250 µg/mL | Negative (+S9)<br>Positive (-S9)<br>Cultured mouse intestinal cells were used as a activation enzyme source:<br>Negative (+cells)<br>Positive (-cells) |
| Stich et al. (1981) | Chromosomal aberration | Chinese Hamster Ovary cells | Up to 40 µg/mL | Negative (+S9)<br>Positive (-S9)<br><br>The positive effect was enhanced by the presence of $Cu^{+2}$ and $Mn^{+2}$ |
| ***In Vivo*** | | | | |
| Hossain et al. (1976) | Micronucleus test | Rat bone-marrow cells | 150 mg/kg orally as 2 doses administered 24 hours apart | Negative |

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