

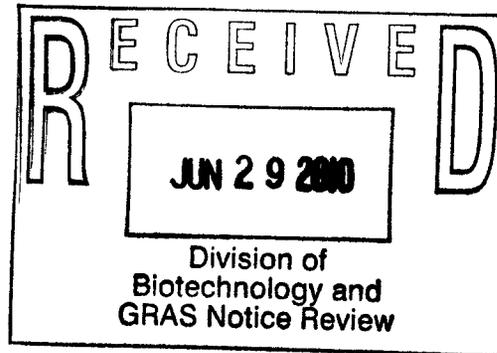
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June 25, 2010

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



**RE: Notice of GRAS Exemption Claim for Use of Caffeine in Alcoholic Beverages**

Dear Dr. Cheeseman:

In accordance with proposed 21 C.F.R. § 170.36 (a notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 Fed. Reg. 18937-18964), I am submitting, as the agent of the notifier, Phusion Projects, LLC, the following information:

**1. GRAS Exemption Claim**

Phusion Projects, LLC, on the advice of a Panel of qualified experts, has determined caffeine to be generally recognized as safe ("GRAS") and, therefore, exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act, under the conditions of its intended use as described below. The basis for this finding and supporting information is provided below.

**(i) Name and Address of the Notifier**

Phusion Projects, LLC  
1658 N. Milwaukee Ave., Suite 424  
Chicago, IL 60647-5651

**Agent of the Notifier:**

George A. Burdock, Ph.D.  
*Diplomate*, American Board of Toxicology  
*Fellow*, American College of Nutrition  
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**(ii) Common Name of the Notified Substance**

The common name of the notified substance is caffeine.

**(iii) Conditions of Use**

Caffeine may be used as an flavoring ingredient in alcoholic beverages at levels of up to 200 parts per million (“ppm”).

**(iv) Basis of GRAS Determination**

Pursuant to 21 C.F.R. § 170.3, the use of caffeine as an ingredient in alcoholic beverages at up to 200 ppm, has been determined generally recognized as safe (GRAS) by scientific procedures.

On the basis of the data and information described in the attached dossier and other publicly available information, a panel of experts qualified by scientific training and experience to evaluate the safety of substances added to food, has determined there is reasonable certainty that caffeine is GRAS under the intended conditions of use.

**(v) Availability of Information**

The basis for this GRAS determination are available for FDA review and copying at reasonable times at 801 N. Orange Ave. Suite 710, Orlando, FL 32801 or will be sent to FDA upon request.

**2. Detailed Information about the Identity of the Notified Substance**

**A. Identity**

Caffeine has a bitter taste, is odorless and occurs as a white powder or as white needles. Caffeine may be compacted or compressed into granular or pellet forms. In its anhydrous form, caffeine contains one molecule of water of hydration; caffeine in solution is neutral in pH. The general descriptive characteristics of caffeine are presented in Table 1.

**Table 1. General description of caffeine**

|                       |   |
|-----------------------|---|
| Appearance            | White powder  |
| Packaging             | Package in containers with tight closure  |
| Storage               | Store hydrous caffeine in tight containers and anhydrous caffeine in well closed containers |
| Stability             | Stable under ordinary conditions of use and storage   |
| Labeling              | Caffeine  |
| Functionality in Food | Flavor ingredient   |

**B. Common or Usual Name:**

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Caffeine is the common name, although synonyms include 1,3,7-trimethyl xanthine; 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- ; and 3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dione.

### C. Composition

Caffeine is an alkaloid with the empirical formula of  $C_8H_{10}N_4O_2$  or  $C_8H_{10}N_4O_2 \cdot H_2O$ . The chemical structure for anhydrous caffeine is shown in Figure 1.

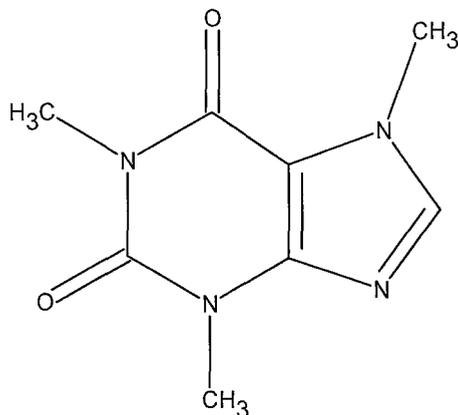


Figure 1. Chemical structure of caffeine (anhydrous)

### D. Method of Manufacture of Caffeine

Caffeine is obtained from tea dust, as a by-product from the manufacture of de-caffeinated coffee or synthetically prepared *via* several methods, including from dimethylurea and malonic acid.

### E. Specifications for Food Grade Caffeine

Specifications provided in Table 2 for bulk caffeine include formula weight, lead, solubility, and residue on ignition.

**Table 2. Specifications for caffeine**

| <b>Characteristics</b>          | <b>Description</b>   |
|---------------------------------|--|
| CAS                             | 58-08-2  |
| Synonyms                        | 1,3,7-Trimethylxanthine  |
| Formula                         | C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> or C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> ·H <sub>2</sub> O |
| Formula weight, anhydrous       | 194.19   |
| Formula weight, monohydrate     | 212.21   |
| Physical properties             | White powder; odorless; bitter taste   |
| Solubility (hydrated caffeine)  | In 50 ml water, 75 ml alcohol, 6 ml chloroform and 600 ml ether  |
| Functional use in foods         | Flavoring agent  |
| Assay                           | Not less than 98.5% and not more than 101.0% of C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>                                   |
| Lead                            | Not more than 1 mg/kg  |
| Melting range                   | Between 235° and 237.5°C   |
| Other alkaloids                 | Passes test  |
| Readily carbonizable substances | Passes test  |
| Residue on ignition             | Not more than 0.1%   |
| Water                           | Anhydrous caffeine: not more than 0.5%; hydrous caffeine: not more than 8.5%   |
| Storage                         | Store hydrous caffeine in tight containers and anhydrous caffeine in well closed containers  |

Thank you very much for your cooperation. If you have any question, please feel free to contact me.

Best regards,

(b) (6)

George A. Burdock, Ph.D.  
*Diplomate, American Board of Toxicology*  
*Fellow, American College of Nutrition*



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**DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED  
AS SAFE (GRAS) STATUS OF CAFFEINE AS AN  
INGREDIENT IN ALCOHOLIC BEVERAGES**

**May 27, 2010**

**FINAL**

**Panel Members:**

**Sidney Green Jr., Ph.D., Fellow ATS**

**Michael P. Holsapple, Ph.D., Fellow ATS**

**Steve Saunders, Ph.D.**

**Thomas N. Thompson, Ph.D.**

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**DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS)  
STATUS OF CAFFEINE AS AN INGREDIENT IN ALCOHOLIC BEVERAGES**

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# DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF CAFFEINE AS AN INGREDIENT IN ALCOHOLIC BEVERAGES

## 1. EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel<sup>1</sup>), qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was requested by Sidley Austin LLP (hereafter referred to as Sidley Austin, on behalf of its client, Phusion Projects, LLC (Phusion)) to determine the Generally Recognized As Safe (GRAS) status of caffeine, based on scientific procedures, when added to alcoholic beverages at up to 200 parts *per* million (ppm). The client assures that all relevant, unpublished information in its possession related to the safety of caffeine has been supplied to Burdock Group and has been summarized in this dossier. A comprehensive search of the scientific literature was conducted by Burdock Group, through March 2010, for safety and toxicity information on caffeine and related substances and has been summarized in this dossier as well. Consumption analysis indicates that the addition of caffeine to alcoholic beverages would result in the consumption of caffeine at mean and 90<sup>th</sup> percentile levels of 156 and 360 mg caffeine/day, respectively. Consumption of caffeine at 360 mg/day from alcoholic beverages (when caffeine is added at 200 ppm or 0.2 mg/ml) would mean that in order to consume 360 mg caffeine, a person would need to consume over 5 12-ounce beers, 7.5 servings of malt liquor, 12 servings of wine, or 40 servings of liquor. Total daily intake of caffeine from all sources at mean and 90<sup>th</sup> percentiles would be not greater than 349 and 746 mg/day (approximately equivalent to 5.8<sup>2</sup> and 12.4 mg/kg bw/day), respectively. Absorption studies indicate that caffeine does not affect ethanol absorption or excretion. Ethanol has been shown to reduce the clearance of caffeine, but this alteration is not expected to cause an unsafe increase in the body burden of caffeine. That information, along with supporting documentation, was made available to the Expert Panel as a dossier. In addition, the Expert Panel independently evaluated materials deemed appropriate and necessary. Following an independent, critical

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<sup>1</sup> Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. *See also* attachments (*i.e.*, *curriculum vitae*) documenting the expertise of the Panel members.

<sup>2</sup> Assumes the average body weight of the consumer is 60 kg.

evaluation, the Expert Panel conferred and unanimously agreed that caffeine is safe when added to alcoholic beverages at up to 200 ppm.

## 2. INTRODUCTION

Caffeine (1,3,7-trimethyl xanthine; CAS No. 58-08-2) is a water soluble plant alkaloid with an empirical formula of  $C_8H_{10}N_4O_2$  and a molecular weight of 194.19. In pure form, it is a white powder with a bitter taste, a characteristic which provides flavoring properties. The chemical structure is provided in Figure 1. There is no difference in chemical structure or characteristics of caffeine whether sourced naturally or when synthesized. Caffeine acts in the body as a central nervous system stimulant (Prothro, 1997; Dews *et al.*, 2002). Caffeine is a methylxanthine naturally found in a variety of plants distributed worldwide. Caffeine is found naturally in many foods, although the primary sources of caffeine include coffee (*Coffea Arabica* and *C. robusta*), kola nuts (*Cola acuminata* and other spp.), tea (*Thea sinensis* and other spp.), and chocolate (*Theobroma cacao* and other spp.) (Apgar and Tarka, 1999). In addition to its natural presence in commonly consumed foods, caffeine is used as a food ingredient, and is a component of several pharmaceutical preparations<sup>3</sup>. As a food ingredient, caffeine is generally considered safe based on a long established history of use and on extensive research conducted over more than a century. The caffeine content of some common food products consumed in the US is provided in Table 1 and Table 2. This dossier is a summary of the scientific evidence that supports the general recognition that caffeine is safe for human consumption as a food ingredient when added to alcoholic beverages.

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<sup>3</sup> Pharmaceutical Online Database, <http://thedrugsinfo.com/?squery=Caffeine> (last visited April 11, 2010).

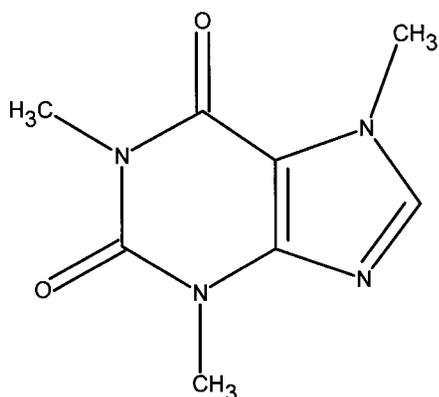


Figure 1. Chemical structure of caffeine (ChemIDplus, 2010)

Table 1. Caffeine content of some common US food products (CSPI, 2009)

| Coffees  | Serving*<br>(oz) | Serving<br>(ml) | Caffeine (mg)          | Caffeine<br>(ppm)        | Dose<br>(mg/kg) |
|--|------------------|-----------------|------------------------|--------------------------|-----------------|
| Coffee, generic brewed                                     | 8                | 236.6           | 133<br>(range:102-200) | 562.13<br>(431 - 845)    | 2.22            |
| Starbucks Brewed Coffee<br>(Grande)                        | 16               | 473.2           | 320                    | 676.25                   | 5.33            |
| Einstein Bros. regular coffee                              | 16               | 473.2           | 300                    | 633.98                   | 5.00            |
| Dunkin' Donuts regular coffee                              | 16               | 473.2           | 206                    | 435.33                   | 3.43            |
| Starbucks Vanilla Latte (Grande)                           | 16               | 473.2           | 150                    | 316.99                   | 2.50            |
| Coffee, generic instant                                    | 8                | 236.6           | 93<br>(range: 27-173)  | 393.07<br>(114 - 731)    |                 |
| Coffee, generic decaffeinated                              | 8                | 236.6           | 5<br>(range: 3-12)     | 21.13<br>(13 - 51)       |                 |
| Starbucks Espresso, doppio                                 | 2                | 59.16           | 150                    | 2535.50                  | 2.50            |
| Starbucks Frappuccino Blended<br>Coffee Beverages, average | 9.5              | 281.01          | 115                    | 409.24                   | 1.92            |
| Starbucks Espresso, solo                                   | 1                | 29.58           | 75                     | 2535.50                  | 1.25            |
| Einstein Bros. Espresso                                    | 1                | 29.58           | 75                     | 2535.50                  | 1.25            |
| Espresso, generic  | 1                | 29.58           | 40<br>(range: 30-90)   | 1352.27<br>(1014 - 3043) | 0.67            |
| Starbucks Espresso decaffeinated                           | 1                | 29.58           | 4                      | 135.23                   | 0.07            |
| Teas   | Serving*<br>(oz) | Serving<br>(ml) | Caffeine (mg)          | Caffeine<br>(ppm)        | Dose<br>(mg/kg) |
| Tea, brewed  | 8                | 236.6           | 53<br>(range: 40-120)  | 224.01<br>(169 - 507)    | 0.88            |
| Starbucks Tazo Chai Tea Latte<br>(Grande)                  | 16               | 473.2           | 100                    | 211.33                   | 1.67            |
| Snapple, Lemon (and diet<br>version)                       | 16               | 473.2           | 42                     | 88.76                    | 0.70            |
| Snapple, Peach (and diet version)                          | 16               | 473.2           | 42                     | 88.76                    | 0.70            |
| Snapple Raspberry (and diet<br>version)                    | 16               | 473.2           | 42                     | 88.76                    | 0.70            |

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|                                 |    |        |    |       |      |
|---------------------------------|----|--------|----|-------|------|
| Arizona Iced Tea, black         | 16 | 473.2  | 32 | 67.62 | 0.53 |
| Nestea                          | 16 | 354.96 | 26 | 73.25 | 0.43 |
| Snapple, Just Plain Unsweetened | 16 | 473.2  | 18 | 38.04 | 0.30 |
| Arizona Iced Tea, green         | 16 | 473.2  | 15 | 31.70 | 0.25 |
| Snapple, Kiwi Teawi             | 16 | 473.2  | 10 | 21.13 | 0.17 |

| Soft Drinks  | Serving*<br>(oz) | Serving<br>(ml) | Caffeine (mg)     | Caffeine<br>(ppm) | Dose<br>(mg/kg) |
|--|------------------|-----------------|-------------------|-------------------|-----------------|
| FDA official limit for cola and pepper soft drinks | 12               | 354.96          | 71                | 200.02            | 1.18            |
| Vault  | 12               | 354.96          | 71 (20 oz. = 118) | 200.02            | 1.18 (1.97)     |
| Jolt Cola  | 12               | 354.96          | 72                | 202.84            | 1.20            |
| Mountain Dew MDX, regular or diet                  | 12               | 354.96          | 71 (20 oz. = 118) | 200.02            | 1.18 (1.97)     |
| Coke Black   | 12               | 354.96          | 69 (20 oz. = 115) | 194.39            | 1.15 (1.92)     |
| Coke Red, regular or diet                          | 12               | 354.96          | 54 (20 oz. = 90)  | 152.13            | 0.90 (1.5)      |
| Mountain Dew, regular or diet                      | 12               | 354.96          | 54 (20 oz. = 90)  | 152.13            | 0.90 (1.5)      |
| Pepsi One  | 12               | 354.96          | 54 (20 oz. = 90)  | 152.13            | 0.90 (1.5)      |
| Mellow Yellow                                      | 12               | 354.96          | 53                | 149.31            | 0.88            |
| Diet Coke  | 12               | 354.96          | 47 (20 oz. = 78)  | 132.41            | 0.78 (1.30)     |
| Diet Coke Lime                                     | 12               | 354.96          | 47 (20 oz. = 78)  | 132.41            | 0.78 (1.30)     |
| TAB  | 12               | 354.96          | 46.5              | 131.00            | 0.78            |
| Pibb Xtra, Diet Mr. Pibb, Pibb Zero                | 12               | 354.96          | 41 (20 oz. = 68)  | 115.51            | 0.68 (1.13)     |
| Dr. Pepper   | 12               | 354.96          | 42 (20 oz. = 68)  | 118.32            | 0.70 (1.13)     |
| Dr. Pepper diet                                    | 12               | 354.96          | 44 (20 oz. = 68)  | 123.96            | 0.73 (1.13)     |
| Pepsi  | 12               | 354.96          | 38 (20 oz. = 63)  | 107.05            | 0.63 (1.05)     |
| Pepsi Lime, regular or Diet                        | 12               | 354.96          | 38 (20 oz. = 63)  | 107.05            | 0.63 (1.05)     |
| Pepsi Vanilla                                      | 12               | 354.96          | 37                | 104.24            | 0.62            |
| Pepsi Twist  | 12               | 354.96          | 38 (20 oz. = 63)  | 107.05            | 0.63 (1.05)     |
| Pepsi Wild Cherry, regular or diet                 | 12               | 354.96          | 38 (20 oz. = 63)  | 107.05            | 0.63 (1.05)     |
| Diet Pepsi   | 12               | 354.96          | 36 (20 oz. = 60)  | 101.42            | 0.60 (1.00)     |
| Pepsi Twist, diet                                  | 12               | 354.96          | 36 (20 oz. = 60)  | 101.42            | 0.60 (1.00)     |
| Coca-Cola Classic®                                 | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Coke Black Cherry Vanilla, regular or diet         | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Coke C2  | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Coke Cherry, regular or diet                       | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Coke Lime  | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Coke Vanilla                                       | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Coke Zero  | 12               | 354.96          | 35 (20 oz. = 58)  | 98.60             | 0.58 (0.98)     |
| Barq's Diet Root Beer                              | 12               | 354.96          | 23 (20 oz. = 38)  | 64.80             | 0.38 (0.63)     |
| Barq's Root Beer                                   | 12               | 354.96          | 23 (20 oz. = 38)  | 64.80             | 0.38 (0.63)     |
| 7-Up, regular or diet                              | 12               | 354.96          | 0                 | 0.00              | 0.00            |
| Fanta, all flavors                                 | 12               | 354.96          | 0                 | 0.00              | 0.00            |
| Fresca, all flavors                                | 12               | 354.96          | 0                 | 0.00              | 0.00            |
| Mug Root Beer, regular or diet                     | 12               | 354.96          | 0                 | 0.00              | 0.00            |

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|  |                          |                         |                      |                           |                         |
|--|--------------------------|-------------------------|----------------------|---------------------------|-------------------------|
| Sierra Mist, regular or free               | 12                       | 354.96                  | 0                    | 0.00                      | 0.00                    |
| Sprite, regular or diet                    | 12                       | 354.96                  | 0                    | 0.00                      | 0.00                    |
| <b>Energy Drinks</b>                       | <b>Serving*<br/>(oz)</b> | <b>Serving<br/>(ml)</b> | <b>Caffeine (mg)</b> | <b>Caffeine<br/>(ppm)</b> | <b>Dose<br/>(mg/kg)</b> |
| Spike Shooter                              | 8.4 oz.                  | 248.5                   | 300                  | 1207.24                   | 5.00                    |
| Cocaine                                    | 8.4 oz.                  | 248.5                   | 288                  | 1158.95                   | 4.80                    |
| Monster Energy                             | 16 oz.                   | 473.2                   | 160                  | 338.12                    | 2.67                    |
| Full Throttle                              | 16 oz.                   | 473.2                   | 144                  | 304.31                    | 2.40                    |
| Rip It, all varieties                      | 8 oz.                    | 236.6                   | 100                  | 422.65                    | 1.67                    |
| Enviga                                     | 12 oz.                   | 354.96                  | 100                  | 281.72                    | 1.67                    |
| Tab Energy                                 | 10.5 oz.                 | 310.59                  | 95                   | 305.87                    | 1.58                    |
| SoBe No Fear                               | 8 oz.                    | 236.6                   | 83                   | 350.80                    | 1.38                    |
| Red Bull                                   | 8.3 oz.                  | 245.51                  | 80                   | 325.85                    | 1.33                    |
| Red Bull Sugarfree                         | 8.3 oz.                  | 245.51                  | 80                   | 325.85                    | 1.33                    |
| Rockstar Energy Drink                      | 8 oz.                    | 236.6                   | 80                   | 338.12                    | 1.33                    |
| SoBe Adrenaline Rush                       | 8.3 oz.                  | 245.51                  | 79                   | 321.78                    | 1.32                    |
| Amp  | 8.4 oz.                  | 248.5                   | 74                   | 297.79                    | 1.23                    |
| Glacéau Vitamin Water Energy<br>Citrus     | 20 oz.                   | 591.6                   | 50                   | 84.52                     | 0.83                    |
| SoBe Essential Energy, Berry or<br>Orange  | 8 oz.                    | 236.6                   | 48                   | 202.87                    | 0.80                    |
| <b>Frozen Desserts</b>                     | <b>Serving*<br/>(oz)</b> | <b>Serving<br/>(ml)</b> | <b>Caffeine (mg)</b> | <b>Caffeine<br/>(ppm)</b> | <b>Dose<br/>(mg/kg)</b> |
| Ben & Jerry's Coffee Heath Bar<br>Crunch   | 8 fl. oz.                | 236.6                   | 84                   | 355.03                    | 1.40                    |
| Ben & Jerry's Coffee Flavored<br>Ice Cream | 8 fl. oz.                | 236.6                   | 68                   | 287.40                    | 1.13                    |
| Haagen-Dazs Coffee Ice Cream               | 8 fl. oz.                | 236.6                   | 58                   | 245.14                    | 0.97                    |
| Haagen-Dazs Coffee Light Ice<br>Cream      | 8 fl. oz.                | 236.6                   | 58                   | 245.14                    | 0.97                    |
| Haagen-Dazs Coffee Frozen<br>Yogurt        | 8 fl. oz.                | 236.6                   | 58                   | 245.14                    | 0.97                    |
| Haagen-Dazs Coffee & Almond<br>Crunch Bar  | 8 fl. oz.                | 236.6                   | 58                   | 245.14                    | 0.97                    |
| Starbucks Coffee Ice Cream                 | 8 fl. oz.                | 236.6                   | 50-60                | 211-254                   | 0.83-1.0                |
| <b>Chocolates/Candies/Other</b>            | <b>Serving*<br/>(oz)</b> | <b>Serving<br/>(ml)</b> | <b>Caffeine (mg)</b> | <b>Caffeine<br/>(ppm)</b> | <b>Dose<br/>(mg/kg)</b> |
| Jolt Caffeinated Gum                       | 1 stick                  | NA                      | 33                   | NA                        | 0.55                    |
| Hershey's Special Dark<br>Chocolate Bar    | 1.45 oz.                 | 42.89                   | 31                   | 722.78                    | 0.52                    |
| Hershey's Chocolate Bar                    | 1.55 oz.                 | 45.85                   | 9                    | 196.29                    | 0.15                    |
| Hershey's Kisses                           | 41g (9 pieces)           | NA                      | 9                    | NA                        | 0.15                    |
|  |                          |                         | 9                    | 38.04                     |                         |
| Hot Cocoa                                  | 8 oz.                    | 236.6                   | (range: 3-13)        | (13 – 55)                 | 0.15                    |
| <b>Over-The-Counter Drugs</b>              | <b>Serving*<br/>(oz)</b> | <b>Serving<br/>(ml)</b> | <b>Caffeine (mg)</b> | <b>Caffeine<br/>(ppm)</b> | <b>Dose<br/>(mg/kg)</b> |
| NoDoz (Maximum Strength)                   | 1 tablet                 | NA                      | 200                  | NA                        | 3.33                    |
| Vivarin                                    | 1 tablet                 | NA                      | 200                  | NA                        | 3.33                    |

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|                           |           |    |     |    |      |
|---------------------------|-----------|----|-----|----|------|
| Excedrin (Extra Strength) | 2 tablets | NA | 130 | NA | 2.17 |
| Anacin (Maximum Strength) | 2 tablets | NA | 64  | NA | 1.07 |

\*Serving sizes are based on commonly eaten portions, pharmaceutical instructions, or the amount of the leading-selling container size. For example, beverages sold in 16-ounce or 20-ounce bottles were counted as one serving.

**Table 2. Caffeine content of some common US food products (IOM, 2004)**

| Food   | Average (mg) | Range (mg) | Average amount (mg) contained in 12 ounces <sup>b</sup> | Average amount (mg/ml) |
|--|--------------|------------|---|------------------------|
| <b>Coffee (5-ounce cup)</b>                          |              |            |   |                        |
| Brewed, drip method                                  | 120          | 90-150     | 288   | 0.81                   |
| Percolated   | 90           | 64-124     | 216   | 0.61                   |
| Instant  | 75           | 30-120     | 180   | 0.51                   |
| Decaffeinated  | 3            | 1-5        | 7.2   | 0.02                   |
| Espresso (6-ounce cup)                               | 240          | 180-300    | 480   | 1.35                   |
| <b>Teas (loose or bags, 5 ounce cup)<sup>a</sup></b> |              |            |   |                        |
| 1-minute brew  | 21           | 9-33       | 50.4  | 0.14                   |
| 3-minute brew  | 33           | 20-46      | 79.2  | 0.22                   |
| <b>Tea products</b>                                  |              |            |   |                        |
| Instant (5-ounce cup)                                | 20           | 12-28      | 48  | 0.14                   |
| Iced (12-ounce glass)                                | 29           | 22-36      | 29  | 0.08                   |
| <b>Carbonated beverage</b>                           |              |            |   |                        |
| <b>Colas and pepper drinks (12 ounce)</b>            |              |            |   |                        |
| National brands, packaged                            | 42           | 36-48      | 42  | 0.12                   |
| National brands, fountain                            | 39           | 32-48      | 39  | 0.11                   |
| Store brands, packaged                               | 18           | 5-29       | 18  | 0.05                   |
| <b>Citrus drinks (12 ounce)</b>                      |              |            |   |                        |
| National brands, packaged                            | 52           | 43-56      | 52  | 0.15                   |
| Store brands, packaged                               | 38           | 26-52      | 38  | 0.11                   |
| <b>Chocolate products</b>                            |              |            |   |                        |
| Cocoa beverage (8 ounce)                             | 6            | 3-32       | 9   | 0.03                   |
| Chocolate milk beverage (8 ounce)                    | 5            | 2-7        | 7.5   | 0.02                   |
| Milk chocolate (1 ounce)                             | 6            | 1-15       | N/A   | N/A                    |
| Dark chocolate, semisweet (1 ounce)                  | 20           | 5-35       | N/A   | N/A                    |
| Baker's chocolate (1 ounce)                          | 35           | 35         | N/A   | N/A                    |
| Chocolate-flavored syrup (1 ounce)                   | 4            | 4          | N/A   | N/A                    |

<sup>a</sup>Note these caffeine amounts are based on a 5-ounce cup of beverage. Servings today are more likely to be 8 or 12 ounces, and caffeine intake has been calculated accordingly; <sup>b</sup>value adjusted to a 12-ounce (355 ml) beverage serving, where appropriate; mg=milligram; N/A = Not appropriate, as the product is commonly sold as a solid product, not a beverage.

## 2.1. Description

Caffeine has a bitter taste, but is odorless, occurring as a white powder or as white needles. Caffeine may be compacted or compressed into granular or pellet forms. Caffeine in its

anhydrous form contains one molecule of water of hydration; caffeine in solution is neutral in pH. General descriptive characteristics and specifications of caffeine are provided in Table 3 (FCC, 2003).

**Table 3. General descriptive characteristics and specifications of caffeine (FCC, 2003).**

| <b>Characteristics</b>          | <b>Description</b>   |
|---------------------------------|--|
| CAS                             | 58-08-2  |
| Synonyms                        | 1,3,7-Trimethylxanthine  |
| Formula                         | C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> or C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> .H <sub>2</sub> O |
| Formula weight, anhydrous       | 194.19   |
| Formula weight, monohydrate     | 212.21   |
| Physical properties             | White powder; odorless; bitter taste   |
| Solubility (hydrated caffeine)  | In 50 ml water, 75 ml alcohol, 6 ml chloroform and 600 ml ether.   |
| Functional use in foods         | Flavoring agent  |
| Assay                           | Not less than 98.5% and not more than 101.0% of C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> ,                                 |
| Lead                            | Not more than 1 mg/kg  |
| Melting range                   | Between 235° and 237.5° C  |
| Other alkaloids                 | Passes test  |
| Readily carbonizable substances | Passes test  |
| Residue on ignition             | Not more than 0.1%   |
| Water                           | <i>Anhydrous caffeine</i> : not more than 0.5%; <i>hydrous caffeine</i> : not more than 8.5%   |
| Storage                         | Store hydrous caffeine in tight containers and anhydrous caffeine in well closed containers  |

## 2.2. History of use

Caffeine is thought to have been discovered in Ethiopia during the third century AD when a shepherd noticed that the goats he was tending became very “frisky” and agitated after eating coffee berries or “beans”. The shepherd tried chewing some of the berries and noted the stimulant effects. An abbot at a nearby monastery brewed the beans in hot water and found that the beverage helped him stay awake during prayer (IOM, 2004). Cultivation of the coffee plant may have begun as early as the sixth century AD, probably in Ethiopia. During this period, coffee beans were also crushed, added to fat, and consumed as a food to stimulate warriors during battle (Nolan, 2001). Coffee as a beverage reached Yemen at approximately 1000 AD, where it was consumed as a popular social ritual beverage among Muslims. From Yemen, it spread to Europe and the Americas. Many major cultures around the world that have had access to caffeine-containing plants developed drinks or food products containing caffeine. In China,

the earliest recorded use of caffeine-containing beverages dates back to the Tang Dynasty (618-907 AD), where tea was a popular drink and believed to increase longevity (IOM, 2004).

### 2.3. Current uses

Caffeine is one of the most widely used central nervous system stimulant substances in the world. Caffeine naturally occurs in some foods, and is also used as a food ingredient and as a drug, or component of, many pharmaceutical preparations. It is used in several over-the-counter cold and allergy medicines, analgesics, appetite suppressants, and nervous system stimulants. Caffeine-containing drinks (e.g., Coca Cola®) and alcoholic beverages have been mixed since the advent of soft drinks, and have assumed a prominent place in our culture. For example, the song “Rum and Coca-Cola®” is the title of a popular calypso song, which became a huge hit in 1945 for the Andrews Sisters, spending ten weeks at the top of Billboard’s U.S. Pop Singles chart. More contemporary uses include the “Jägerbomb”, a cocktail that is mixed by dropping a shot of Jägermeister into a glass of Red Bull (a caffeine- containing “energy” drink).<sup>4</sup>

Even coffee has been used as the vehicle for alcoholic beverages. So-called “Spanish coffee” is made with brandy<sup>5</sup> and “Irish coffee” is made with Irish Whiskey.<sup>6</sup>

Kahlua, a coffee-flavored liqueur, was first created in 1936,<sup>7</sup> and a website lists 60 drinks made with Kahlua.<sup>8</sup> Other coffee-flavored alcoholic beverages include: Allen’s Coffee Brandy, Aruba Arehucas, Vibe Robusta Coffee Liqueur, Bols Coffee Liqueur, Café Britt Coffee Liqueur, Café Oriental, Caffé Borghetti, Coloma, Kona Gold, De Kuyper Crème de Café, Lauterer Luft, Leroux Coffee-Flavored Brandy, Mr. Boston Coffee-Flavored Brandy and Patron XO Café and Tia Maria, to name a few.<sup>9</sup> Even the iconic coffee vendor Starbucks® markets a coffee liqueur.<sup>10</sup>

Non-coffee based, but caffeine/methylxanthine containing, liqueurs include: “Everglo” a liqueur that combines tequila and vodka for the alcoholic component and caffeine, along with

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<sup>4</sup> Definition of a “Jägerbomb”, <http://en.wikipedia.org/wiki/J%C3%A4gerbomb> (last visited April 12, 2010).

<sup>5</sup> Definition of “Spanish coffee”, <http://ineedcoffee.com/09/spanish-coffee/> (last visited April 11, 2010).

<sup>6</sup> Definition of “Irish coffee”, <http://www.drinksmixer.com/drink4414.html> (last visited April 12, 2010).

<sup>7</sup> Kahlua coffee-flavored liqueur, <http://www.kahlua.com>, (last visited April 13, 2010).

<sup>8</sup> List of drinks made with Kahlua, <http://www.drinksmixer.com/cat/1640/> (last visited April 12, 2010).

<sup>9</sup> For a complete list of coffee liqueurs, see [http://en.wikipedia.org/wiki/List\\_of\\_liqueurs#Coffee\\_liqueurs](http://en.wikipedia.org/wiki/List_of_liqueurs#Coffee_liqueurs) (last visited April 12, 2010).

<sup>10</sup> Starbucks Coffee Liqueur, <http://news.starbucks.com/news/starbucks+products/starbucks+coffee+liqueur/> (last visited April 12, 2010).

ginseng.<sup>11</sup> Everglo is imported and distributed by Carolina One, a company in North Carolina. Moreover, distilled spirits companies produce caffeinated and/or stimulant-enhanced vodkas, such as p.i.n.k.<sup>®</sup> vodka<sup>12</sup> and Belvedere IX<sup>®</sup> vodka<sup>13</sup>.

Caffeine content of commonly used beverages and other products vary, from as low as 0.0004% (*e.g.*, chocolate milk containing 1 mg/8 oz serving) to as high as 0.169% (*e.g.*, a strong espresso coffee containing 240 mg/6 oz serving) (IOM, 2004).

#### 2.4. Regulatory status

Caffeine has been approved for use in food by the Food and Drug Administration (FDA) as a multi-purpose GRAS food substance, when added to cola-type beverages current good manufacturing practice ("cGMP") with an upper limit at 0.02% (200 ppm) (Table 4). In 1958, caffeine was included in the US FDA GRAS list; however, in 1980, FDA proposed that caffeine no longer be considered GRAS, but that caffeine should be placed in an interim food additive status prior to completion of additional studies (Prothro, 1997; Deshpande, 2002).<sup>14</sup>

FDA published a proposed rule on the use of caffeine in nonalcoholic carbonated beverages in 1987. FDA proposed to codify a prior sanction for the use of added caffeine in nonalcoholic carbonated beverages, based on comments received in response to an earlier proposal.<sup>15</sup> The agency proposed applying a provision of the Food, Drug and Cosmetic Act that exempts any substance from the requirement of being defined as a food additive, if that substance was used in accordance with an approval that was granted prior to the Food Additives Amendment Act of 1958 (*i.e.*, "prior sanction"). It was FDA's conclusion that existing data did not demonstrate that a level of 0.02% caffeine added to nonalcoholic beverages presented any risk to humans. In 1992, FDA reanalyzed the issue, reviewing scientific articles published from 1986 – 1991 that had bearing on the potential health effects of caffeine, which included animal and clinical studies on developmental, reproductive, behavioral, carcinogenic, cardiovascular, and other effects. FDA concluded that there was no evidence to show a human health hazard

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<sup>11</sup> Everglo vodka and tequila, <http://www.everglo.com/home.html> (last visited April 12, 2010).

<sup>12</sup> p.i.n.k. vodka, <http://www.pinkspirits.com/> (last visited April 14, 2010).

<sup>13</sup> Belvedere IX vodka, <http://www.belvedereix.com/> (last visited April 14, 2010).

<sup>14</sup> "Caffeine; Deletion of GRAS Status; Proposed Declaration That No Prior Sanction Exists and Use on an Interim Basis Pending Additional Study", 45 Fed. Reg. 69817, 69818 (1980).

<sup>15</sup> Caffeine in Nonalcoholic Carbonated Beverages, 52 Fed. Reg. 18923 (1987).

resulting from the consumption of caffeine through the use of cola beverages at 100 mg/person/day or less (IOM, 2004).

The Flavor and Extract Manufacturers' Association (FEMA) has evaluated caffeine and determined that caffeine is GRAS as a flavor ingredient in several food categories. The FEMA-reported average maximum level of caffeine usage is 120 ppm for beverages (Hall and Oser, 1965). Caffeine use in cola-type beverages is permitted by FDA for flavor use (21 CFR § 182.1180) at a 0.02% concentration, which is equivalent to 20 mg in 100 ml beverage, or 71 mg in a 355 ml (12 ounce) beverage.

Pertinent to the regulatory use of caffeine is the fact that caffeine may be added to over-the-counter (OTC) drugs when adhering to the restriction put into place by FDA in 21 CFR 340.10. This restriction limits the amount of caffeine in an OTC drug to 100-200 mg *per* dose (limited only to "not more often than every 3 to 4 hours"), and must include cautionary labeling. The regulation is specific to caffeine content, and states that label directions should also include the phrase "too much caffeine may cause nervousness, irritability, sleeplessness, and, occasionally, rapid heart beat".

Although there are restrictions to limit the amount of caffeine consumed from carbonated beverages, as indicated above, many beverages (*e.g.*, processed and fresh-brewed coffee and tea drinks, and energy drinks) contain caffeine at concentrations over the 0.02% (*i.e.*, 0.2 mg/ml) that was permitted for non-alcoholic beverages. The rationale for these increased levels of caffeine concentration is based on the long history of debate over the properties of caffeine. Following the chemical identification of caffeine in the early part of the twentieth century and the determination of caffeine's pharmacological stimulation property, discussion focused on the properties of caffeine as a food ingredient. Starting in the 1960s, debate intensified around the supposition that caffeine produced birth defects, and although disproved, the evidence has not been universally accepted (IOM, 2004). The controversies with caffeine have resulted in many proposed regulations, although only a few are in effect. These proposed regulations were mostly aimed at labeling products, such as "No-Doz"<sup>®</sup> and similar OTC stimulant products, followed by caffeine-containing weight-loss products.

Although initial concerns about high-level consumption of caffeine was targeted at OTC products, the amount of caffeine from food sources consumed by an individual may greatly exceed what is considered a pharmacologically “excessive” dose. The reason that more “overdoses” are not reported is that the tolerance to the side effects of caffeine consumption (e.g., nervousness, sleeplessness) varies greatly with the individual; a tolerance is acquired to caffeine and consumers are able to titrate their intake because of the familiarity with coffee and caffeinated products. Therefore, the claim often made by manufacturers of caffeine-added products is that the product contains no more caffeine than an ordinary cup of coffee (e.g., 5-Hour Energy<sup>®16</sup>). Of course, bottled or canned coffee products, such as “Frappuchino” or “Doubleshot” sold by Starbucks<sup>®</sup>, contain caffeine that comes from the actual brewing of the coffee product. Because it is common knowledge that coffee contains caffeine, no additional labeling is required for this type of product.

In recent years, a number of drinks have been marketed that contain high levels of caffeine, such as “Jolt Cola<sup>®</sup>”, that was marketed as having twice the sugar and caffeine as an ordinary soft drink. Because the concentration of caffeine in Jolt exceeded the established limit, FDA requested that a ‘caffeine warning’ statement be added to the label. The Jolt Cola<sup>®</sup> producers countered that the stimulant properties promised by the contents of the product were obvious because of the following product characteristics: (1) the name “Jolt”; (2) the yellow color of the can; and (3) the presence on the label of a prominent “lightning bolt.” FDA agreed, and no additional labeling was required. Subsequently, many additional products that contain elevated concentrations of caffeine have entered the market and have found safe harbor with the “obvious” nature of a label containing words such as “energy” to indicate a stimulant nature. Companies have marketed several additional cola-type beverages that contain higher caffeine levels than permitted by FDA, such as Planet Java Tremble (0.046%; 0.46 mg caffeine/ml), SoBe No Fear (0.033%; 0.33 mg caffeine/ml), and Red Bull (0.032%; 0.32 mg caffeine/ml).

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<sup>16</sup> 5-Hour Energy, <http://www.5hourenergy.com/> (last visited April 14, 2010).

**Table 4. Regulatory status of caffeine**

| <b>Agency</b> | <b>Permitted functionality</b>    | <b>Use limits (maximum level of use in food)</b>  | <b>Reference</b>  |
|---------------|-----------------------------------|---|-------------------|
| FDA           | Multi-purpose GRAS food substance | Permitted to be used as a flavoring ingredient in cola-type beverages in accordance with cGMP. The tolerance (upper limit) is 0.02% | 21 CFR § 182.1180 |

CFR = Code of Federal Regulations; FDA = United States Food and Drug Administration; cGMP = current good manufacturing practice; GRAS = Generally Recognized as Safe

## 2.5. Proposed use or uses

Caffeine is to be added to alcoholic beverages at up to 200 ppm as a flavoring ingredient. The use equals 0.02% (0.2 mg/ml) caffeine concentration. Alcoholic beverages are for use by adults only, as they contain alcohol; alcoholic beverages are prohibited from purchase and use by persons under age 21 in the United States. In the United States, a standard alcoholic drink has approximately 13.7 grams of pure alcohol, as stated by the Centers for Disease Control and Prevention (CDC). Generally, this amount of alcohol is found in: (1) 12 ounces (355 ml) of regular beer or wine cooler, (2) 8 ounces (235 ml) of malt liquor, (3) 5 ounces (147 ml) of wine, and (4) 1.5 ounces (44 ml) of 80-proof distilled spirits or “liquor”.<sup>17</sup> The amount of caffeine to be added to each of these standard drinks is (1) 71 mg caffeine in 255 ml of regular beer or wine cooler, (2) 47 mg caffeine in 235 ml of malt liquor, (3) 29.4 mg caffeine in 147 ml of wine, and (4) 8.8 mg caffeine in 44 ml liquor, respectively.

Caffeine is an added ingredient in approximately 70% of soft drinks consumed in the US, and is commonly consumed to improve performance in both brief, intense effort and in endurance exercise. Caffeine has been classified as a stimulant by the International Olympic Committee (IOC). Although the IOC does not ban caffeine use, caffeine is regulated nonetheless. Therefore, an athlete with a urine caffeine concentration greater than 12 mg/l is considered to have committed a doping offense. Comparatively, one would need to consume 800 mg of caffeine to reach the caffeine urine level to generate a disqualification (depending on body mass, gender, fluid mass, *etc.*).

<sup>17</sup> <http://www.cdc.gov/alcohol/terms.htm>; (last visited May 3, 2010)

### 3. ESTIMATED DAILY INTAKE

Caffeine has been widely consumed by most segments of the population worldwide. Caffeine consumption from coffee and other sources has been calculated by several different investigators. Coffee, tea and carbonated beverages are the major sources of caffeine in the diet. The amount of caffeine in a cup (typical cup volume of 8 ounces or 240 ml) of coffee is approximately 80 mg (Stavric *et al.*, 1988), and can range between 5 – 190 mg/cup (IOM, 2004).<sup>18</sup> Stavric *et al.* (1988) reported that the size of a commercially available cup of coffee ranges from 130 – 280 ml. Coffee drinkers ingest on average 3.2 cups (assuming 6 ounces *per* cup) of coffee *per* day (National Coffee Association, 2008). Therefore, the average coffee consumer may consume approximately 256 mg caffeine *per* day (approximately 4.3 mg caffeine/kg bw/day for a 60 kg person), with 90<sup>th</sup> percentile drinkers consuming approximately 5-7 mg caffeine/kg bw/day (Mandel, 2002). Freholm *et al.* (1999) reported that worldwide (*i.e.*, the 42 countries included in the survey), caffeine consumption from all sources evaluated (coffee, tea, mate and cocoa) has been estimated at approximately 70-76 mg/person/day (~1.1 mg/kg). The Netherlands reported the highest daily caffeine consumption at 414 mg/person/day (6.9 mg/kg), followed by Sweden (407 mg/person/day; 6.7 mg/kg) and Norway (400 mg/person/day; 6.6 mg/kg). Caffeine consumption in the US was estimated at 168 mg/person/day (2.8 mg/kg); however, consumption of soft drinks, which can be a significant source of caffeine in the diet of people in developed countries, was not included in this caffeine consumption analysis. Bruce and Lader (1986) reported the amount of caffeine intake in the UK at 359 – 621 mg/person/day (5.9 – 10.3 mg/kg).

In addition to coffee, tea, and cocoa, consumption of caffeinated, cola-type carbonated beverages may provide a significant source of caffeine. As a GRAS substance, caffeine is permitted for addition to cola-type beverages at levels not to exceed 0.02% (0.2 mg/ml). The caffeine content found in currently marketed cola-type beverages varies widely (Table 1). In addition, there are several cola-type beverages on the market with higher levels of caffeine (Table 1), as well as caffeine added to over-the-counter (OTC) medications. Therefore, daily intake of caffeine from all sources may vary.

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<sup>18</sup> <http://www.ico.org/caffeine.asp>; last visited March 26, 2010.

Caffeine is widely consumed in the US population, and has been the subject of scientific study and public interest. Knight *et al.* (2004) evaluated the consumption of caffeine in the US (sample size of 10,712 caffeinated beverage consumers) from the major dietary sources (*i.e.*, coffee, tea and carbonated beverages), and found that mean caffeine intakes in adult consumers (*i.e.*, eater's only)<sup>19</sup> ranged from 106 – 170 mg/day (1.76 – 2.8 mg/kg), while the 90<sup>th</sup> percentile intake ranged from 227 – 382 mg/day (3.7 – 6.3 mg/kg). Pregnant women consumed an average of 58 mg/day (0.9 mg/kg) and 157 mg/day (2.6 mg/kg) at the 90<sup>th</sup> percentile. Women of reproductive age consumed 91-109 mg caffeine/day (1.5 – 1.8 mg/kg) and 229-247 mg caffeine/day (3.8 – 4.1 mg/kg) at average and 90<sup>th</sup> percentile intakes, respectively.

To determine the overall daily caffeine intake from both current consumption of caffeine and when caffeine is added to alcoholic beverages (*i.e.*, current intake plus intake from caffeinated alcoholic beverages), the current intake of caffeine must first be calculated. Recently, Frary *et al.* (2005) found that, utilizing the US Department of Agriculture (USDA) Continuing Surveys of Food Intakes by Individuals (CSFII) food survey 1994 – 1996 and 1998, nearly 90% of adults consumed caffeine. The mean caffeine intake for all consumers of caffeine was 193 mg/day (2.64 mg/kg bw/day),<sup>20</sup> with the highest intake among men aged 35 – 54 at 336 mg/day (stated at 3.96 mg/kg bw/day) (Frary *et al.*, 2008). This information is provided in Table 5. Frary *et al.* (2005) found that coffee was the primary source of caffeine, providing 136.4 mg caffeine/day (2.2 mg/kg/day for a 60 kg person) in the diets of people in the US two years of age and older. Soft drinks provide 30.6 mg/day (0.51 mg/kg) and tea provides 23.4 mg/day (0.39 mg/kg). It is generally accepted that the majority of coffee is consumed in the morning.

To calculate the amount of caffeine consumed when added to alcoholic beverages, the average and 90<sup>th</sup> percentile consumption of alcoholic beverages must be first calculated. The consumption of caffeine from alcoholic beverages can be derived from the intake profile (amount and frequency) by individuals that were queried in USDA's What We Eat in America

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<sup>19</sup> "Eater's Only" is a term to indicate that the individuals evaluated for quantifiable consumption were only those individuals that indicated consuming caffeine, as opposed to utilizing all individuals in the population, whether they consumed any caffeine or not.

<sup>20</sup> The body weights utilized in these calculations were not stated in the document and the ranges of caffeine intake were not provided.

(WWEIA) Continuing Survey of Food Intakes by Individuals 2003-2004 dietary intake survey (Dwyer *et al.*, 2003). This survey data on the consumption of alcoholic beverages by this sample population was extrapolated to the US population to calculate the estimated daily intake (EDI) at the mean and 90<sup>th</sup> percentile consumption of caffeine when added to alcoholic beverages for individuals consuming alcoholic beverages (*i.e.*, “eaters only”). The client wishes to add caffeine to alcoholic beverages at up to 200 ppm (0.2 mg/ml). Based on the survey data, the volume of alcoholic beverages consumed, and the concentration of caffeine added to alcoholic beverages (0.2 mg/ml), the consumption of caffeine from alcoholic beverages was calculated at a mean and 90<sup>th</sup> percentile consumption of 156 and 360 mg caffeine *per day*, respectively (Table 5).

All alcoholic beverages have been utilized in the calculations as indicated in the 2005-2006 WWEIA food code database (APPENDIX I); however, certain alcoholic beverages may have a standard of identity, which prohibits the addition of ingredients to the food not identified as mandated or optional ingredients under the regulation. Therefore, addition of caffeine to a food for which a standard of identity exists would demand that the food product be named other than that as indicated under the standard of identity or a waiver of that standard be obtained.

Many types of liquor are not consumed as manufactured, but are diluted with water or other beverages or beverage mixes. The drinks listed in APPENDIX I were assumed to contain 200 ppm caffeine in each serving; however, the 200 ppm level of caffeine only relates to the alcohol product as manufactured. Thus, for example, much of a margarita utilizes bottled tequila (the alcohol is assumed to contain 200 ppm caffeine as manufactured), and the addition of a non-alcoholic margarita mix would reduce the actual assumed consumption of caffeine from these types of drinks. Therefore, the amount of caffeine consumed from alcoholic beverages diluted with water or other beverages or beverage mixes is an overestimation of the actual caffeine consumption. If the alcoholic beverage were manufactured as a pre-mixed alcoholic beverage, it would be assumed that the total beverage would contain a 200 ppm caffeine concentration.

The total caffeine consumption at the mean and 90<sup>th</sup> percentile intake levels would be 349 and 746 mg/day, respectively (typically consumed over the course of the day), as indicated in Table 5, when added to the 90<sup>th</sup> percentile consumption of caffeine from current uses at a mean level indicated by Frary *et al.* (2005) (386 mg/day). It is assumed that the 90<sup>th</sup> percentile

consumption is approximately two-times the mean<sup>21</sup>. This is equivalent to 5.8 and 12.4 mg/kg bw/day, for a 60 kg person, and approximately equivalent to two and a half servings of brewed coffee for a “mean” consumer and five and a half cups of brewed coffee for the “90<sup>th</sup> percentile” consumer, respectively (Table 1).

**Table 5. Caffeine: current intake, predicted intake following supplementation of alcoholic beverages with caffeine at 200 ppm and total intake (predicted + current) for individuals consuming caffeinated alcoholic beverages**

| <b>Daily caffeine intake from:</b>   | <b>Per User (mg/day)</b> |                                    |
|--|--------------------------|------------------------------------|
|  | <b>Mean</b>              | <b>90<sup>th</sup> Percentile*</b> |
| Current consumption from food  | 193                      | 386                                |
| Possible maximum consumption of caffeine as an added ingredient to alcoholic beverages | 156                      | 360                                |
| Total from conventional food (current + added)   | 349                      | 746                                |

\*The 90<sup>th</sup> percentile is typically estimated as two times the mean consumption

The mean and 90<sup>th</sup> percentile of caffeine consumed is over the course of the day. Typically, coffee is consumed as a morning beverage, while most alcohol is consumed in the afternoon or evening. This scenario would limit the quantity of caffeine from typical food intake (the majority of caffeine is consumed in the morning) consumed concurrently with caffeinated alcoholic beverages, which are consumed much later in the day. The 90<sup>th</sup> percentile calculation is likely a significant overestimate of the actual 90<sup>th</sup> percentile consumption of caffeine with the additional proposed use, as consumption of caffeine at the 90<sup>th</sup> percentile when added to alcoholic beverages (360 mg/day) at the concentration of 200 ppm would require consumption of approximately 1.8 liters of the alcoholic beverage. Consumption of 1.8 liters of an alcoholic beverage would be approximately equivalent to five 12-ounce servings of beer, 7.5 servings of malt liquor, 12 servings of wine, or 40 servings of liquor, as calculated from CDC’s statement on standard drink volumes.

<sup>21</sup>The 90<sup>th</sup> percentile is typically estimated as two times the mean consumption, <http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/ucm074725.htm#ftn9> (last visited March 31, 2010).

#### 4. ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION (ADME)

Caffeine is probably one of the most studied natural dietary ingredients. In 2004, the Institute of Medicine (IOM) (2004) prepared an extensive review on caffeine, discussing several issues pertaining to its safety. At approximately the same time, Health Canada released a review of the many studies dealing with caffeine and its health effects, including the pharmacokinetics of caffeine (Nawrot *et al.*, 2003). In addition to these reviews, several articles and other reviews (Dalvi, 1986) related to the safety of caffeine are discussed below to determine the safety-in-use of caffeine in alcoholic beverages.

##### 4.1. Absorption, Distribution and Bioavailability

Caffeine is rapidly and almost entirely absorbed, with 99% of caffeine being absorbed within 45 minutes of ingestion (Carrillo and Benitez, 2000). After ingestion, caffeine is readily distributed throughout the body, as it is retained in the aqueous fraction (*i.e.*, total body water) (Beach *et al.*, 1984). Newton *et al.* (1981) reported that the oral bioavailability of caffeine is essentially complete (following an apparent first-order elimination rate constant) and does not seem to be influenced by dose level, with the rate of elimination also independent of dose level.

Caffeine reaches a peak plasma level between 30 – 75 minutes after ingestion (Mandel, 2002). Maximum plasma caffeine concentrations were reached within one hour of a single oral dose in humans (Beach *et al.*, 1984; Bonati *et al.*, 1984; Collomp *et al.*, 1991). In humans, caffeine absorption from the GI tract is consistent, with no changes due to age, gender, physical exercise, liver cirrhosis, vehicle for the caffeine (coffee, cola or capsules), or the concomitant consumption of ethanol or use of oral contraceptive steroids (Patwardhan *et al.*, 1980; Mitchell *et al.*, 1983).

*In vivo* concentrations of 5 – 10  $\mu$ M caffeine are required to cause mild central nervous system (CNS) stimulation, while higher concentrations in the range of 50  $\mu$ M are associated with the adenosine receptor blockade (as quantified by *in vitro* receptor binding assays) necessary for cardiac stimulation (Green and Stiles, 1986). Human studies (Table 6) suggest that caffeine consumption at approximately 50 – 600 mg/kg can result in maximum concentrations of caffeine in the blood of 5 – 12 mg/l (20 – 57  $\mu$ M), which may cause CNS or cardiac stimulation (serum caffeine levels greater than 100 mg/l are considered lethal in humans). Caffeine at one mg/kg in

humans (considered by some to be equivalent to a cup of coffee) produces peak plasma drug concentrations (C<sub>max</sub>) of 1 – 2 mg/L (or 5 – 10 µmol/L) (Carrillo and Benitez, 2000). The levels reported in the studies outlined in Table 6 are the maximum concentrations reported in blood after caffeine administration. Mandel (2002) summarized studies indicating that additional factors, such as metabolism and excretion, play important roles in blood levels of caffeine that would be considered stimulatory. Caffeine half-life<sup>22</sup> varies in different species; in healthy humans the half-life is approximately four hours (Kaplan *et al.*, 1997), while impaired liver functions increase the half-life, and cigarette smoking reduces caffeine half-life (Mandel, 2002). Siegers *et al.* (1972) reported that caffeine in rats delays stomach emptying due to relaxation of gastric musculature which, when consumed prior to or concurrently with alcohol, retards ethanol absorption and consequently depresses blood alcohol concentrations.

#### 4.2. Metabolism

The metabolism of caffeine has been well-studied in both humans and other mammalian species. Caffeine is metabolized primarily in the liver to dimethyl- and monomethyl-xanthines, dimethyl and monomethyl uric acids, trimethyl- and dimethylallantoin- and uracil-derivatives (Figure 2). The majority of enzymes involved in caffeine metabolism have been well-characterized, but questions still remain concerning some intermediary steps and specific enzymes utilized in the degradation of secondary and tertiary metabolites (McQuilkin *et al.*, 1995). Although some variations exist between mammalian species in the principal metabolic pathways for caffeine, in healthy humans, approximately 69-80% of a single dose of caffeine is N3-demethylated into paraxanthine, with 10-15% N7-demethylated to theophylline and 3-7% N1 demethylated to theobromine (Bonati *et al.*, 1982; Tang-Liu *et al.*, 1983; Bonati *et al.*, 1984; Blanchard *et al.*, 1985; Lelo *et al.*, 1986a; Scott *et al.*, 1989; Ullrich *et al.*, 1992; Tassaneeyakul *et al.*, 1994; McQuilkin *et al.*, 1995; Rodopoulos *et al.*, 1995; Rodopoulos and Norman, 1996).

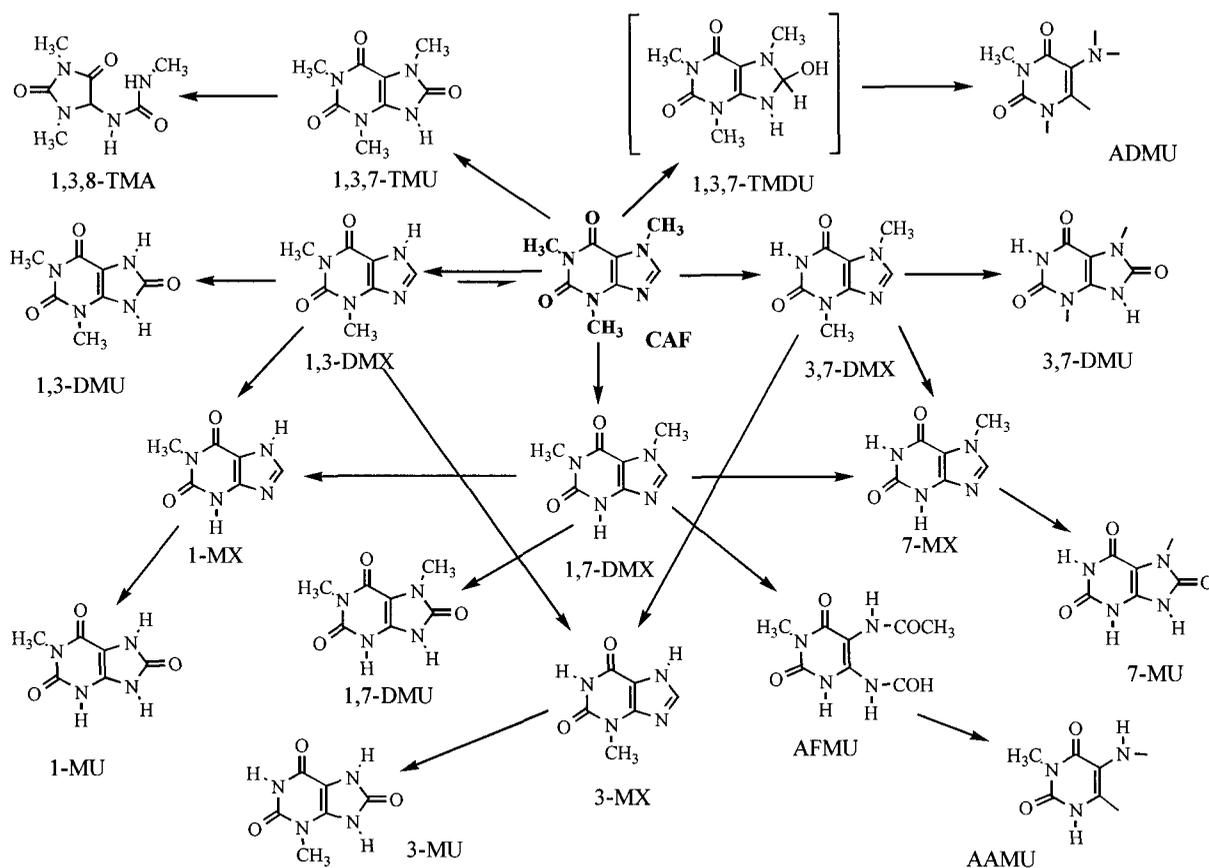
In humans, caffeine (CAF; 1,3,7-trimethylxanthine) N-demethylation (primarily N1-, N3- and N7-demethylation) occurs principally by cytochrome P450 1A2 (CYP1A2) to derive the dimethyl and monomethyl metabolites, as indicated in Figure 2 (Pelkonen *et al.*, 2008). Paraxanthine (1,7-DMX) pathway accounts for approximately 80% of caffeine metabolism in

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<sup>22</sup>A term describing the time required to remove one-half of an administered dose.

humans (Miners and Birkett, 1996). 8-Hydroxylation of caffeine is catalyzed by CYP3A4, whereas xanthine oxidase catalyses 8-hydroxylation of mono-methylxanthines to monomethyluracils. In rats, C-8-hydroxylation of caffeine is the major metabolic reaction in rat liver microsomes (~70%) and liver slices compared to 1-N- and 7-N-demethylation (8–9%) and 3-N-demethylation (~13%) (Kot and Daniel, 2008). Dimethylaminouracil formation, arising from C8-N9 bond scission of 1,7-DMX and 3,7-DMX (not drawn in Figure 2) is believed to be the product of the polymorphic N-acetyltransferase (NAT2) enzyme and results in the production of 5-acetylamino-6-formylamino-3-methyluracil (AFMU) (Miners and Birkett, 1996). Further deacylation of AFMU into 5-acetyl-6-amino-3-methyluracil (AAMU) is likely a spontaneous, non-enzymatic reaction.

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**Figure 2. Major metabolic pathways of caffeine metabolism in mammals (adapted from Miners and Birkett (1996)). Not all enzymes involved in caffeine metabolism have been clearly identified and some steps are still in question.**

A variety of substances modulate CYP1A2 activity, including cigarette smoke and consumption of coffee, charcoal-broiled meat or cruciferous vegetables such as broccoli or Brussels sprouts, while grapefruit juice and alcohol inhibit CYP1A2 activity (Carrillo and Benitez, 1996; Nekvindova and Anzenbacher, 2007). In addition to CYP1A2, CYP1B1 also has catalytic activity that overlaps CYP1A2 activity for caffeine. However, overall CYP1A2 and CYP1B1 metabolic enzymes are not known to alter caffeine clearance to a great extent, unless the enzymes have been induced (caffeine is a low-affinity and low-clearance substance for CYP1A2 metabolism) (Carrillo and Benitez, 2000). Ethanol is metabolized by the CYP2E1 liver enzyme, and not CYP1A2 or CYP1B1 (Pawan, 1972; Tanaka *et al.*, 2000).

Caffeine metabolism *via* the CYP1A2 pathway is considered the primary step in the metabolic pathway (accounting for greater than 95% of the primary caffeine metabolism),

although a large number of enzymes and intermediate products are noted in the complete metabolism of caffeine (Carrillo and Benitez, 2000). Gender and smoking may influence the potential toxicity of caffeine, as reported by Carrillo and Benitez (1996), who reported the following symptoms and frequency of adverse effects in subjects ( $n = 82$ ) that consumed 300 mg caffeine (5 mg/kg) as a bolus dose: restlessness or muscle tremor, 69; palpitation, 30; dizziness, 27; headache, 22; diarrhea, 20; wakefulness, 14; polyuria, 11; increased sweating, 8; abdominal pain, 7; tinnitus or photopsia, 5; vomiting or nausea, 3; and delirium, 2. In this study, females and nonsmokers had a higher symptom rate ( $P < 0.01$ ), which may be attributed to a lower body weight among the female subpopulation, and cigarette smoke may increase CYP1A2 activity.

Azcona *et al.* (1995) evaluated the dynamic and kinetic interactions of alcohol and caffeine in a double-blind placebo controlled, cross-over trial. The subjects ( $n = 8$ ) were healthy males 23 – 27 years of age, with a mean body weight of 71.6 kg. The treatments were administered randomly according to a cross-over, Latin square design, keeping one week between each experimental session, and received single oral doses of the following: 1) placebo-alcohol + placebo-caffeine; 2) alcohol (800 mg/kg bw); 3) caffeine (400 mg); and alcohol (800 mg/kg bw) + caffeine (400 mg). The test battery consisted of a critical flicker fusion (CFF) test to measure the level of cortical activity or arousal (*i.e.*, psychomotor performance), a visual stimuli test to determine simple reaction time (SRT), a tapping test (TT) to measure reflex rate, neurophysiological measures *via* a long latency checkerboard pattern-reversal visual evoked potential (VEP)-COMPACT FOUR program, subjective assessments, profile of mood states, clinical evaluations and alcohol and caffeine plasma concentrations.

Alcohol increased SRT, while caffeine decreased SRT. Alcohol + caffeine and placebo had a similar profile, with no significant difference reported. There were no significant differences between treatments in the TT or CFF tests. In the VEP test, alcohol produced a significant decrease in the response, while caffeine significantly increased the response, and the placebo and caffeine + alcohol responses were in the middle. Subjectively, alcohol and alcohol + caffeine treatments produced feelings of drunkenness, as expected, and were different from the caffeine and placebo treatment groups. None of the treatments affected subjective feelings of depression, anxiety or drowsiness.

The plasma concentrations of alcohol and caffeine indicated that alcohol inhibited caffeine metabolism, while caffeine consumption had no effect on alcohol metabolism (Figure 3), as was previously reported (Pawan, 1972). The area under the curve (AUC) for the plasma concentrations for caffeine and alcohol indicated that, for alcohol, there was no statistical difference between alcohol and caffeine + alcohol, but the AUC for caffeine was significantly greater after caffeine + alcohol than after caffeine. Increased caffeine half-life during alcohol intake was also reported by George *et al.* (1986), who reported that alcohol intake of 50 g/day (approximately equivalent to 3.5 standard drinks) prolonged caffeine half-life by 72% and diminished caffeine clearance by 36%, compared to those individuals that were not consuming caffeine on a daily basis. The increase in caffeine half-life and decrease in caffeine clearance was less apparent when compared to individuals who consumed caffeine on a daily basis.

Overall, when alcohol (0.8 mg/kg bw) and caffeine (400 mg) were administered together, the objective assessments suggested an antagonistic effect, as the results of both the placebo and the combination were between alcohol and caffeine alone, and reached statistical significance when the assessments were able to clearly show effects by caffeine. This study indicated that caffeine can antagonize some of the effects of alcohol, but not all. No changes were found in any of the clinical safety parameters evaluated (Azcona *et al.*, 1995).

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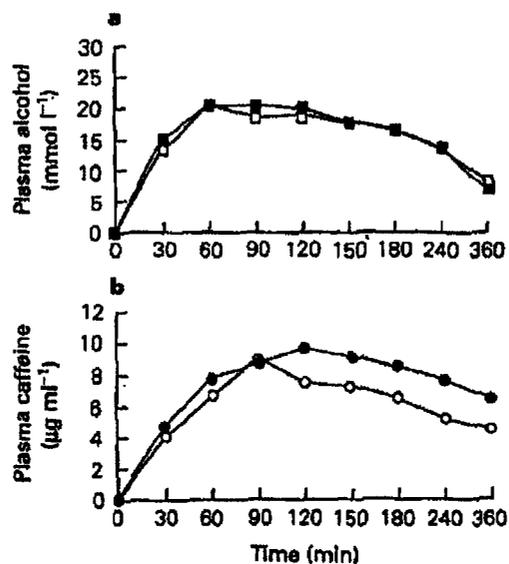


Figure 3. Mean alcohol (a) and caffeine (b) plasma concentrations after single oral doses either alone (open symbols) or in combination (closed symbols) ( $n = 8$ ) (Azcona *et al.*, 1995).

#### 4.3. Elimination

Clearance values for low-dose caffeine intake in both men and women are approximately 1 – 3 mg/kg/min (Kaplan *et al.*, 1997). Higher caffeine doses diminish caffeine clearance because of the saturable metabolism of paraxanthine and its resultant decreased clearance. In this clearance mechanism, paraxanthine accumulates in plasma, which leads to a reduction in caffeine clearance, leading to prolonged high caffeine plasma levels. The half-life of caffeine in humans is approximately four hours (Kaplan *et al.*, 1997). Birkett and Miners (1991) reported a strong correlation between urine and plasma concentrations and clearance values of caffeine in volunteers consuming 150 mg caffeine every eight hours for six days, although there was a high inter-individual variability. The authors concluded that caffeine is reabsorbed from the renal tubule to equilibrium with free (unbound) caffeine in the plasma.

Caffeine has been detected in human sweat at concentrations paralleling ingested dose levels (Kovacs and Brouns, 1998), with a strong correlation between caffeine concentrations in sweat with those in plasma and urine. As a whole, there does not appear to be any significant difference in the pharmacokinetic parameters of caffeine elimination between men and women (Patwardhan *et al.*, 1980), although among women, sex hormone profiles may affect caffeine

elimination. Lane *et al.* (1992) reported a small but significant decrease in the plasma clearance of caffeine during the luteal phase of menstruation (the authors indicate that the effect may be too small to be of clinical significance to the majority of women), and oral contraceptive steroids were found to increase the elimination time of caffeine from the plasma.

Consumption of ethanol was found to change the pharmacokinetics of caffeine elimination (Table 6). Ethanol consumption at 0.8 mg/kg in eight subjects significantly increased caffeine's plasma half-life (*i.e.*, the amount of time caffeine remains in the plasma), and decreased plasma clearance (Mitchell *et al.*, 1983). Neither the volume of distribution or the peak time to maximum plasma concentration were significantly different following ethanol consumption, suggesting that the absorption of caffeine from the GI tract was not changed during ethanol consumption.

In liver cirrhosis conditions, pharmacokinetic parameters evaluated after caffeine consumption (5 mg caffeine/kg bodyweight) indicated significant reductions in caffeine elimination (Holstege *et al.*, 1993). In six patients requiring biliary decompression (but non-cirrhotic), caffeine consumption (5 mg caffeine/kg body weight) resulted in maximum blood and bile caffeine concentrations by one hour post-dose. There was a high correlation of caffeine, theobromine, theophylline and paraxanthine concentrations in the blood and bile at all time points, but there was a lower bile-to-blood ratio of metabolites, suggesting a much slower entry of caffeine and its metabolites into the bile (Holstege *et al.*, 1993).

Caffeine generally follows first-order elimination kinetics in most mammals, except for rats, which have a capacity-limited elimination process at doses greater than 10 mg/kg (Bortolotti *et al.*, 1990).

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**Table 6. Pharmacokinetics of caffeine elimination from plasma following a single oral dose in patients and healthy adult groups**

| Subjects                      | n        | Dose                      | Conc. (max) | Clearance      | t <sub>1/2</sub> (hr) | Vd        | AUC             | Reference                |
|-------------------------------|----------|---------------------------|-------------|----------------|-----------------------|-----------|-----------------|--------------------------|
| Healthy men                   | 5        | 200 mg                    | 3.36 µg/ml  | 156.12 ml/min  | 3.46                  | 47.3 l    | 23.46 µg/ml hr  | Beach <i>et al.</i>      |
| Healthy men                   | 4        | 400 mg                    | 7.40 µg/ml  | 161.39 ml/min  | 3.67                  | 50.24 l   | 46.12 µg/ml hr  | Beach <i>et al.</i>      |
| Healthy men                   | 4        | 0.22 mg/kg <sup>1</sup>   | 0.4 µg/ml   | 104 ml/min     | 3.4                   | 33.7 l    | 170 mg/l min    | Bonati <i>et al.</i>     |
| Healthy men                   | 4        | 1.00 mg/kg                |             | 133 ml/min     | 4.2                   | 48.7 l    | 544 mg/l min    | Bonati <i>et al.</i>     |
| Healthy men                   | 4        | 1.54 mg/kg <sup>1</sup>   | 2.0 µg/ml   | 119 ml/min     | 5.0                   | 53.5 l    | 977 mg/l min    | Bonati <i>et al.</i>     |
| Healthy men                   | 4        | 5.00 mg/kg                | 8.3 µg/ml   | 71 ml/min      | 6.3                   | 39.4 l    | 5164 mg/l min   | Bonati <i>et al.</i>     |
| Healthy men                   | 2        | 10.00 mg/kg               |             | 103 ml/min     | 5.2                   | 46.5 l    | 6815 mg/l min   | Bonati <i>et al.</i>     |
| Subjects at rest              | 6♀<br>6♂ | 250 mg                    | 7.28 mg/l   | 6.82 l/hr      | 4                     | 0.6 l/kg  |                 | Collomp <i>et al.</i>    |
| Subjects with exercise        | 6♀<br>6♂ | 250 mg                    | 10.45 mg/l  | 6.59 l/hr      | 2.29                  | 0.34 l/kg |                 | Collomp <i>et al.</i>    |
| Non-cirrhosis patients        | 6        | 5 mg/kg                   |             | 6.8 ml/min     |                       | 104 l/kg  | 297 µmol/l hr   | Holstege <i>et al.</i>   |
| Liver cirrhosis patient       | 1        | 5 mg/kg                   |             | 107 ml/min     |                       | 2.4 l/kg  | 16266 µmol/l hr | Holstege <i>et al.</i>   |
| Women in follicular phase     | 10       | 250 mg                    |             | 0.99 ml/kg/min | 5.3                   | 0.41 l/kg | 4522 µg/ml min  | Lane <i>et al.</i>       |
| Women in late luteal phase    | 10       | 250 mg                    |             | 0.89 ml/kg/min | 5.4                   | 0.33 l/kg | 5178 µg/ml min  | Lane <i>et al.</i>       |
| Healthy men                   | 6        | 270 mg                    | 5 mg/l      | 2.07 ml/min/kg | 4.1                   | 1.06 l/kg | -----           | Lelo <i>et al.</i>       |
| Healthy adults                | 17       | Daily source <sup>2</sup> |             | 1.2 ml/min/kg  |                       |           |                 | Lelo <i>et al.</i>       |
| Healthy adults no ethanol;    | 5♀<br>3♂ | 250 mg                    | 7.00 µg/ml  | 96.6 ml/min    | 4.03                  | 31.1 l    | -----           | Mitchell <i>et al.</i>   |
| with ethanol (0.8 mg/kg)      | 5♀<br>3♂ | 250 mg                    | 9.99 µg/ml  | 60.6 ml/min    | 6.04                  | 29.1 l    | -----           | Mitchell <i>et al.</i>   |
| Healthy men                   | 13       | 250 mg                    |             | 1.3 ml/min/kg  | 5.5                   | 0.54 l/kg | -----           | Patwardhan <i>et al.</i> |
| Women – OCS                   | 9        | 250 mg                    |             | 1.3 ml/min/kg  | 6.2                   | 0.69 l/kg | -----           | Patwardhan <i>et al.</i> |
| Women + OCS                   | 9        | 250 mg                    |             | 0.79 ml/min/kg | 10.7                  | 0.72 l/kg | -----           | Patwardhan <i>et al.</i> |
| Healthy male volunteers       | 2        | 400 mg <sup>1</sup>       | 6.18 mg/l   | 2.66 ml/min/kg | 3.9                   | 0.83 l/kg | -----           | Rump <i>et al.</i>       |
| Healthy adults                | 8        | 400 mg                    |             | 1.3 ml/min/kg  | 4.4                   | 0.44 l/kg | -----           | Scott <i>et al.</i>      |
| Cirrhosis patients (Pugh < 7) | 10       | 400 mg                    |             | 1.4 ml/min/kg  | 5.2                   | 0.38 l/kg | -----           | Scott <i>et al.</i>      |
| Cirrhosis patients (Pugh ≤ 7) | 5        | 400 mg                    |             | 0.4 ml/min/kg  | 39.5                  | 0.48 l/kg | -----           | Scott <i>et al.</i>      |

| Subjects                      | n      | Dose                | Conc. (max) | Clearance      | t <sub>1/2</sub> (hr) | Vd        | AUC           | Reference              |
|-------------------------------|--------|---------------------|-------------|----------------|-----------------------|-----------|---------------|------------------------|
| Healthy adults                | 10     | 400 mg              | 7.1 mg/l    | 1.47 ml/min/kg | 4.0                   | 0.41 l/kg | 78.3 mg/L hr  | Scott <i>et al.</i>    |
| Cirrhosis patients (Pugh < 7) | 10     | 400 mg              | 10.9 mg/l   | 1.19 ml/min/kg | 3.9                   | 0.38 l/kg | 76.2 mg/l hr  | Scott <i>et al.</i>    |
| Cirrhosis patients (Pugh ≤ 7) | 9      | 400 mg              | 12.4 mg/l   | 0.22 ml/min/kg | 25.8                  | 0.48 l/kg | 333.4 mg/l hr | Scott <i>et al.</i>    |
| Healthy men                   | 8      | 4 mg/kg             |             | 0.11 l/hr/kg   | 3.98                  | 0.58 l/kg | -----         | Shi <i>et al.</i>      |
| Healthy adults                | 1F, 5M | 7.5 mg/kg           |             | 0.096 l/hr/kg  | 5.4                   | 0.52 l/kg | -----         | Tang-Liu <i>et al.</i> |
| Healthy adult male            | 1      | 153 mg <sup>1</sup> | 5 mg/l      | 5.34 l/hr      | 3.8                   | 30.7 l    | 28.7 mg.h/l   | Teeuwen <i>et al.</i>  |

Abbreviations: AUC = area under the plasma concentration curve; F = female; M = male; OCS = oral contraceptive steroid; t<sub>1/2</sub> = plasma half-life; Vd = volume of distribution; <sup>1</sup> Caffeine provided as coffee or cola drink; <sup>2</sup> Patients consumed their regular daily intake of caffeine containing beverages

## 5. SAFETY EVALUATION

### 5.1. Acute Studies

Acute oral toxicity studies of caffeine are summarized in Table 7. Overall, toxic effects (including lethality) *via* oral administration occurred in the dose range of 100 – 150 mg/kg in cats and dogs, and 200 – 360 mg/kg in mice, rabbits and rats. Several reports have described the acute toxic effects of caffeine that result in lethality. Intravenous or oral doses of caffeine in CD2F1/Crl BR mice resulted in death following clonic and tonic seizures (Bonati *et al.*, 1985). Clonic convulsions were almost immediate following intravenous administration, but animals that did not exhibit a subsequent tonic phase survived. Animals with tonic seizures showed muscle rigidity, extended limbs and died from respiratory arrest. If a state of seizure did not occur, the mice had reduced activity for several hours, but the animals fully recovered (Bonati *et al.*, 1985). Intraperitoneal administration of caffeine at 100 mg/kg also induced intermittent clonic convulsions in adult male Wistar rats (Bhattacharya *et al.*, 1997). The concentration of caffeine in the organs after caffeine administration (intravenous (*i.v.*) or oral) differed between animals that succumbed to caffeine toxicity, compared to those that survived the same administered mean lethal dose, even when of the same species/strain. The amount of caffeine in the blood, brain and heart was significantly lower in the mice that did not die from caffeine

toxicity, compared to those mice that died (Bonati *et al.*, 1985). In matched-pair studies of CD2F1/Crl BR mice, animals that died from caffeine toxicity had significantly higher concentrations of caffeine in the blood, brain and heart tissues, compared to the mice that survived. The oral LD<sub>50</sub><sup>23</sup> (339 mg/kg) was stated to be approximately five times the *i.v.* route, with the lethal brain concentration for caffeine in this mouse strain at approximately 1 µmol/g (Bonati *et al.*, 1985).

**Table 7. Acute oral toxicity studies of caffeine**

| Species | Route | Dose*            | Dosage (mg/kg) | Reference            |
|---------|-------|------------------|----------------|----------------------|
| Cat     | Oral  | MLD              | 100 - 150      | Spector              |
| Dog     | Oral  | LD <sub>50</sub> | 140 - 150      | Salant and Rieger    |
| Hamster | Oral  | LD <sub>50</sub> | 230 - 249      | Palm <i>et al.</i>   |
| Mouse   | Oral  | LD <sub>50</sub> | 339            | Bonati <i>et al.</i> |
|         | Oral  | LD <sub>50</sub> | 127 - 137      | Palm <i>et al.</i>   |
| Pigeon  | NR    | LD <sub>50</sub> | 140 - 150      | Salant and Rieger    |
| Rabbit  | Oral  | LD <sub>50</sub> | 350 - 360      | Salant and Rieger,   |
|         | Oral  | LD <sub>50</sub> | 224 - 246      | Palm <i>et al.</i>   |
| Rat     | Oral  | LD <sub>50</sub> | 247 - 355      | Palm <i>et al.</i>   |
|         | Oral  | LD <sub>50</sub> | 233            | Scott and Chen       |
|         | Oral  | LD <sub>50</sub> | 200            | Smith and Hamburger  |
|         | Oral  | LD <sub>50</sub> | 192 ±18        | Boyd (1959)          |

\*Abbreviations: LD<sub>50</sub>, dose which is lethal to 50% of animals; MLD, mean lethal dose; NR = Not reported

The acute toxic effect levels of caffeine differs for various species, and Seale *et al* (1984) found that acute toxic effects even differ between mouse strains when analyzing the toxic behavioral effects of caffeine in adult males from seven inbred mouse strains. The physiological effects of various caffeine doses were scored according to the following characteristics: locomotor activity, righting ability, clonic seizure induction, stress-induced lethality, and death without external stress. Different mouse strains had markedly different responses to toxic

<sup>23</sup> LD<sub>50</sub> = the dose that produces 50 percent lethality in the test population

caffeine doses, evaluated by any single behavioral criterion or a combination. For example, administration of 250 mg/kg (*i.v.*) caffeine was lethal to all C3H/HeJ mice, but not to other strains of mice. Inhibition of locomotor activity by 55% following *i.v.* administration occurred in CBA and C3H/He mice, but this same dose inhibited locomotor activity by greater than 89% in C57BL/6, SWR, DBA/2, A/j and BALB/c mice. Overall, this study indicates that behavioral toxicity testing of caffeine in one mouse strain may be misleading, and suggests that the nervous system response to caffeine ingestion is genetically influenced in mammals (Seale *et al.*, 1984). Boyd (1959) found that the median lethal dose of caffeine in female albino rats ( $n \geq 8$ ) was calculated to be  $192 \pm 18$  mg/kg bw, with clinical signs of the survivors that included hyperreflexia, loose stools or slight diarrhea, and anorexia by 24 hours post dose.

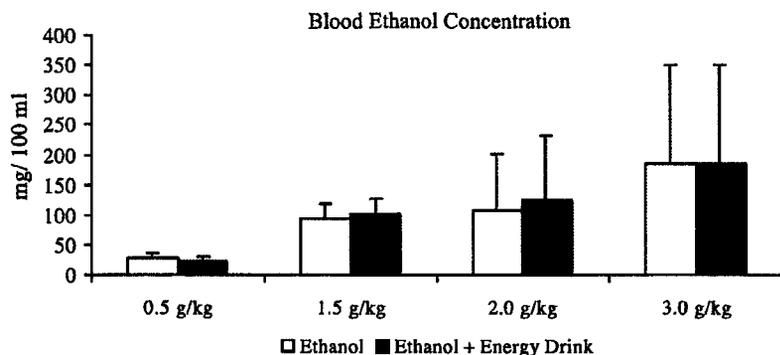
Ferreira *et al.* (2004) utilized the male Albino Swiss mouse model to evaluate the toxicity and the behavioral effect of an acute oral administration of energy drinks containing caffeine combined with ethanol, utilizing locomotor activity and blood ethanol concentration endpoints. Different doses of ethanol (500, 1000, 1500 and 2500 mg/kg)<sup>24</sup>, combined or not with 10.71 ml/kg energy drink (approximately 3.4 mg caffeine/kg bw), were administered *via* gavage, and animals immediately placed in locomotor activity cages and locomotor activity recorded for 45 minutes. The blood ethanol levels reached approximately 0.180 g/100 ml in the high dose group (Figure 4).<sup>25</sup> The administration of the energy drink dose-dependently increased locomotion. Low doses of ethanol did not depress locomotion, although the high dose of ethanol (2.5 g/kg) did depress locomotion ( $P < 0.05$ ). Energy drink consumption did not alter the effects of ethanol at 500, 1000 or 1500 g/kg ethanol, but reduced the 2500 g/kg ethanol depressant effect, noted by increasing the locomotor effects back to control values. The authors concluded that “the data obtained suggest that the dose of 10.71 ml/kg of energy drinks antagonized the depressant effects of ethanol on the locomotor activity of mice. Considering mice metabolism is at least several times faster than that of humans, the administration of about one or two cans (3.57-7.4 ml/kg) to

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<sup>24</sup> The authors indicated that 500 mg/kg ethanol was equivalent to 6% ethanol in liquid, and 2500 mg/kg ethanol was equivalent to 23% ethanol in liquid.

<sup>25</sup> The American Medical Association has defined the blood alcohol concentration level of impairment for all people to be 0.04 grams/100 milliliters of blood. See <http://www.intox.com/physiology.asp> (last visited March 26, 2010).

a person could exert similar effects.” However, the authors did indicate that locomotor activity may not be indicative of a total reversal of all ethanol effects (Ferreira *et al.*, 2004).



**Figure 4. Blood ethanol concentration 30 min after the administration of 500, 1000 2000 or 3000 mg/kg ethanol and 10.71 ml/kg energy drink or water (control). Results are mean ± SD (Ferreira *et al.*, 2004).**

These studies indicate that the LD<sub>50</sub> of caffeine varies with the mammalian model utilized, with LD<sub>50</sub> ranging from a low of 100 mg/kg bw in the cat, to close to 360 mg/kg bw in the rat and rabbit.

## 5.2. Short term/Subchronic Studies

Caffeine (250 mg/kg bw/day; 0.5% of the diet) or 0.8% theobromine, administered in the diet for up to eight weeks to male CD Sprague-Dawley rats, significantly decreased weight gain when compared to control animals (Gans, 1984). This dose was near or at the LD<sub>50</sub> for caffeine in the rat. Caffeine or theobromine administration resulted in a decrease in thymus gland weight gain, and a significant decrease in testicular weight (but the relative weights of these organs were similar in treated and control rats). Histological examination of the testes revealed scattered areas of vacuolar degeneration in spermatogenic cells of the caffeine-treated rats, although the morphology of the testes was maintained and there were clearly defined stages of spermatogenesis (Gans, 1984). Fears (1978) administered caffeine (2.5 mg/kg bw/day) in the diet to male CFY rats for four months (similar to caffeine levels consumed by humans), and evaluated atherosclerogenic lesion formation in the aorta or cardiac vessels. Caffeine at the dose provided did not increase atherosclerogenic lesion formation in the aorta or cardiac vessels.

Caffeine was administered to Syrian golden hamsters for 90 days at 0, 91.3, 274 and 822 mg/liter as drinking water provided *ad libitum* (providing 0, 14.7, 50.8 and 104.8 mg/kg bw/day in females and 0, 9.0, 24.6 and 65.2 mg/kg bw/day in the males, respectively) to evaluate the effects of caffeine on the thyroid (Bartsch *et al.*, 1996). No treatment-related changes were found in thyroxine (evaluated on Days 3, 24, and 91 of caffeine administration), or other clinical chemistry analyses,<sup>26</sup> as well as absolute and relative adrenal weight, gross pathology and thyroid histopathology evaluated at the end of the study (Day 91). A transient increase in T3 was noted after three days of treatment, but not at any other time points in the study. The authors concluded that “no signs of thyroid stimulation or toxicity due to caffeine, even in the high-dose group were observed in the Syrian golden hamster” (Bartsch *et al.*, 1996). However, at approximately 20 mg/kg bw/day, 15-week administration of caffeine in the drinking water significantly increased relative liver weight and increased liver RNA polymerase I activity (Shields *et al.*, 1981). The authors indicated that this level of caffeine consumption would be in the range of 12-14 cups of coffee *per day* in humans (approximately 1200 mg/day for a 60 kg person).

The short-term and subchronic studies conducted in animals indicate that caffeine does not induce toxic responses at levels typical of human consumption.

### 5.3. Chronic Studies

The effects of chronic caffeine consumption in rats and mice have been evaluated in several studies (*i.e.*, greater than one year) (Table 8). Wurzner *et al.* (1977) conducted a two-year study in which male and female Sprague Dawley rats ( $n = 40/\text{sex}/\text{group}$ ) were administered regular or decaffeinated instant coffees in the diet at 6% of the diet. The average daily intake of coffee was approximately 2900 mg/kg bw/day for the males and 3500 mg/kg bw/day for the females.<sup>27</sup> The caffeine consumption was approximately 168 and 200 mg/kg bw/day, respectively. The body weights of the coffee-treated groups were generally lower than controls, occasionally statistically significant and were inversely proportional to the caffeine content of the coffee. However, rats that received decaffeinated coffee also had decreased body weights that

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<sup>26</sup> Thyroid hormones (T4 and T3), total protein, albumin, glucose, cholesterol and triglyceride levels.

<sup>27</sup> The authors noted that this level of consumption corresponds to approximately 80 cups for a 70 kg man and 70 cups for a 50 kg woman.

were occasionally statistically significant. The authors also noted that there were significant differences between certain groups in blood chemistry and hematology, but they were not considered to indicate toxic or ill effects, since historical controls exhibited similar values. This interpretation was substantiated by the histological findings (Wurzner *et al.*, 1977). Overall, the authors concluded that the various instant coffee samples were tolerated by the rats and the instant coffee (which included caffeine) did not induce any significant toxic effects, although there was occasionally statistically significant weight loss in males but less so in females.

Chronic caffeine consumption was non-carcinogenic at the administered dose levels (ranging from 55 mg caffeine/kg bw to approximately 192 mg caffeine/kg bw in the diet or drinking water) in several different studies (Macklin and Szot, 1980; Johansson, 1981b; Takayama and Kuwabara, 1982; Mohr *et al.*, 1984). The levels utilized in these studies are approximately 18 – 60-fold greater than the daily caffeine amount consumed by humans. An inverse dose-response effect was noted in one study with respect to both frequency and multiplicity of tumor formation (Mohr *et al.*, 1984). Caffeine consumption led to decreased weight gain in rats (Johansson, 1981a; Takayama and Kuwabara, 1982; Mohr *et al.*, 1984), although, in most studies, mortality rates in both mice and rats were not affected by chronic administration of caffeine. Chronic administration of caffeine (approximately 50 mg/kg bw/day) in the diet to male Sprague-Dawley rats for 117 weeks resulted in a significant increase in cardiovascular lesions, and the author (Johansson, 1981a; Johansson, 1981b) noted that the average life span of male Sprague Dawley rats was significantly shorter in treated animals, with the mean survival at 78 weeks in caffeine-treated animals, compared to 94 weeks in control animals. The caffeine group was found to have consumed significantly less diet than the control group. Moderately severe myocardial fibrosis of both left and right chambers of the heart and in the atria were noted in 20 of 29 caffeine-treated rats, compared to 5 of 29 control animals. Signs of cardiac insufficiency were exhibited as enlarged, dilated hearts, acute and chronic lung, liver and spleen congestion, and vascular changes consisting of dilated vessels of the mesenteric artery in conjunction with severe necrotizing inflammatory changes with fibrinoid necrosis. Four rats in the caffeine group also had signs of acute myocardial infarction and four additional animals had signs of scarification, presumably from previous infarctions (Johansson, 1981a). There was no significant increase in carcinogenesis in the caffeine group compared to the control group, but an

increased number of rats in the caffeine group died of cardiac insufficiency ( $P < 0.01$ ). This study is of questionable significance as other studies have not replicated this finding.

Caffeine consumption by C57BL/6 mice did not affect serum sulfhemoglobin, urea nitrogen, creatinine, or serum LDH values, with no gross histopathological changes noted; the only significant finding was an increase in the mean urinary specific gravity (Macklin and Szot, 1980). Mohr *et al.* (1984) reported that caffeine administration to rats (at up to 2,000 mg caffeine/liter, providing up to 170 mg caffeine/kg bw/day) did not induce a significant change in tumor frequency or multiplicity, with the exception of mammary fibroadenomas, which comprised 50% of all tumors in controls and only 26% in the rats administered 2,000 mg caffeine/liter drinking water (approximately equivalent to 102 and 170 mg/kg bw/day in male and female rats, respectively).

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**Table 8. Dosing regimens used in chronic caffeine consumption studies**

| Species/Strain/Sex                  | Dosing Protocol   | Animals (#/group) | Results   | Reference                    |
|-------------------------------------|---|-------------------|---|------------------------------|
| Male and female C57BL/6 mice        | 55 mg caffeine/kg/day for 72 weeks  | 40/sex/group      | No evidence of carcinogenic effect by caffeine; no toxicity was reported in the treatment group   | Macklin and Szot (1980)      |
| Male and female Sprague-Dawley rats | 200, 430, 930, 2000 mg caffeine/l drinking water (~20, 43, 93, and 200 mg/kg bw/day) for 104 weeks*   | 50/sex/group      | No treatment-related trend in any of the parameters measured, including hematology, clinical chemistry and histology; no increase in tumor incidence        | Mohr <i>et al.</i> (1984)    |
| Male and female Wistar rats         | 0.1 and 0.2% caffeine in drinking water (~100 and 200 mg/kg bw/day) for 78 weeks  | 50/sex/group      | No increase in tumor incidence above control values. No difference in mortality between treated and control rats. No toxicity reported in treatment groups. | Takayama and Kuwabara (1982) |
| Male and female Sprague Dawley rats | Regular and decaffeinated coffee added to the diet at 6%, providing an average of 133 and 161 mg caffeine/kg bw/day in a two-year study in male and female rats, respectively | 40/sex/group      | No toxicity noted in any of the dose groups that received regular coffee, decaffeinated coffee, or decaffeinated coffee with added caffeine                 | Wurzner <i>et al.</i> (1977) |

\*Equivalent to: 12, 26, 49 and 102 mg caffeine/kg bw/day in males and 15, 37, 80 and 170 mg/kg bw/day in females

#### 5.4. Mutagenicity/genotoxicity studies

Several investigators have evaluated the ability of caffeine to induce mutagenic/genotoxic effects. The studies utilized a number of prokaryotic, eukaryotic and mammalian cell culture systems, as well as whole animal studies. Summaries of studies evaluating the potential mutagenic effects of caffeine in mammalian cell systems are summarized in Table 9. Caffeine was not mutagenic in human peripheral blood lymphocytes (Weinstein *et al.*, 1973; Aeschbacher *et al.*, 1985) or Chinese hamster V79 cells (Sivak *et al.*, 1982). In rat breast MCT<sub>1</sub> cells incubated with 40 – 160 µg caffeine/ml<sup>28</sup> continuously for four weeks, minor chromosomal effects were noted, but only at the highest concentrations. However, treatment of HeLa cells with caffeine resulted in a significant increase in the frequency of terminal breaks at all concentrations tested (40 – 160 µg/ml), as well as dicentric and chromosomal breaks (Bishun *et al.*, 1974). Using the AraR mutagenicity assay in *Salmonella typhimurium* BA13 cells, Ariza *et al.* (1988) reported that caffeine was not mutagenic at the concentrations evaluated.

**Table 9. Mutagenic effects of caffeine on mammalian cell systems**

| Caffeine concentration | Cell type  | Results/Notes   | Reference                        |
|------------------------|--|---|----------------------------------|
| 250 – 750 µg/ml        | Human peripheral blood lymphocytes   | Non-mutagenic   | Weinstein <i>et al.</i> (1973)   |
| 5 – 100 µg/ml          | Human peripheral blood lymphocytes   | Non-mutagenic   | Aeschbacher <i>et al.</i> (1985) |
| 40 – 160 µg/ml         | Rat breast MCT <sub>1</sub> cells  | Minor chromosomal aberrations at highest concentrations | Bishun <i>et al.</i> (1974)      |
| 40 – 160 µg/ml         | Human HeLa cells   | Gross chromosome terminal breaks                        | Bishun <i>et al.</i> (1974)      |
| 1.0 mM                 | Chinese hamster V79 cells  | Inhibited post-replication repair mechanism             | Sivak <i>et al.</i> (1982)       |
| 1.0 mM                 | Human fibroblasts, SGL xeroderma pigmentosa fibroblasts, BALB/c-3T3 mouse cells, Syrian hamster embryo cells | Non-mutagenic   | Sivak <i>et al.</i> (1982)       |

D'Ambrosio (1994) critically reviewed many research studies evaluating the potential genotoxic effects of caffeine alone or in combination with other agents on various parameters of

<sup>28</sup> This concentration is 8 – 32-times the transitory peak blood caffeine physiological level of 1 – 5 µg/ml.

cell division, chromosome stability, toxicity and mutagenicity. A number of effects by caffeine were observed, but these effects were usually noted at very high caffeine concentrations (> 1 mM; 212 mg/l), were in combination with genotoxic agents, or were usually specific to a certain cell type and/or cellular parameters. There is some evidence of caffeine being mutagenic when evaluated in mammalian cell systems *in vitro*, while *in vivo* mammalian test systems indicate that caffeine is not mutagenic. The author concluded that “it is difficult to implicate caffeine, even at the highest levels of dietary consumption, as a genotoxin to humans” (D'Ambrosio, 1994). Contrary to most reports, Sen *et al.* (1994) found that caffeine (2.0, 4.0 and 6.0 mg/kg bw/day *via gavage*) administered for 7, 14 and 21 days induced chromosomal aberrations (*i.e.*, clastogenicity) in Swiss albino mice. The relevance to the potential for carcinogenesis has not been elucidated. Nawrot *et al.* (2003) concluded that although the evidence of the potential for caffeine to be mutagenic is conflicting, “it appears to be unlikely that at normal, physiologically relevant levels of consumption (*i.e.*, at less than systemic toxicity ranges), caffeine would result in mutagenic effects in humans.”

In a chronic study designed to evaluate the tumorigenic effects of caffeine, administration of caffeine at 50 mg/kg bw/day in the diet for 117 weeks to Sprague-Dawley rats ( $n=30$ /group) did not affect the type, distribution or frequency of tumor formation and did not induce chromosomal aberrations, but did induce a statistically significant increase in the frequency of blood cell sister chromatid exchanges (Granberg-Ohman *et al.*, 1980).

The EPA evaluated the genetic toxicity data for a variety of chemicals (including caffeine), as part of a Gene-Tox Carcinogen database that describes the analysis of 506 chemicals for their ability to induce tumors in experimental animals (Waters *et al.*, 1988). The Gene-Tox program identified 61 chemicals that were found to be inactive when tested in chronic rodent carcinogenesis studies, and these studies were compared to the genotoxicity studies conducted on these same substances, one of which was caffeine. Caffeine was considered a “limited negative” by the Gene-Tox Carcinogenesis Panel (Waters *et al.*, 1988).<sup>29</sup> In analyzing

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<sup>29</sup> A chemical was placed in the Sufficient Negative category if it did not induce significant numbers of benign or malignant tumors in at least one independent lifetime study in more than one species performed at or near a dose which induced minimal toxicity without appreciably altering the normal lifespan. Also required was that the chemical not demonstrate evidence of tumor induction in any other carcinogenesis bioassay. Chemicals were placed

gene mutations, *in vitro* tests provided mixed results, with some assays providing negative results in bacterial and mammalian cell assays *in vitro*, while assays for chromosome aberrations in plant, insect and mammalian cell systems were positive. All mammalian *in vivo* assays for caffeine were negative, including the mouse spot test, mouse specific locus test, unscheduled DNA synthesis in mouse germ cells, and altered mouse sperm morphology. Waters *et al.* (1988) concluded “[O]verall, while there is evidence for genotoxicity (especially chromosome aberration and aneuploidy) for this compound in *in vitro* mammalian systems, it does not appear to cause genotoxicity in mammals *in vivo*.”

In summary, the potential for caffeine to induce genotoxicity has been evaluated in both *in vitro* and *in vivo* studies, with *in vitro* assays claiming both genotoxic and nongenotoxic results, while overall, *in vivo* studies indicate that caffeine is not genotoxic.

### 5.5. Carcinogenesis

VanderPloeg *et al.* (1991) evaluated the influence of caffeine on benign and carcinomatous mammary gland tumor formation in female Sprague-Dawley rats administered caffeine at 500 mg/l in drinking water (approximately equivalent to 75 mg/kg bw/day) from 27 to 59 days of age. The exposed rats showed no apparent morphological differences in mammary gland ductal branching or lobuloalveolar development. In contrast to the above study, Welsch *et al.* (1988b) reported that caffeine at 250 or 500 mg/l in the drinking water (approximately equivalent to 62.5 or 125 mg/kg bw/day, respectively) significantly increased the multiplicity of mammary tumors by 20% and 40% in DMBA-treated female BD2F<sub>1</sub> mice. In female C3H mice, the multiplicity of tumors was increased by 13 and 117% in the 250 and 500 mg/l dose groups. C3H mice are known to exhibit a high incidence of spontaneous mammary tumors. There was no effect of caffeine on the latency period or percent of mice developing mammary tumors in either of these models (Welsch *et al.*, 1988b).

Several short-term carcinogenicity studies evaluated the effects of caffeine on DMBA-induced mammary tumor formation in female Sprague-Dawley rats (Table 10). In general, caffeine administered *via* the drinking water (100 – 860 mg/l; approximately equivalent to 10 –

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in the Limited Negative category using the same criteria as those for Sufficient Negative, except that only one study in one species was required (Waters *et al.*, 1988).

86 mg/kg bw/day, respectively) significantly decreased the multiplicity of mammary tumors (by up to 50%) (Welsch and DeHoog, 1988; Welsch *et al.*, 1988a; Wolfrom *et al.*, 1991; VanderPloeg *et al.*, 1991), but no effect by caffeine was shown on the *percent* of rats with mammary tumors or an effect on mean latency period to the onset of the first detectable neoplasm. The anti-carcinogenic effect of caffeine was most apparent when the caffeine treatment was initiated several weeks prior to administration of the carcinogen, but was usually ineffective when caffeine administration was started concurrent with or after DMBA administration. This anti-carcinogenic effect was specific to DMBA, as it was not reported to occur in rats induced with N-methyl-N-nitrosourea (MNU). Nagasawa and Sakurai (1986) found that caffeine at 500 mg/l (approximately equivalent to 125 mg/kg bw/day) for six weeks did not affect mammary gland end-bud system growth or mammary gland DNA content in C3H mice.

Administration of 0.25% caffeine in drinking water to male Wistar rats (approximately equivalent to 250 mg/kg bw/day) for a 32-week period had no effect on MNNG-induced glandular stomach carcinogenesis (Nishikawa *et al.*, 1995). Fundic and pyloric mucosa cell proliferation and gastric mucosa lipid peroxidation levels were also unaffected by caffeine treatment. The authors hypothesized that the absence of an anti-carcinogenic effect may be due to caffeine administration after the induction process, rather than before the MNNG treatment.

Johansson (1981b) noted that chronic treatment of male Sprague-Dawley rats with caffeine had no significant effect on the type, distribution or frequency of tumors when treated for 117 weeks at approximately 50 mg/kg bw/day.

**Table 10. Short-term carcinogenicity studies of caffeine**

| Duration   | Species<br>(#/dose group)         | Dose/Route  | Results/Notes  | Reference                       |
|--|-----------------------------------|---|--|---------------------------------|
| Approximately 26 weeks<br>(endpoint was number of days to tumor formation. | Sprague-Dawley rat (F) (20/group) | Dietary study <sup>1,*</sup>                              | Improved survival and increased mean latency time to onset of mammary tumors.                              | Minton <i>et al.</i> (1983)     |
| Approximately 26 weeks<br>(endpoint was number of days to tumor formation. | Sprague-Dawley rat (F) (20/group) | High-fat (20% vegetable fat) dietary study <sup>1,*</sup> | Reduced survival time, reduced mean latency time to mammary tumor onset; increased multiplicity of tumors. | Minton <i>et al.</i> (1983)     |
| 117 weeks  | Sprague-Dawley rat (M)            | 0.102% in the diet (approximately 50 mg/kg bw/day).       | No increase in tumor formation; decreased lifespan due to increased number of cardiovascular lesions.      | Johansson <i>et al.</i> (1981b) |

<sup>1</sup>Rats were pre-treated with 20 mg dimethylbenz(a)anthracene (DMBA); \*The amount of caffeine each rat received per day was equivalent to 500 mg caffeine in a 50-kg woman, based on surface area.

## 5.6. Teratogenicity and developmental toxicity effects

Studies that evaluated the potential teratogenicity of caffeine have been discussed in a review (Christian and Brent, 2001) and elsewhere (Nehlig and Debry, 1994), and only will be summarized here. It is not expected that a significant portion of the female population will be consuming caffeinated alcoholic beverages during pregnancy, as alcohol has been strongly contraindicated during pregnancy.<sup>30</sup> However, for completeness, a short overview of the issue of caffeine consumption and potential teratogenic or developmental adverse effects will be described herein. In addition, in a review of the impact of lifestyle factors on reproductive performance (Homan *et al.*, 2007) it was concluded that “the summation of evidence of associations between psychological stress, caffeine, alcohol consumption and reproductive performance is inconclusive,” although it is biologically plausible that these factors may affect reproductive performance. The authors concluded by stating, “some reports regarding the effect

<sup>30</sup> See <http://www.cdc.gov/ncbddd/fasd/alcohol-use.html> (last visited March 23, 2010); see also <http://www.surgeongeneral.gov/pressreleases/sg02222005.html> (last visited March 23, 2010).

of alcohol and caffeine consumption on fertility are conflicting and there is potential for error in the recall of consumption of exact dosage and residual confounding” (Homan *et al.*, 2007).<sup>31</sup>

Epidemiological studies of caffeine consumption have been conducted to determine the potential to produce congenital malformations. Two studies (Fredrick, 1974; Borlee *et al.*, 1978) reported a significant association with the consumption of caffeinated beverages during pregnancy and the birth of anencephalic or other various malformations occurring among the infants. This association was weak in both studies, and the studies were considered to have serious methodological limitations. In a well-controlled cohort study (Linn *et al.*, 1982), the frequency of congenital anomalies was not increased in the births of women ( $n = 595$ ) that consumed four or more cups of coffee *per* day during the first trimester of pregnancy. Christian and Brent (2001) concluded that “the epidemiological studies in the medical literature that reported statistical associations to many of the reproductive parameters lack consistency, contain methodological errors, are subject to multiple confounding factors, and are counterbalanced by many negative studies. Furthermore, pharmacokinetics data, when available, do not support the concept that toxic levels can be achieved under normal use conditions.”

In animal studies, caffeine administration to pregnant animals has been found to induce teratogenicity and toxic effects on the development of the fetuses only at doses that also caused toxic effects in the dams (Christian and Brent, 2001). In a teratogenicity study conducted to assess the teratogenic potential of coffee and caffeine in the Sprague Dawley rat, the rats were administered coffee as their sole beverage to provide caffeine intakes of approximately 9, 19, and 38 mg/kg bw/day, caffeine in drinking water at 30 mg/kg bw/day, caffeine (30 mg/kg bw/day *via* gavage) or control water (Palm *et al.*, 1978).<sup>32</sup> The authors found that there were no dose-related teratogenic effects due to coffee consumption, and no treatment related differences in body weight gain, food or water consumption or reproductive performance in the F<sub>1</sub> animals. The incidence of cleft palate was increased in the coffee-treated groups, but was inversely proportional to coffee and caffeine consumption. Teratogenic differences between gavage-

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<sup>31</sup> Residual confounding is confounding by unmeasured variables, or by measurement errors in variables. <http://aje.oxfordjournals.org/cgi/reprint/155/7/622> (last visited March 31, 2010).

<sup>32</sup> The authors stated that for a 60-kg person these caffeine levels would be comparable to the consumption of about 6, 14, and 27 cups of coffee daily (assuming 84 mg caffeine/150 ml cup of coffee).

versus water-provided caffeine were not detected, and there were no teratogenic effects compared to controls, although both groups differed from the control group in cryptorchism and irregular (possibly delayed) ossification of ribs and vertebrae. The delayed ossification was considered an aspect of delayed calcification of the bones and not a teratogenic effect (Palm *et al.*, 1978). Whitby *et al.* (1986) reported that caffeine, administered twice daily *via gavage* in a total dose of 40 or 80 mg/kg bw/day (providing a bolus dose of only 20 and 40 mg/kg bw/day, respectively), did not adversely affect the reproductive performance of male Osborne-Mendel rats. Christian and Brent (2001) concluded that animal studies that evaluated the potentially teratogenic or developmental effects of caffeine ingestion helped address contradictions noted when evaluating the epidemiological studies:

“...they revealed that the mode of administration is critical to the blood levels attained and the effects produced. Bolus (gavage) dosages of caffeine result in substantially higher blood levels and greater maternal and developmental toxicity, as compared with blood levels and effects produced by administration of caffeine in the drinking water or diet. The probable blood level of caffeine required to produce teratogenic effects in rats is in excess of 60 µg/ml, which can only be reached in rodents by administration of large bolus dosages achieving peak short-term exposure. Neither rodents nor humans can attain such peak exposures by consuming solutions of caffeine over several hours, the usual mode of human caffeine consumption.”

The aspect that bolus doses of caffeine are not representative of the consumption of caffeine in humans was provided earlier by Sullivan (1988), who indicated that pharmacokinetic studies are necessary in the interpretation of teratology studies for the extrapolation to humans, in that bolus doses of approximately 75 mg/kg produces a peak blood level of about 60 mg/l, while caffeine provided at up to 200 mg/kg/day in the diet or water result in plasma caffeine concentrations that may never exceed 10 mg/l. Nehig and Derby (1994) summarized the concept succinctly when they noted that when a bolus dose known to induce malformations was divided into several administrations throughout the day (thereby providing maternal and fetal caffeine blood concentrations lower than that obtained *via* a bolus dose), no malformations were observed, even when drinking water was replaced by 100% roasted coffee (providing 85 mg caffeine/kg bw/day). Nehig and Derby (1994) concluded that “the levels of caffeine must be very high (330 mg/kg) for the fractioned administration of caffeine to have an effect on the rate of malformations in the rat.”

Caffeine has been evaluated for its potential to produce teratogenic or developmental effects, with no indication of teratogenic effects at serum caffeine levels that could occur in humans consuming caffeinated beverages. Studies that reported positive teratogenic effects were administering large doses of caffeine equivalent to 15 – 23 cups of coffee *per* day in humans, and were providing the caffeine *via* gavage, which results in much higher peak serum caffeine levels and therefore overstates toxicity potential. In addition, epidemiological studies in humans have not indicated a positive association with high caffeine consumers and increases in teratogenic effects.

### 5.7. Cardiovascular effects

The effects of caffeine on the cardiovascular system have been examined in several experimental animal models. In an early study, Sollman and Pilcher (1911) evaluated the circulatory, cardiovascular and respiratory effects of a wide range of caffeine levels (2 – 800 mg/kg bw) in dogs. Small doses (up to 20 mg/kg) had little effect on the circulatory system, while doses ranging from 20 to 150 mg/kg decreased blood pressure, increased heart and respiration rates, and also produced some cardiac arrhythmias. Higher doses led to death, which was stated to have occurred from cardiac “paralysis” (Sollman and Pilcher, 1911).

Administration of approximately 50 mg/kg bw/day caffeine<sup>33</sup> in the diet for 117 weeks to male Sprague-Dawley rats induced significant cardiovascular lesions and reduced the mean survival time, compared to control animals (Johansson, 1981a). Sixty-four *percent* of the caffeine-treated animals died from cardiac insufficiency, compared with 17% of the control animals. Rats that exhibited cardiac insufficiency had enlarged and dilated hearts with signs of acute and chronic congestion of the lungs, liver and spleen. In four rats, acute myocardial infarction was noted, and an additional four had scarification, which is an indication of old myocardial infarcts. Twenty out of 29 rats had moderately severe myocardial fibrosis of both chambers and atria. In addition, twelve of the animals in the caffeine dose group had prominent vascular changes in the mesenteric arteries and in the arteries near the pancreas, including thromboses and severe necrotizing inflammation with fibrinoid necrosis (Johansson, 1981a).

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<sup>33</sup> This dose would be equivalent to 3000 mg caffeine/day in a 60 kg person.

These findings were not replicated in other chronic studies (Macklin and Szot, 1980; Takayama and Kuwabara, 1982; Mohr *et al.*, 1984) at comparable dose levels.

## 5.8. Observations in Humans

The effects of caffeine on the human body in regard to long-term health has been a source of debate for decades, and recent, convincing research has narrowed the list of potential adverse effects, removing the hypothesis that caffeine consumption may be related to several diseases, which included various cancers and benign breast disease. Extensive research has also been conducted to analyze the effect of caffeine consumption on the incidence of cardiovascular disease, fluid homeostasis, osteoporosis, and reproduction and pregnancy outcomes. Caffeine may increase calcium excretion in the urine in women, which could lead to bone maintenance issues and, eventually, osteoporosis, if overall calcium intake is low (Thomas, 1997; Nolan, 2001). However, this is not expected to occur when adequate calcium intake is maintained. Recent reviews have indicated that caffeine does not have a clinically significant effect on reproduction, osteoporosis and calcium metabolism, addiction/withdrawal or cardiovascular health (Nolan, 2001; Heaney, 2002).

The hypothesis that caffeine may affect metabolic parameters was studied after consumption of caffeine in a double-blind, placebo-controlled crossover clinical trial in which subjects ( $n = 16$ ; 7 males and 9 females) consumed a supplement containing 600 mg black tea extract (60% polyphenols, 20% caffeine (2 mg/kg caffeine)) and 442 mg guarana extract (36% caffeine; 2.6 mg/kg caffeine) or matching placebo (Roberts *et al.*, 2005). The total amount of caffeine consumed was 279 mg (4.6 mg/kg) during this study, and the parameters evaluated were the resting metabolic rate (RMR), respiratory quotient (RQ), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, and temperature. Metabolic rate and respiratory quotient were measured for 30 minutes after the resting period by indirect calorimetry. RMR increased ( $P < 0.05$ ) after supplementation, compared to placebo, and peaked one hour after consumption. The AUC for SBP increased ( $P < 0.01$ ) over a two hour period, while there were no differences in RQ, DBP, pulse rate or temperature between supplement and placebo groups. The authors concluded that “the modest significant rise in systolic blood pressure can be attributed to the known pressor effect of caffeine” (Roberts *et al.*, 2005). However, this effect may not have been

solely due to caffeine, as there were additional ingredients (polyphenols, epigallocatechins, *etc.*) in the supplement.

Very high doses of caffeine may induce caffeine toxicity in humans, defined by the specific symptoms that occur as a direct result of the consumption of caffeine, which include the following: anxiety, gastrointestinal upset, insomnia, nervousness, psychomotor agitation, restlessness, tachycardia, tremors and, in rare cases, death (IOM, 2004). Ingestion of caffeine at doses up to 10 g was found to cause convulsions and vomiting, with complete recovery in six hours (Dreisbach, 1974). Caffeine at 1,000 mg (approximately 15 mg/kg bw) may induce insomnia, restlessness, and agitation (IOM, 2004). The estimated lethal dose in adults is 150-200 mg/kg (10 – 14 g for a 70 kg person) (Hodgman, 1998). Mrvos *et al.* (1989) reported of a case where a 22-year-old female consumed an unknown quantity of caffeine in pill form, resulting in a caffeine serum level of 1,560 µg/ml (lethal > 100 µg/ml). No other drugs were identified by a drug screen. The woman died of cardiac arrest. No other information on the quantity of caffeine consumed was provided.

#### **5.8.1. Cardiovascular disease risk**

Nawrot *et al.* (2003) conducted a review of the effects of caffeine on human health and concluded that “for the healthy adult population, moderate daily caffeine intake at a dose level up to 400 mg/day (equivalent to 6 mg/kg body weight/day in a 65-kg person) is not associated with adverse effects such as general toxicity, cardiovascular effects, effects on bone status and calcium balance (with consumption of adequate calcium), changes in adult behavior, increased incidence of cancer and effects on male fertility.” It was indicated that habitual daily use of caffeine at greater than 500-600 mg/day (8.3 – 10 mg/kg) (4-7 cups of coffee or 7-9 cups of tea) could be considered a health risk. For women, caffeine intake greater than 400 mg/day (6.7 mg/kg) “may increase the risk of detrusor instability (unstable bladder) development in women” (Nawrot *et al.*, 2003).

In the review, Nawrot *et al.* (2003) also summarized clinical trials that investigated the effects of caffeine or coffee on cardiac arrhythmia, heart rate, serum cholesterol and blood pressure, as well as epidemiological studies, which have primarily investigated any potential associations between coffee intake and cardiovascular risk factors, such as blood pressure and

serum cholesterol levels, or the incidence of cardiovascular disease. Clinical studies evaluating single caffeine doses of less than 450 mg (7.5 mg/kg) indicate that caffeine does not increase the frequency or severity of cardiac arrhythmia in healthy people, patients with ischemic heart disease or serious ventricular ectopia. Caffeine at greater than 150 mg (2.5 mg/kg) (acute) may actually decrease heart rate (James, 1991; Green *et al.*, 1996; Myers, 1998). Current literature indicates that caffeine does not increase total and low-density lipoprotein cholesterol levels, but that possibly two diterpenoid alcohols found in coffee may have potential hypercholesterolemic actions (Thelle, 1995). Mukamal *et al.* (2004) also found that coffee consumption was not associated with an overall change in long-term post-infarction mortality rate.

Wiklund *et al.* (2009) investigated the changes in the electrocardiogram (ECG) and heart rate variability after intake of an energy drink, taken in combination with alcohol and exercise. The subjects ( $n = 5/\text{sex}/\text{group}$ ) performed a physical and laboratory baseline screening, followed by four tests, performed in the same order with 1-3 months between each test: (1) consumption of 750 ml of an energy drink (containing 240 mg caffeine; 4 mg/kg) after an overnight fast; (2) consumption of 750 ml energy drink mixed with vodka, to provide 400 mg ethanol/kg bw, and a maximal bicycle ergometer exercise 30 minutes later (ED/ET); (3) consumption of 750 ml energy drink followed by a maximal bicycle ergometer exercise 30 minutes later (ED); (4) maximal bicycle ergometer exercise after 30 minutes rest (EX). Electrolytes, electrocardiogram measurements and heart rate variability and recovery were analyzed on each subject. The subjects developed blunted cardiac autonomic modulation after exercising when they had consumed energy drinks mixed with alcohol. No subject developed any clinically significant arrhythmias. The post-exercise recovery in the heart rate and heart rate variability was slower when the subjects consumed the energy drink/alcohol combination before exercise, than when consumed after exercise. The final heart rate was also increased in the subjects that consumed energy drink alone, and no statistical analysis was conducted to determine if there were different effects between energy drink consumption and energy drink plus alcohol consumption. This study does not provide an analysis to determine significant differences between caffeine and caffeine plus alcohol consumption (Wiklund *et al.*, 2009).

### 5.8.2. Reproduction, teratology and pregnancy outcome

Caffeine consumption has been suggested as a cause of several adverse reproductive effects, including reduced conception, delayed implantation, premature births, low infant birth weight, congenital malformations, spontaneous abortions, and shortened menstrual cycles. However, the totality of the data indicate that caffeine consumption has not been consistently linked to adverse effects on conception, pregnancy or lactation (Leviton, 1988; Christian and Brent, 2001; Leviton and Cowan, 2002). Studies conducted to evaluate the effects of caffeine intake on fertility, birth weight, premature births, or congenital malformations have certain methodological inadequacies and are conflicting in their conclusions. Overall, human studies indicate that caffeine consumption does not have an effect on reproductive parameters (Leviton, 1988; 1998; Christian and Brent, 2001; Leviton and Cowan, 2002).

The effect of caffeine on fertility has been evaluated in several studies, with the outcomes at times contradictory. Some results indicate no significant effect of caffeine on fertility or risk of delayed conception (Curtis *et al.*, 1997; Caan *et al.*, 1998; Hakim *et al.*, 1998), while others indicated reduced fecundity or delayed conception in those who consume caffeine (Wilcox *et al.*, 1988; Hatch and Bracken, 1993; Bolumar *et al.*, 1997). In one study, total caffeine intake did not affect fecundity among smokers ( $n = 430$  couples), except when caffeine consumption exceeded 700 mg/day (Jensen *et al.*, 1998).

The effects of caffeine consumption on spontaneous abortion has been evaluated in several epidemiological studies, with some results indicating a small or nonsignificant increase in risk (Armstrong *et al.*, 1992; Mills *et al.*, 1993), while other studies find a stronger correlation between caffeine consumption and spontaneous abortion (Srisuphan and Bracken, 1986; Fenster *et al.*, 1991; Mills *et al.*, 1993; Dlugosz *et al.*, 1996; Fenster *et al.*, 1997). The effect of caffeine on pregnant women is difficult to ascertain because consumption is often curtailed during pregnancy as the result of the nausea associated with early pregnancy, as well as an abandonment of certain lifestyle practices perceived harmful to the fetus. One study reported that pregnant women suffering from nausea and consuming >300 mg caffeine/day had a significantly greater risk for spontaneous abortion than those who abstained from caffeine. However, the study also found that coffee consumption reduced the risk for spontaneous abortion in women who did not

experience nausea during their pregnancy (Fenster *et al.*, 1991). Fenster *et al.* (1997) also found that high levels of caffeine, or coffee, consumption was not associated with spontaneous abortion; however, consumption of  $\geq 3$  cups decaffeinated coffee *per* day doubled the risk. Klebanoff *et al.* (1999) found that women who had spontaneous abortions also had significantly higher serum paraxanthine levels (a marker for caffeine exposure), and the paraxanthine levels that correlated with an increased risk of spontaneous abortion were extremely high ( $> 1,845$  ng/ml). The authors concluded that moderate consumption of caffeine was not likely to increase the risk of spontaneous abortion.

### 5.8.3. Fluid homeostasis

It is generally known that caffeine is a diuretic, increasing urinary excretion within one hour of consumption. Wemple *et al.* (1997) reported that consumption of a caffeinated beverage (2500 ml, providing 1 mg caffeine/kg bw) led to a greater mean three-hour urine output, when compared to a non-caffeinated beverage. However, exercise decreased the effect to a nonsignificant level. Administration of 250 mg caffeine to healthy subjects ( $n = 8$ ) resulted in an increase in diuresis, with increased potassium, sodium, and osmol excretion within one hour post-treatment, although aldosterone and vasopressin concentrations were unchanged (Nussberger *et al.*, 1990). Compared to mineral water consumption, coffee (providing 642 mg caffeine over the course of a day) significantly increased the 24-hour urine output, resulting in a negative fluid balance and a decrease in total body water by 2.7%. Sodium and potassium excretion increased by 66% and 28%, respectively. Overall, caffeine consumption has been found to increase the potential for total water body deficits to occur (Gonzalez-Alonso *et al.*, 1992; Maughan and Leiper, 1994; Neuhauser-Berthold *et al.*, 1997), but that this effect depends on the amount of caffeine consumed, the individual's history of acute and chronic caffeine use, and the total solute load of the beverage plus accompanying meals (Wemple *et al.*, 1997; Brouns *et al.*, 1998). Kiyohara *et al.* (1999) evaluated the effects of caffeine consumption on serum uric acid concentrations, a possible indicator of increased urination. Men that consumed less than one cup coffee/day had a mean serum uric acid concentration of 60 mg/l, while men that consumed  $\geq 5$  cups/day had a mean concentration of 56 mg/l. Overall, although moderate to high caffeine intake (600 - 900 mg/day) may increase fluid and electrolyte losses in urine, for the general population, a typical diet will replace these losses (Maughan and Leiper, 1994).

#### 5.8.4. Behavioral effects

There has been a longstanding use of caffeine in alcoholic beverages, and as a result, there have been studies exploring the use of the two together on behavior, and while not related specifically to the safety-in-use of caffeine in alcoholic beverages, the reported behavioral effects will be briefly discussed here. Recent on-line surveys and anecdotal reports have indicated that the ingestion of caffeinated drinks and alcohol are increasing (Oteri *et al.*, 2007; O'Brien *et al.*, 2008; Reissig *et al.*, 2009).<sup>34</sup> However, research efforts to date focus mainly on the behavioral aspects of caffeine and alcohol consumption, as opposed to any toxic effects. Thombs *et al.* (2010)<sup>35</sup> assessed event-level associations in a U.S. college bar district<sup>36</sup> between energy drink consumption, alcohol intoxication, and the intent to drive a motor vehicle. The authors reported that approximately six percent of all bar patrons in this report ( $n = 693$ ) consumed energy drinks mixed with alcohol. The consumption of energy drinks mixed with alcohol was associated with an approximately three-fold increased risk of leaving a bar with a blood alcohol level (BrAC) of greater than 0.08 g/210 L (the legal limit BrAC for driving in the state where the study was conducted). The authors also reported that patrons who had consumed energy drinks mixed with alcohol were four times more likely than other patrons to leave the bar intending to drive. However, those patrons who had consumed energy drinks mixed with alcohol, and who intended to drive, had a mean BrAC of 0.07 g/210 L, while those who did not intend to drive had a mean BrAC of 0.09 g/210 L. Thombs *et al.* (2010) hypothesized that "[T]he habitual practice of ordering alcoholic drinks in bars that are mixed with energy drinks may be a manifest feature of an underlying syndrome of problem behavior" and that drinking energy drinks mixed with

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<sup>34</sup> Exaggerated claims of caffeine effects are not new: In 1911, cola became the focus of one of the earliest documented health scares when the US government seized 40 barrels and 20 kegs of Coca-Cola<sup>®</sup> syrup in Chattanooga, Tennessee, alleging that the caffeine in its drink was "injurious to health". On March 13, 1911, the government initiated *United States v. Forty Barrels and Twenty Kegs of Coca-Cola*, 241 U.S. 265 (1916), hoping to force Coca-Cola<sup>®</sup> to remove caffeine from its formula by making claims, such as that the excessive use of Coca-Cola<sup>®</sup> at one girls' school led to "wild nocturnal freaks, violations of college rules and female proprieties, and even immoralities." The judge ruled in favor of Coca-Cola<sup>®</sup>. <http://en.wikipedia.org/wiki/Caffeine> (last visited April 12, 2010).

<sup>35</sup> Thombs *et al.* (2010) was published and available after the comprehensive literature search was conducted for this GRAS dossier, but was noted as a manuscript relevant to this GRAS dossier. No new literature search has been conducted since March, 2010.

<sup>36</sup> The interview, self-report survey, and BrAC data were collected from patrons exiting seven drinking establishments in a college bar district in Gainesville, FL adjacent to a public university.

alcohol “may be a marker of difficulties in psycho-social development.” The authors stated that their findings suggest that “patrons who consume drinks mixed with energy drinks may have somewhat distinct behavioral or psychological characteristics, and it tends to weaken pharmacological explanations for the observed associations” between combined consumption of energy drinks and alcohol.

Ferreira *et al.* (2006) analyzed the effects of consumption of an energy drink containing caffeine with alcohol on the perception of motor coordination impairment, compared to alcohol consumption alone. The authors stated that “[W]hen compared with the ingestion of alcohol alone, the ingestion of alcohol plus energy drink significantly reduced subjects’ perception of headache, weakness, dry mouth, and impairment of motor coordination. However, the ingestion of the energy drink did not significantly reduce the deficits caused by alcohol on objective motor coordination and visual reaction time.” A review of the data indicated that the effects of alcohol plus energy drink were still substantially above the energy drink alone measurements (an indication that the perception of impairment was still apparent), and that the difference between the means for these effects were, for many of the parameters, greater than the mean value (*e.g.*, the alteration in motor coordination for the energy drink session was  $6 \pm 12$  points, while consumption of alcohol increased the perception to  $15 \pm 15$  ( $P < 0.05$ ) and energy drink plus alcohol reduced the perception of impairment to  $11 \pm 12$ ). Although the data provided indicate that energy drink consumption did not substantially affect the perception of alcohol-induced impairment, the authors evaluated the data and concluded that “[E]ven though the subjective perceptions of some symptoms of alcohol intoxication were less intense after the combined ingestion of the alcohol plus energy drink these effects were not detected in objective measures of motor coordination and visual reaction time, as well as on the breath alcohol concentration.” Since caffeine itself is known to affect motor coordination and visual reaction time, the study groups probably should have been compared to a naïve control group.

Behavioral effects of caffeine and alcohol consumption have been conducted in both preclinical and clinical studies. Preclinical studies have reported conflicting results, as indicated in the following studies. The effect of the administration of caffeine on ethanol-induced motor incoordination was investigated in mice (Dar and Wooles, 1986). In the study, mice ( $n \geq 15$  mice/group) received caffeine (approximately 45 and 90 mg/kg/24 h *ad libitum* in the drinking

water) or tap water (control) for ten days, then administered ethanol (1500 mg/kg bw, *i.p.*) one hour or 24 hours after caffeine withdrawal. Each mouse served as his own control and was tested for motor coordination by utilizing a standard mouse rotorod treadmill and determining the ability of each mouse to remain on the rotorod for an arbitrarily assigned time of 180 seconds at a rotational speed of 18 rpm. Significant motor incoordination was produced by the acute dose of ethanol and occurred approximately 15 minutes after ethanol administration. Caffeine alone had no effect on motor incoordination, but chronic caffeine administration potentiated (*i.e.*, increased) the ethanol-induced motor incoordination at both caffeine doses. The authors concluded that “acute ethanol-induced motor incoordination was markedly potentiated in animals chronically (defined as ten days) fed caffeine or isobutyl-methylxanthine (IBMX) after 1 and 24 h of their withdrawal” (Dar and Wooles, 1986). This study indicates that caffeine may potentiate the effects of motor in coordination induced by ethanol consumption. Conversely, Spinetta *et al.* (2008) reported that caffeine administration (*i.p.* at 5 mg/kg bw), delivered either one hour after ethanol (3000 mg/kg bw; *i.p.*), or 20 minutes prior to habituation to a novel odor, negated ethanol-induced impairment of memory, indicating that caffeine may influence various areas of cognitive function during ethanol consumption.

Gulick and Gould (2009) utilized a plus-maze discriminative avoidance task (PMDAT) that allows within-subject measurement of learning, anxiety, and locomotion in the mouse model to evaluate behavior modulation of caffeine and alcohol. In this study, male C57BL/6 mice ( $n = 8-10/\text{group}$ ) were administered caffeine (5-40 mg/kg) *i.p.* 30 minutes before training and ethanol (1000 or 1400 mg/kg) was administered (*i.p.*) 15 minutes before training. For training, each mouse was placed in the center of the plus-maze<sup>37</sup> for five minutes, and each time the mouse entered an aversive enclosed arm, a light and white noise were initiated. During the testing phase, each mouse was returned to the center of the maze for three minutes. No cues were initiated during testing. Gulick and Gould (2009) found that ethanol alone (1000 – 1400 mg/kg bw) decreased anxiety and learning and increased locomotion in a dose-dependent fashion. Caffeine alone (5 – 40 mg/kg bw) dose-dependently decreased locomotion and learning, but increased anxiety. Ethanol (1400 mg/kg bw) inhibited the anxiogenic effect of caffeine, while

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<sup>37</sup> Indicating the shape of the maze.

caffeine did not reverse the ethanol-induced learning deficits. The authors concluded that “caffeine has been shown to reverse some of the behavioral effects of ethanol, including sedation and deficits in attention, but the current study demonstrates that caffeine was unable to reverse ethanol-induced deficits in avoidance learning. However, ethanol reduced caffeine-induced anxiogenesis” (Gulick and Gould, 2009).

Caffeine and ethanol are known to affect human behavior, but the mechanisms specific to the interaction between caffeine and alcohol to modulate behavior is difficult to ascertain. According to Fundin and Nicastro (1988), reports in the published literature of investigations on the mutual effects of caffeine and ethyl alcohol (*i.e.* alcohol), may reach as far back as 1894 and; as early as 1924, caffeine was offered as an antidote to alcohol poisoning (Cushny, 1924). Fundin and Nicastro (1988) concluded that the variations in caffeine and alcohol doses and timing provided in various studies, and the exact function evaluated, will influence whether caffeine will antagonize (Nash, 1966; Osborne and Rogers, 1983) or potentiate (Mozkowitz and Burns, 1981) alcohol-induced decreases in physical motor coordination. In their seminal review of the subject, published in 1988, Fundin and Nicastro (1988) examined the published literature of the time, and found a number of incongruities and identified a number of issues that may have led to the reported conflicting results (Table 11).

**Table 11. Summary of findings of Fudin and Nicastro (1988)**

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| <ul style="list-style-type: none"><li>• No conclusive evidence of a difference in sex in caffeine and alcohol interaction, but there may be a difference in performance on tests.</li><li>• No attempt in several studies to adjust or select for subjects with a history of caffeine or alcohol consumption.</li><li>• No attempt was made to describe how much alcoholic beverage was administered in terms of absolute alcohol (<i>e.g.</i>, 80-proof vodka is approximately 40% alcohol).</li><li>• Some testing required hand steadiness, which was often difficult to maintain, especially for non-habitual caffeine users.</li><li>• The interval of caffeine and alcohol ingestion was ignored by most investigators, with some providing alcohol and caffeine together, and others dosing with caffeine prior to or following alcohol.</li><li>• Investigator speculation that tasks such as arithmetic tests were a surrogate to more complex tasks, such as driving.</li></ul> | <ul style="list-style-type: none"><li>• Several studies disregarded weight differences between subjects and dosed on a mg/person basis, as opposed to mg/kg for caffeine, alcohol or both.</li><li>• Alcohol and caffeine doses varied considerably; some caffeine doses were up to 500 mg/person (11.6 mg/kg).</li><li>• No standard for testing for alcohol, although some later studies reported blood alcohol levels (BrAC).</li><li>• Investigators often focused on the decrement of performance as the result of alcohol, but did not discuss improvement of performance with caffeine and alcohol.</li><li>• Some conclusions were not supported by statistical analysis (<i>i.e.</i>, caffeine potentiation of alcohol's negative effects).</li><li>• Despite early evidence that the effect of caffeine on performance may differ according to phase of alcohol distribution and excretion (a greater effect on the descending portion of the curve), there was often no correction for time of administration.</li></ul> |
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Although more recent studies have taken the criticisms of Fudin and Nicastro (1988) into account, some newer studies include the use of energy drinks (ED) as the caffeine source, but ED often include other ingredients, such as taurine and sugar, which may have their own effects, and, guarana, which is as much as 36% caffeine and would result in additive effects to the caffeine dose. Lastly, most ED do not disclose concentrations of these other ingredients. However, despite the deficiencies of these studies, some provided insightful information regarding alcohol and caffeine interactions.

Franks *et al.* (1975) analyzed the effect of caffeine (300 mg/70 kg) on cognitive, perceptual and motor functions both alone (C) and in combination with ethanol (C&E) (750 mg/kg) in healthy volunteers ( $n = 68$ ; 20-28 yrs of age; 31M and 37F), over an eight-week period. The subjects were divided into four groups: Ethanol + caffeine, ethanol + caffeine placebo, ethanol placebo + caffeine, and a double placebo. Caffeine was administered in decaffeinated coffee immediately after consuming an alcoholic beverage (over a 20-minute period). The test battery was composed of the following tests: standing steadiness (eyes open and

eyes closed), simple auditory and visual reaction time, complex reaction time, manual dexterity, numerical reasoning, perceptual speed, and verbal fluency. The tests were conducted at 40, 100 and 160 minutes post beverage administration. Plasma ethanol concentrations were evaluated at 40, 100, and 160 minutes post ethanol/caffeine consumption.

Caffeine consumption did not affect blood ethanol levels during the 160 minute evaluation period, nor did it affect mean blood lactate concentrations. Compared to a double placebo, a significant decrease in standing steadiness (eyes open) occurred in the ethanol groups, with or without caffeine consumption. Overall, Franks *et al.* (1975) concluded that “there was no clear pattern of antagonism of the ethanol-induced performance decrements by caffeine.” Caffeine tended to reduce the alcohol-induced reductions in simple auditory and complex reaction times, as well as the simple visual reaction time. Caffeine did not antagonize ethanol-induced reductions in performance in numerical reasoning, manual dexterity, or verbal fluency. In general, caffeine had a positive effect (usually at the 160 minute interval – the descending curve of intoxication), which improved some of the poor scores achieved by ethanol alone. At times, the caffeine + alcohol group was no different from double placebo controls or controls alone.

In a clinical study that evaluated the reinforcing and physical dependence producing effects of caffeine, subjects ( $n = 9$ ; the subjects reported consuming a mean of twelve cups of coffee *per day* and all subjects were smokers) consumed caffeinated (100 mg/cup) or decaffeinated coffee ten consecutive days, and were then switched to the opposite drink (Griffiths *et al.*, 1986). This occurred for a total of 55 days, with three rounds of consuming caffeinated coffee for 10-day periods, and two rounds of consumption of the decaffeinated coffee. When the subjects switched between caffeinated and decaffeinated coffee, the daily amount of coffee consumed remained constant. When subjects were caffeine tolerant/dependent (based on previous caffeine consumption), caffeinated coffee was rated as being better liked and preferred to decaffeinated coffee. Those subjects that were not caffeine tolerant/dependent had no preference to the type of coffee consumed. This study demonstrated that caffeine has behavioral reinforcing properties in human coffee consumption, and that caffeinated coffee withdrawal resulted in an orderly caffeine withdrawal syndrome that peaked on Day 1 or 2 of decaffeinated coffee consumption, then, gradually decreased. The withdrawal syndrome was

characterized by increased headache, sleepiness and laziness and decreased alertness and activeness. The authors stated that the present study “suggests that the reinforcing effects of caffeine may be related functionally to caffeine tolerance/dependence (*i.e.*, background condition)” (Griffiths *et al.*, 1986).

Grattan-Miscio and Vogel-Sprott (2005) conducted a clinical trial to evaluate the effect of caffeine or an environmental incentive (*i.e.*, monetary incentive) on the reduced intentional control observed in subjects that consumed alcohol. Social drinkers ( $n = 11/\text{group}$ ) were provided either placebo (two drinks of carbonated soda; P); caffeine (4.4 mg/kg bw) consumed at the same time as alcohol (620 mg/kg bw; AC); alcohol alone (620 mg/kg bw absolute alcohol in a beverage containing two parts carbonated beverage to one part alcohol; A); or alcohol plus a monetary incentive (AR) for correct responses on the word stem completion task that was the study test to determine intentional control.<sup>38</sup> AC and AR did not significantly affect controlled responses, compared to the placebo group that did not consume caffeine or alcohol. Controlled responses were depressed in the alcohol group. The most inappropriate responses were displayed under alcohol alone, while fewer were noted with the addition of caffeine or the incentive, and the least were noted in the placebo group. No treatment significantly affected automatic processes. The authors concluded that “the current study shows that the depressing effect of alcohol on controlled processes can be counteracted by a stimulant drug, caffeine, and by an environmental incentive.”

In a social setting, a large component of the effects of alcohol or caffeine may be influenced by the drinkers’ expectations about the behavioral effect of the drink. Fillmore and Vogel-Sprott (1995) investigated the effect of expectation on predicting psychomotor performance when subjects are expecting both substances. Male social drinkers ( $n = 50$ ; 19-27 years of age) were divided into 1 of 4 treatment groups or a placebo group (10/group); all had a history of alcohol and caffeine consumption. The test was a “pursuit rotor task” where the subjects were required to use a computer mouse to keep a cross-hair on a moving target on a

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<sup>38</sup> The participants received a word list and were asked to remember them, then the word stem completion task was conducted, in which half of the word stems were colored green and the participants were told to complete these stems with the familiar word from the list they had seen. If they could not think of the list word, they were to complete the stem with the first word that came to mind that fit the stem.

screen. The four treatment groups expected and received alcohol (560 mg/kg), while the expectation and receipt of caffeine was the independent factor; alcohol and caffeine were received within six minutes of each other. Poorer performance was displayed when alcohol was administered alone or with placebo caffeine, and performance was better when caffeine was administered with alcohol, regardless of whether caffeine was expected. Fillmore and Vogel-Sprott (1995) concluded that, “[a]s predicted, individual difference in expected effects predicted the participants’ performance when they expected to receive caffeine in combination with alcohol. Regardless of whether caffeine was actually received, those who expected the most impairment from the drug combination performed most poorly.” However, the authors failed to discuss the fact that the group expecting caffeine and receiving it, scored near equally to the untreated control (an improvement over training sessions), despite having also received the same amount of alcohol as the other groups. Other groups performed less well than their baselines. It was unclear if the test subjects were apprised of the investigators’ expectations (*i.e.*, caffeine’s exaggeration of alcohol effects) or if the subjects were not segregated according to beliefs held. This indicates that the expected type of effect influences the behavioral effect of alcohol combined with caffeine, and is largely dependent on the individual, and may not largely be a physiological effect.

In a subsequent test performed by Fillmore *et al.* (2002), 42 social drinkers (mean age 22.3 yrs, with a range in age of 21 – 32) were administered 650 mg/kg alcohol and 4 mg/kg caffeine. Some of the subjects were told to expect that caffeine would have an antagonistic effect on alcohol and others were not. The pursuit rotor task was employed and performance measured as percentage of time on target. Average BrAC during the test was 0.08%. The results were counter-intuitive: groups lead to expect caffeine antagonism to alcohol displayed levels of impairment that were comparable to an alcohol (only) control group that received no expectancy or caffeine treatment. In contrast, groups told caffeine would have no counteracting effect were essentially unimpaired under alcohol.

Fillmore (2003) went on to test the hypothesis that a history of drug-induced antagonism of alcohol impairment would enhance alcohol tolerance in humans ( $n = 21$ ; mean age = 23.5 yrs), and found that a history of combined alcohol and caffeine administrations increased alcohol

tolerance compared with an exposure history to either substance alone. He found that “the coadministration of caffeine antagonized the psychomotor-impairing effects of alcohol (peak BrAC = 0.07%) on repeated sessions (pursuit rotor task was used), and this behavioral history resulted in tolerance to an alcohol challenge dose. Those with a history of combined alcohol-caffeine administration showed no significant impairment in response to the challenge dose.” Fillmore noted that tolerance was displayed by drinkers who received reinforcement, while those that did not receive rewards did not develop tolerance, and the tolerance effects were specific to psychomotor impairment. The author also indicated that certain compensatory strategies to counteract alcohol impairment might also be enhanced by the caffeine, which can alter the conditions of performance.

In a clinical trial designed to investigate the interactive effects of caffeine and alcohol on rapid information processing (RIP), Hasenfratz *et al.* (1993) provided the subjects ( $n = 9$ ; males ages 23-29 years) 3.3 mg/kg bw caffeine (contained in a cup of decaffeinated coffee) and a 300 ml orange juice beverage that provided 700 mg ethanol/kg bw. The placebos were decaffeinated coffee, and orange juice that contained only one ml of alcohol on the surface, respectively. The task to evaluate mental performance was a RIP task, in which the subjects had to press a response key as rapidly as possible after the detection of a target. The processing rate (number of digits processed per time unit) and the reaction times for hits were analyzed as indices of performance. The reaction time and processing rate were affected by alcohol alone and caffeine alone ( $P < 0.05$ ), but the combination of alcohol and caffeine was not different from the control values (Figure 5). The authors concluded that “qualitatively, caffeine improved and alcohol impaired both assessed performance parameters of the RIP task. The combination of the two treatments led to an addition of these two effects, indicating that caffeine was able to offset the debilitating effects of the alcoholic beverage under these conditions” (Hasenfratz *et al.*, 1993). This work confirmed earlier work by Kerr *et al.* (1991) in which female subjects ( $n = 10$ ) performed a choice reaction time task (CRT), a compensatory tracking task (CTT), a short-term memory task (STM) and a critical flicker fusion (CFF) threshold parameter, when consuming placebo, caffeine (300 mg; approximately 5 mg/kg bw), alcohol (30 g 80 proof vodka; approximately 500 mg alcohol/kg bw), or the combination of caffeine and alcohol. Alcohol consumption impaired the performance to some extent in all parameters evaluated, while

caffeine plus alcohol consumption antagonized the CFF and STM parameters ( $P < 0.05$ ). The authors noted that the results were group effects, and that the effects of caffeine and alcohol on an individual varies according to a number of factors, including age, weight, gender, genetic susceptibility, and normal consumption habits (Kerr *et al.*, 1991).

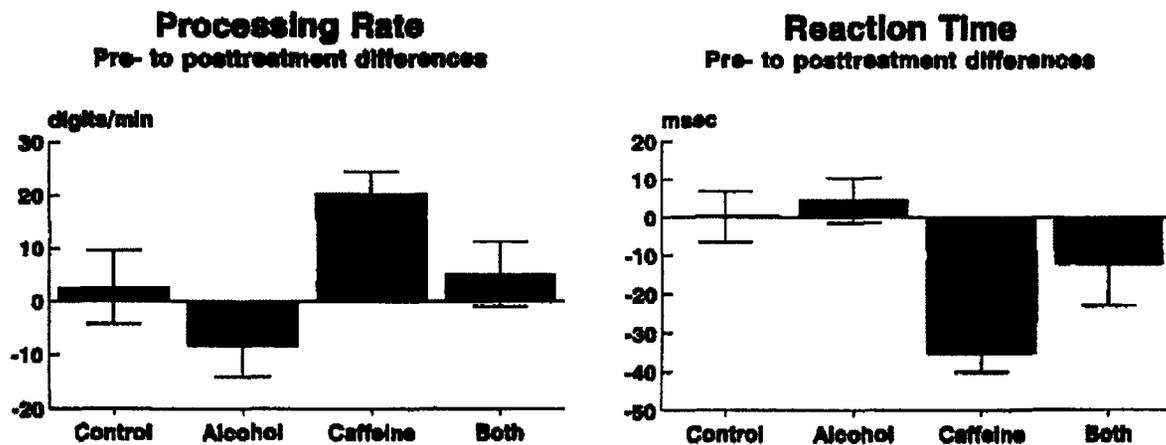


Figure 5. Mean pre- to post-treatment differences for processing rate and reaction time of the RIP task (Hasenfratz *et al.*, 1993).

Following on the concept of Kerr *et al* (1991), a paper published by Maczinski and Fillmore (2006) indicated that previous studies had tended to focus on a single task (such as the pursuit rotor task), which did not replicate a more complex and probably more naturalistic model of human information process, such as driving. Twelve adults (6 M and 6F) were used in a 2X3 factorial designed that crossed two doses of alcohol (0.0 and 650 mg/kg) with three doses of caffeine (0.0, 2.0 and 4.0 mg/kg); both alcohol and/or caffeine were administered simultaneously. The design was such that the investigators felt they could examine the extent to which the behavioral effects of alcohol could be counteracted by two active doses of caffeine. BAC was measured at 30, 45, 60 and 90 minutes post dose. The tests were a psychological refractory period task (a go/no-go visual stimulus visible for 2,000 milliseconds (ms) or upon occurrence of task) and an auditor discrimination task (tone presented for 500 ms) and 2,000 ms allowed for response. Measurements consisted of the following: (1) time to respond and how the

treatments would affect timing; and (2) accuracy of response. BAC levels were found to be 0.07 at 30 min, 0.084 at 45 min, 0.08 at 60 min (when testing ended) and continued to decline to 0.064 at 90 min. In summary, caffeine improved response time in the absence or presence of alcohol; caffeine did not improve accuracy of response. Tasks plotted against time showed that various cognitive processes differentially recover over the course of the descending limb of the BAC curve. These results generally compare to the findings of Liguori and Robinson (1997), who employed dynamic posturography, critical flicker fusion, choice reaction time, divided attention (Stroop test) and simulated driving. Both experiments suffered from the small number of subjects used (12 for Marczinski and Fillmore (2006) and 15 for Liguori and Robinson (1997)).

Kerr and Hindmarch (1998) reviewed the data on the effects of alcohol on human psychomotor performance and cognitive function, and found that the effects are very variable at low doses (under 1000 mg/kg bw), due to the different measures and methods employed in the various study designs, and the large interindividual and interoccasional differences in the effects of alcohol. Specifically, Kerr and Hindmarch (1998) stated that “alcohol affects different people in different ways and it affects the same person differently on separate occasions. Greater performance deficits are observed as the dose increases and as the tasks become more complex. Although results vary, both nicotine and caffeine appear to antagonize the detrimental effects of alcohol on performance.”

Caffeine and ethanol are known to affect behavior, but the interaction of caffeine and alcohol to modulate behavior in many different parameters, and, the overall effects of these interactions are equivocal, in large measure because behavior is exceedingly complex and cannot be reduced to a single, or even two or three, additional tasks simultaneously. It is plain there is no reversal of effect by caffeine on alcohol at levels of intoxication and the investigators cited above have shown there are at times, improvement on performance, but, this is dependent on the test performed. There is no true pharmacologic reversal of effect, simply because caffeine and alcohol act on different pathways within the central nervous system. Importantly, it is likely that personal expectations and/or a certain personality type, according to Thombs *et al* (2010), may play a large role in the behavioral effects of caffeine and alcohol.

## 6. EVALUATION

Caffeine (CAS No. 58-08-2) is a water soluble plant alkaloid that is consumed from many different foods, such as coffee, tea, cola and chocolate, as well as many over the counter analgesics, appetite suppressants and stimulants. A cup of coffee contains approximately 27 – 200 mg caffeine, and caffeine content in tea ranges from 40 – 120 mg. Analgesics contain approximately 60 – 130 mg caffeine, while stimulants contain up to 200 mg caffeine *per* serving. Caffeine is approved as a multipurpose GRAS food ingredient by the FDA, thereby indicating that the agency concluded that there is no evidence of human health hazard when caffeine is consumed in foods and cola beverages at current levels.

Caffeine is consumed worldwide by most life stages (*i.e.*, young, old, pregnant) of the population. Consumption of caffeine has been evaluated by several different investigators, with US consumption of caffeine in adult users at a mean of 193 mg/day, UK caffeine intake at 359 – 621 mg/day, and the highest consumption reported in the Netherlands at 414 mg/day. The highest caffeine consumers in the US are adult men aged 35 – 54 at 336 mg/day (approximately 5.6 mg/kg bw/day). Caffeine consumption at the 90<sup>th</sup> percentile has been reported as high as 382 mg/day (approximately 6.4 mg/kg for a 60 kg person), although some studies indicate that some individuals may consume up to 15 mg caffeine/kg bw (900 mg caffeine for a 60 kg person).

Caffeine is to be added to alcoholic beverages at up to 200 ppm (0.2%). Recent evaluation of caffeine consumption indicates mean caffeine daily intake in caffeine consumers at 193 mg/day, with estimated 90<sup>th</sup> percentile intake calculated at 382 mg/day. Addition of caffeine to alcoholic beverages indicated in this GRAS would provide a mean and 90<sup>th</sup> percentile caffeine intake at 156 and 360 mg/day, respectively. The estimated daily intake of caffeine from food and alcoholic beverages at the mean and 90<sup>th</sup> percentile levels would be 349 and 746 mg caffeine *per* day, respectively. This estimation is most probably a gross overestimation of the actual mean and 90<sup>th</sup> percentile consumption levels.

Caffeine is rapidly and completely absorbed in humans, with approximately 99% absorbed within 45 minutes of consumption. Plasma caffeine levels may be influenced by the diet or route of exposure, but peak plasma levels occur approximately 15 – 120 minutes after consumption. Caffeine is water soluble and is rapidly distributed throughout the body, detected

in all bodily fluids, including saliva, breast milk, urine and semen. Caffeine elimination follows first-order kinetics, with the plasma half-life of caffeine at approximately 3 – 6 hours in healthy adults and does not accumulate in body fat or other tissues. Caffeine is rapidly metabolized and excreted (1 – 3 mg/kg/minute) in the urine, and varies between species, with a slightly different metabolic route noted in rats. Caffeine does not affect ethanol absorption or excretion. Ethanol does decrease the clearance rate of caffeine, but the amount of caffeine consumed from caffeinated alcoholic beverages is not expected to result in unsafe caffeine blood levels.

The LD<sub>50</sub> of caffeine in rodents ranges from 200 – 355 mg/kg bw, depending on the species and strain, and the LD<sub>50</sub> in cats and dogs is 100 – 150 mg/kg bw. In humans, the fatal acute oral dose is estimated at 10 and 14 g (approximately 160 – 230 mg/kg for a 60 kg person). The serum caffeine concentration is the most reliable indicator of potential caffeine toxicity, with a serum caffeine concentration greater than 100 µg/ml considered lethal in humans. Human caffeine consumption at up to 10 g has caused convulsions and vomiting, with recovery in six hours. An acute dose of one gram caffeine can cause adverse effects, progressing from restlessness, nervousness and irritability to delirium, emesis, neuromuscular tremors and convulsions. However, consumption of caffeine throughout the day at up to 900 mg has been reported without adverse effects.

Consumption of high levels of caffeine in chronic studies in rodents led to decreased weight gain, but in most studies caffeine administration in the diet or in the drinking water did not affect mortality rates of mice and rats, with the exception of two studies that reported a significant reduction in the average life span of rats consuming caffeine. In chronic toxicity and carcinogenicity studies, administration of caffeine was non-carcinogenic. The potential for caffeine to induce genotoxicity has been evaluated in both *in vitro* and *in vivo* studies, with *in vitro* assays claiming both genotoxic and nongenotoxic results, while overall, *in vivo* studies indicate that caffeine is not genotoxic.

Caffeine is a stimulant, and has been studied for its physiological and behavioral effects. Caffeine increases heart rate and blood pressure, increases diuresis, increases locomotion and alertness, and decreases sleepiness. Clinical studies indicate that doses less than 450 mg do not increase the risk or severity of cardiac arrhythmia, while acute doses of 150 mg caffeine may

decrease heart rate. Studies evaluating effects of caffeine on cardiovascular health or serum cholesterol levels have not provided a consistent adverse effect with caffeine consumption.

Caffeine consumption does not affect fertility or fecundity, although caffeine is known to cross the placenta and enter the fetus. Moderate consumption of coffee by humans does not appear to influence birth weight, gestational period, time to delivery or overall pregnancy. Caffeine has been evaluated for its potential to produce teratogenic or developmental effects, with no indication of teratogenic effects at serum caffeine levels that could occur in humans consuming caffeinated beverages. Studies that reported positive teratogenic effects were administering large doses of caffeine equivalent to 15 – 23 cups of coffee *per* day in humans, and were providing the caffeine *via* gavage, which results in much higher peak serum caffeine levels and therefore overstates toxicity potential. In addition, epidemiological studies in humans have not indicated a positive association with high caffeine consumers and increases in teratogenic effects.

Based on animal and clinical studies, and the long history of caffeine use, moderate daily consumption of caffeine does not cause an irreversible adverse effect on human health. Caffeine is a stimulant with varied effects throughout the population, but this effect is readily noticeable and consumption adjusted. Caffeine is continuously being evaluated for its various health effects, and recent reviews on the overall health of caffeine consumers have indicated that for the average adult, moderate daily caffeine intake is not associated with any adverse effects including general toxicity, bone or cardiovascular effects, behavioral changes or increased incidence of cancer or reduced incidence of fertility or developmental or teratogenic toxicity.

In summary, on the basis of scientific procedures, and history of exposure and use, the consumption of caffeine, manufactured according to current Good Manufacturing Practices (cGMP) as an added food ingredient into alcoholic beverages at up to 200 ppm is considered safe when consumed as intended.

## 7. CERTIFICATION

The undersigned authors of this document—a dossier in support of GRAS status determination for use of caffeine added to alcoholic beverages—hereby certify that, to the best of their knowledge and belief, this document is a complete and balanced representation of all available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

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## 8. CONCLUSION

After critically evaluating the information available, the Expert Panel has determined that, based on common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food, there is reasonable certainty that caffeine, produced in accordance with current Good Manufacturing Practice (cGMP), is safe under the intended conditions of use, and is therefore Generally Recognized As Safe (GRAS), by scientific procedures, when used as an ingredient when added to alcoholic beverages, such that total daily consumption of caffeine from all sources is calculated at the 90<sup>th</sup> percentile consumption to be not greater than 746 mg/day. In particular, the Expert Panel has evaluated the proposed use of caffeine in alcoholic beverages at up to 200 ppm (0.02% caffeine), and has concluded that such use is Generally Recognized As Safe (GRAS).

## 9. SIGNATURES

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## 11. APPENDIX I

**Table 12. Alcoholic beverages for the potential addition of caffeine as caffeinated alcoholic beverages**

| Alcoholic beverage                      | Intended use level<br>(ppm) |
|---|-----------------------------|
| Beer                                    | 200                         |
| Beer, lite                              | 200                         |
| Cordial or liqueur                      | 200                         |
| Cocktail, NFS                           | 200                         |
| Alexander                               | 200                         |
| Bacardi cocktail                        | 200                         |
| Bloody Mary                             | 200                         |
| Canadian Club and soda                  | 200                         |
| Cape Cod                                | 200                         |
| Daiquiri                                | 200                         |
| Gimlet                                  | 200                         |
| Gin and Tonic                           | 200                         |
| Grasshopper                             | 200                         |
| High ball                               | 200                         |
| Kamikaze                                | 200                         |
| Manhattan                               | 200                         |
| Margarita                               | 200                         |
| Martini                                 | 200                         |
| Mint julep                              | 200                         |
| Old fashioned                           | 200                         |
| Rob Roy                                 | 200                         |
| Rusty Nail                              | 200                         |
| Salty Dog                               | 200                         |
| Screwdriver                             | 200                         |
| Seabreeze                               | 200                         |
| Seven and Seven                         | 200                         |
| Tom Collins                             | 200                         |
| Whiskey sour                            | 200                         |
| Bourbon and soda                        | 200                         |
| Mixed Drinks (for recipe modifications) | 200                         |
| Rum and cola                            | 200                         |
| Pina Colada                             | 200                         |
| Coquito, Puerto Rican (coconut, rum)    | 200                         |
| Sloe gin fizz                           | 200                         |
| Black Russian                           | 200                         |
| White Russian                           | 200                         |
| Fruit punch, alcoholic                  | 200                         |
| Singapore Sling                         | 200                         |
| Stinger                                 | 200                         |
| Gibson                                  | 200                         |
| Mai Tai                                 | 200                         |
| Tequila Sunrise                         | 200                         |
| Gin Rickey                              | 200                         |
| Golden Cadillac                         | 200                         |
| Long Island iced tea                    | 200                         |
| Fuzzy Navel                             | 200                         |

| Alcoholic beverage            | Intended use level<br>(ppm) |
|-------------------------------|-----------------------------|
| Irish Coffee                  | 200                         |
| Liqueur with cream            | 200                         |
| Frozen daiquiri               | 200                         |
| Frozen margarita              | 200                         |
| Eggnog, alcoholic             | 200                         |
| Gin fizz                      | 200                         |
| Rum, hot buttered             | 200                         |
| Zombie                        | 200                         |
| Wine, table, red              | 200                         |
| Wine, table, white            | 200                         |
| Wine, rice                    | 200                         |
| Wine, cooking (assume cooked) | 200                         |
| Wine, dessert, sweet          | 200                         |
| Wine, light                   | 200                         |
| Wine cooler                   | 200                         |
| Sangria                       | 200                         |
| Sangria, Puerto Rican style   | 200                         |
| Wine spritzer                 | 200                         |
| Glug                          | 200                         |
| Brandy                        | 200                         |
| Whiskey                       | 200                         |
| Gin                           | 200                         |
| Rum                           | 200                         |
| Rum cooler                    | 200                         |
| Vodka                         | 200                         |

The beverages were taken from the 2005-2006 WWEIA USDA Continuing Survey of Food Intakes by Individuals; ppm=parts *per* million

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