

## TOXICOLOGY NEWSLETTERS

*“The powers of a man’s mind are directly proportional to the quantity of coffee he drank.”*

- Sir James Mackintosh

August 2019

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### Toxicity of Caffeine

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*In the past, my newsletters covered various aspects of basic toxicity, organ toxicity, risk assessment, etc. I do not know the information need in the toxicology community on the toxicity aspects of various types of chemicals. Just as an example and a prototype, I ventured this time to cover the most widely used “chemical” caffeine through various drinks and foods, which may be of interest to our readers. Please write to me if such need exists, which will guide me to consider writing in future on selected such widely used chemicals, e.g. alcohol, etc. There is no way anyone can cover hundreds and thousands of industrial chemicals, drugs, pesticides, nutraceuticals, etc. Moreover, I do not see a need for such comprehensive monograph coverage in these informational and educational newsletters. Also, as always, I encourage our readers to take*

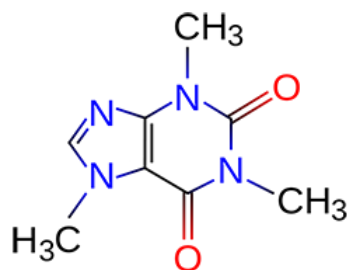
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*initiative in writing yourself a solo newsletter to share it with fellow toxicologists. There is no hierarchy in ToxGurukul, it is a voluntary group helping each other for community growth.*

#### A. Preamble

Caffeine (1,3,7-trimethylxanthine; see the image below) is the most widely consumed stimulant drug in the world.



Caffeine has been utilized globally for centuries secondary to its ability to improve mental alertness. As many as 80% or more of world population consume caffeine daily with the average cup of coffee containing anywhere from 40 to 150 mg of caffeine. Caffeine can be found in many over-the-counter preparations (energy drinks, appetite suppressants, stimulants, exercise supplements, decongestants, bronchodilators, and mental stimulants), increasing the risk of toxicity with inadvertent overuse or severe toxicity with an intentional overdose. *Lethal doses of caffeine have been reported at blood concentrations of 80 to 100 micrograms/ml which can be reached with ingestion of approximately 10 grams (equivalent to 65 cups of coffee) or greater.*

#### B. History Use of Caffeine

Caffeine (1,3,7-trimethylxanthine) is a psychostimulant purine-like alkaloid, which is found naturally in coffee, tea, cacao beans, and in more than 60 plant species. It has been consumed for thousands of years by humans. Recent evidence demonstrates tea consumption in China as far back as 2,100 years ago during the Western Han Dynasty which ruled from 207 BCE to 9 BCE. The first confirmed historical consumption of tea was 750 CE. Consequently, the shepherd began consuming them and experienced what is now in modern times recognized as the central nervous system (CNS) stimulation from the caffeine in the berries. Caffeine was isolated as the active constituent of coffee's stimulating effects in 1819 followed by its first total synthesis in 1895. By the 14th century, the roasting of coffee beans had been discovered and by the 15th and 16th centuries, knowledge of its stimulating effect had apparently led to widespread consumption and commercialization in coffee houses in Arabia and Constantinople. In the 17th century, once shipped from overseas the consumption of coffee in Europe became more common and consequently spread to the colonies in North America. Tea and coffee have since served as the major beverage sources of caffeine but in the late 1800s, caffeinated soda entered the marketplace in the branded products, Dr. Pepper, Coca-Cola, and

Pepsi-Cola becoming extremely popular during the second half of the 20th century. Since then, the latest iteration of caffeinated beverages that have become popular are the so-called “energy drinks”, which entered the market in the late 20th century and have since grown in popularity.

Nowadays, caffeine is the most widely consumed psychostimulant in the world. It is estimated, that caffeine is being consumed by more than 80% of the world’s population. The average daily consumption of caffeine varies depending upon the survey, years conducted, and sources considered but has most recently (i.e., 2011–2012) been reported as 142 mg per day for adults and children in the United States. Coffee purchased from the grocery store and tea remain the largest contributors to caffeine intake.

While caffeine is generally thought to be safe in moderate amounts (i.e.,  $\leq$  400 mg per day) in healthy adults, it is clearly not an innocuous compound and can cause significant toxicity and even lethality (i.e., most commonly via myocardial infarction or arrhythmia) if sufficient quantities are consumed. Some sensitive individuals may also experience toxicity and lethality at doses not normally associated with such outcomes.

### C. Physical and Chemical Properties

Caffeine is soluble in water with 20 g/l at 20°C (BASF AG, 2001a) and has a calculated vapor pressure of 0.0000047 Pa at 25°C (BASF AG, 2000a). Henry’s law constant has been estimated to  $1.9 \times 10^{-19}$  atm\*m<sup>3</sup>/mole (Swann et al., 1983). The partition coefficient log Pow is measured to  $-0.091$  at 23°C. The density of caffeine is higher than that of water (1.23 g/cm<sup>3</sup> at 18°C). The melting point is 235 – 239 °C, Caffeine sublimates at 178°C.

### D. Pharmacokinetics

#### 1. Absorption

*Caffeine has rapid and complete (i.e., 99%) absorption from the small intestine after oral administration due to its weakly basic nature and pKa of 14 at 25 °C, favoring an un-ionized/lipophilic state in the more basic environment of the small intestine where it may more easily partition into the lipid bilayer of cells, as compared to the acidic environment of the stomach where it is more ionized and less lipophilic.*

*Caffeine is not known to undergo significant first-pass metabolism and generally reaches peak plasma concentrations within 30–120 min after administration.*

#### 2. Distribution

Caffeine is distributed throughout the body after being absorbed from the gastrointestinal tract (the small intestine in particular), entering all tissues via cell membranes (i.e., due to its lipophilic moiety or moieties and limited plasma protein binding) and entering intracellular tissue water. It readily penetrates the blood-brain barrier as well. The average volume of distribution is about 0.7 L/kg. It has rather low protein binding with around 10–35% reported. Caffeine is also not known to accumulate in tissues. *Caffeine is often referred to as being lipophilic, but it is more accurately characterized as an amphiphilic molecule*

(i.e.,  $\log P = -0.07$ ), which due to certain lipophilic moieties is able to partition into the lipid bilayer and diffuse across into the cell.

### 3. Metabolism

Caffeine is described by a single-compartment model where it follows first-order, linear kinetics. *Caffeine is primarily metabolized to 1,7-dimethylxanthine (paraxanthine) in the liver via the CYP isozyme CYP1A2, which causes 3-demethylation of caffeine. Paraxanthine is the major metabolite (approximately 80%) of caffeine biotransformation.* Interestingly, paraxanthine itself is also pharmacologically active albeit with potentially lower toxicity than caffeine. CYP1A2 is also responsible for, along with to some extent CYP2E1, the 1 and 7-demethylation of caffeine to 3,7-dimethylxanthine (theobromine) and 1,3-dimethylxanthine (theophylline), respectively, which are also pharmacologically active. Theobromine accounts for approximately 11%, while theophylline is around 5% of caffeine metabolites. These metabolites may then be further demethylated via CYP1A2 primarily, acetylated via N-acetyltransferase 2, and oxidized via xanthine oxidase or CYP3A4 to yield the major metabolites which are excreted primarily in the urine including 1-methyluric acid, 5-acetylamino-6-formylamino-3-methyluracil, 1-methylxanthine. Overall, more than 25 metabolites have been identified in humans after caffeine administration, demonstrating a rather complex metabolism. It is important to note that the involvement of other CYP isozymes (e.g., CYP3A4/3A5 and CYP2D6) is only important at rather high (i.e., millimolar) concentrations rather than those normally encountered after typical caffeine ingestion. Less than 5% of ingested caffeine is excreted unchanged.

### 4. Elimination

*Caffeine is eliminated from plasma via CYP1A2-mediated clearance in which paraxanthine is the main metabolite.* Elimination occurs mainly via renal excretion in urine (~85–88%), although fecal excretion also takes place to a limited extent (i.e., around 2–5%). The typical clearance value is between 1 to 3 mL/kg/min. The elimination half-life is approximately 3–6 hours in healthy humans.

### 5. Toxicokinetics

Toxicological symptoms begin above concentrations of 15 mg/L (i.e., generally more mild psychological side effects such as irritability and nervousness but also potentially palpitations, nausea, tremor, perspiration and paresthesia), *while a concentration of 50 mg/L is considered “toxic” and concentrations of 80 mg/L or greater are considered lethal.*

### E. Mechanism of Action

*Caffeine has been shown to be a non-selective adenosine receptor antagonist* with  $K_i$  values of 44 and 40  $\mu\text{mol}$  (around 8.5 and 7.8 mg/L) for the adenosine A1 and A2A receptor subtypes, respectively. However, the threshold for initial adenosine antagonism with caffeine is less than 10  $\mu\text{mol}$  (1.94 mg/L), and potentially as low as 2  $\mu\text{mol}$  (0.38 mg/L). The A1 subtype is mainly localized to the brain, spinal cord, eye, adrenal gland, heart and to a lesser extent, tissues

such as skeletal muscle and adipose. The A2A subtype is mainly localized to the spleen, thymus, striatopallidal GABAergic neurons and to a lesser extent the heart, lung, and blood vessels.

### **F. Preclinical Safety of Caffeine**

*In animal studies caffeine showed acute toxicity LD50 rat oral 200-400 mg/kg bw, LD50 mouse oral 185 mg/kg bw, LC50 rat inhalation ca. 4.94 mg/l/4h; LD50 rat dermal > 2000 mg/kg bw).* The undiluted substance was not irritating to the eyes of rabbits, the substance in a 50% aqueous dilution was not irritating to the skin of rabbits. In a 90-day-drinking water study in rats and mice, a slight decrease in body weight gain was observed. No clinical signs of toxicity and significant gross lesion or microscopic findings were seen in either rats or mice. The NOAEL for rats was 1500 ppm (ca. 151-174 mg/kg bw/day) and for mice 1500 ppm (ca. 167-179 mg/kg bw/day). *In all dose groups, effects on salivary glands were observed, which were regarded as an adaptive and reversible response to the sympathomimetic effect of caffeine.* There are numerous studies available concerning genetic toxicity *in vitro* and *in vivo*. In the majority of the studies, caffeine produced negative results. Several positive responses were obtained only in studies which used extreme culture conditions, lethal doses or non-validated methods. There was no statistically significant increase in the tumor incidence in treated animals as compared to controls even at doses exceeding the maximum tolerated dose and given to rats over a major portion of their lifespan.

Caffeine resulted in reproductive effects occurring in the presence of general toxicity in parental rats and mice. A NOAEL in rats was not established. NOAEL: mouse 22 mg/kg bw/d (F0 parental, F1 offspring), 88 mg/kg bw/d (F1 parental, F2offspring). *Gross malformations were observed in rats and mice only after bolus administration (i.p. or gavage) of very high maternally toxic doses. Fetotoxicity without maternal toxicity was observed in one drinking water study.* NOAEL: 360 ppm (51 mg/kg bw/d)(maternal), 70 ppm (10 mg/kg bw/d) (fetotoxicity), 2000 ppm (205 mg/kg bw/d) (teratogenicity). However, in two other gavage studies with lower doses, this finding was not confirmed. No NOAEL for maternal toxicity could be established; the NOAEL for developmental toxicity was 40 mg/kg bw/d; no teratogenic effects were observed.

### **G. Human Toxicity**

#### **1. Toxic Doses of Caffeine**

*Low doses (up to 2 µg/ml in the blood) stimulate the central nervous system, while high blood concentrations (10-30 µg/ml) produce restlessness, excitement, tremor, tinnitus, headache, and insomnia.* Caffeine can induce alterations in mood and sleep patterns, increase diuresis and gastric secretions. Acute toxicity is rare and is the result of an overdose. *The lethal dose is estimated to be 5 g.* Caffeine and coffee consumption are highly correlated in most populations studied. No association between moderate consumption of coffee/caffeine and cardiovascular diseases was demonstrated. In short-term clinical trials, an increase in blood pressure was seen, whereas in other surveys no relationship

between caffeine consumption and elevation of blood pressure was observed. Caffeine consumed in moderate amounts did not cause a persistent increase in blood pressure in normotensive subjects. Withdrawal symptoms, although relatively limited with respect to severity, do occur, and may contribute to the maintenance of caffeine consumption. There is little evidence for an association of caffeine intake and benign breast disease. A cohort study with a short follow-up period showed no association between caffeine consumption and mortality from cancers at all sites. Case-control studies of breast cancer showed no association with caffeine intake. Weak positive associations between caffeine intake and lung, bladder or pancreas cancer as well as a weak inverse association between caffeine intake and colon cancer may be due to bias or confounding. IARC evaluated that there is inadequate evidence of carcinogenicity in humans. No clear dose-related reproductive effects in humans have been documented. A teratogenic effect has not been proven. While caffeine intake up to 3-4 cups/day or 300 mg caffeine/day is unlikely to be causally related to spontaneous abortions or relevant reduction of birth weight, an association between higher daily caffeine intake and these endpoints cannot be excluded.

*Conflicting results exist regarding a potential relationship between caffeine/coffee consumption and delayed conception or infertility.*

*Caffeine is technically a drug. The recommended amount of caffeine is up to 400 milligrams per day for healthy adults.* Caffeine overdose may occur if you ingest more than this amount. Adolescents should limit themselves to no more than 100 mg of caffeine per day. Pregnant women should limit their daily intake to less than 200 mg of caffeine per day since the effects of caffeine on the baby are not fully known. However, what constitutes a safe amount of caffeine differs for everyone based on age, weight, and overall health.

Lethal overdoses of caffeine in adults are rare but when encountered are commonly caused by an intentional overdose of medications. Conversely, caffeine toxicity in children is typically caused by accidental ingestion. Toxic ingestions can be seen after ingestion of energy drinks but are rarely seen from ingestion of coffee or tea secondary to the excessive amount of fluid that would have to be ingested to reach toxic levels. Over-the-counter drugs containing large doses of caffeine purchased for their stimulant properties can also complicate caffeine exposure. Some examples include health food products, diet aids decongestants, bronchodilators, or stay-awake pills.

## **2. What are the symptoms of caffeine overdose?**

Several types of symptoms occur with this condition. Some symptoms may not immediately alert you that you've had too much caffeine because they may not seem serious. *For example, you may experience dizziness, diarrhea, increased thirst, insomnia, headache, fever, irritability*

Babies can also suffer from caffeine overdose. This can happen when breast milk contains excessive amounts of caffeine. Some mild symptoms include nausea and muscles that continually tense and then relax.

More serious signs of caffeine overdose can accompany these symptoms, including vomiting, rapid breathing, and shock.

### 3. Diagnosing caffeine overdose

If you suspect a caffeine overdose, let your doctor know of any caffeinated items you consumed prior to having symptoms. Your breathing rate, heartbeat, and blood pressure will also likely be monitored. Your temperature may be taken, and you may be given a urine or blood test to identify the drugs in your system.

### 4. Treatment for caffeine overdose

Treatment is meant to get the caffeine out of your body while managing the symptoms. You may be given activated charcoal, a common remedy for a drug overdose, which often prevents the caffeine from going into the gastrointestinal tract.

If the caffeine has already entered your gastrointestinal tract, you may be offered a laxative or even a gastric lavage. Gastric lavage involves using a tube to wash the contents out of your stomach. Your doctor will likely choose the method that works fastest to get the caffeine out of your body. During this time, monitored of EKG (electrocardiogram) is recommended. If needed, breathing support may be offered.

## H. Risk Characterization

*Caffeine is listed in GRAS Food Substances.* The tolerance in foods is 0.02%. “The substance is generally-recognized as safe when used in cola-type beverages in accordance with good manufacturing practice” (Code of Federal Regulations Title 21-Food and drugs revised as of April 1, 2001). Caffeine in pharmaceutical use is described in a specific regulation.

### **Workplace:**

Taking worst-case assumptions, the maximum exposure of workers during the filter changes is 1.2mg/m<sup>3</sup>. Considering this high exposure, which is unrealistic for 8 hours, the maximum uptake (absorption: 100 %; ventilation rate: 1.8 m<sup>3</sup>/h) is 17.28 mg/person (ca. 0.3 mg/kg bw/day).

With respect to the NOAEL in subchronic studies - NOAEL for rats was 1500 ppm (ca. 150 – 174mg/kg bw/day) and for mice 1500 ppm (ca. 167-179 mg/kg bw/day) - there are safety factors of 500 and higher.

NOAELs for developmental toxicity, the most sensitive endpoint are: NOAEL: 360 ppm (51 mg/kg bw/d)(maternal toxicity), 70 ppm (10 mg/kg bw/d) (fetotoxicity), 2000 ppm (205 mg/kg bw/d) (teratogenicity).

Even under this worst-case assumption, there is still a safety factor of 33 for fetotoxicity. This safety factor is substantiated by a new publication of Christian and Brent, 2001 in which up to 5 –6 mg/kg caffeine uptake per day is regarded to have no reproductive risk for humans.

## I. Conclusion

Caffeine is an interesting molecule with diverse physiological effects in humans. While it has a long history of consumption around the world and continues to be consumed in significant quantities. *The main molecular target considered is the adenosine receptor.* Despite having a general understanding of the toxic and lethal doses of caffeine there is clearly a need for more data, especially with

respect to determining safe doses in sensitive populations.

Caffeine is a widely recognized psychostimulant compound. While it has received a significant amount of attention there is still much to be learned with respect to its toxicology in humans, especially in cases of overdose.

The diagnosis of caffeine toxicity is based largely upon reported ingestion and symptoms, although serum caffeine concentrations can be obtained through quantitative chemical analysis. Caffeine's toxicological symptoms vary according to the dose and individual, with psychological side effects generally manifesting at lower dosed-intoxications and more serious side effects occurring in the cardiovascular and muscular tissues with higher doses. Treatment generally involves supportive therapy along with decontamination and increased elimination techniques, although there is no standard treatment regimen.

*In summary, there is absolutely no issue encountered with normal consumption of 2 to 4 cups of coffee every day.* However, most of you know Dose Makes the Poison, any chemical if abused, including caffeine can always harm in any organism.

### Questions

1. Lethal doses of caffeine have been reported at blood concentrations of \_\_\_\_\_ to \_\_\_\_\_ micrograms/ml which can be reached with ingestion of approximately 10 grams (equivalent to \_\_\_\_\_ cups of coffee) or greater
2. Caffeine has rapid and complete (i.e., 99%) absorption from the \_\_\_\_\_ after oral administration due to its weakly \_\_\_\_\_ nature and pKa of 14 at 25 °C
3. Caffeine is not known to undergo significant first-pass metabolism and generally reaches peak plasma concentrations within \_\_\_\_\_ to \_\_\_\_\_ min after administration
4. Caffeine is often referred to as being \_\_\_\_\_, but it is more accurately characterized as an \_\_\_\_\_ molecule
5. Caffeine is primarily metabolized to \_\_\_\_\_ in the liver via the CYP isozyme \_\_\_\_\_, which causes 3-demethylation of caffeine.
6. While a concentration of \_\_\_\_\_ is considered "toxic" and concentrations of \_\_\_\_\_ mg/L or greater are considered lethal.
7. Caffeine has been shown to be a non-selective \_\_\_\_\_ receptor antagonist
8. Low doses (up to \_\_\_\_\_ µg/ml in the blood) stimulate the central nervous system, while high blood concentrations (\_\_\_\_\_ to \_\_\_\_\_ µg/ml) produce restlessness, excitement, tremor, tinnitus, headache, and insomnia.
9. What are the symptoms of caffeine overdose?
10. Caffeine is listed in GRAS Food Substances - true or false

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