

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-793

PHARMACOLOGY REVIEW(S)

**DIVISION OF PULMONARY DRUG PRODUCTS  
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA  
Amendment 28.01, Review No. 2**

NDA 20-793

Reviewer: Misoon Y. Chun, Pharm.D., D.A.B.T.

Date of Submissions: 3/22/99

Date of Review: 8/27/99

Information to conveyed to Sponsor: Yes ( ), No ( x )

Sponsor: O.P.R. Development, L.P.  
425 Volker Blvd.  
Kansas City, Missouri 64110

Drug Name: Caffeine Citrate Injection (CAFCIT™)

**Background:**

NDA 20-793 (CAFCIT) was submitted on August 25, 1997, and the review for non-clinical portion was finalized on December 18, 1997. An approvable letter was sent on February 23, 1998 with a list of request for sponsor to respond. Subsequently, Amendment No.24.01 (June 30, 1998) in response to Clinical/Labeling issues, Amendment 27.01 (December 10, 1998) in response to CMC and Amendment 28.01 (March 22, 1999) were submitted in response to a teleconference with the Division on February 8, 1999. At the teleconference, Dr. Anne Trontell requested the sponsor to address four principal queries. To address the third query specifically asking to provide any new safety updates, all additional studies that have been done with caffeine either by itself or published in the literature, sponsor submitted the previous safety literature update submitted to FDA in Amendment No. 24.01 and a current safety update.

**Review:**

Among the list of literature citation which is mostly for clinical, there are only three articles (abstracts only) from animal studies worth noting. A brief summary from the three articles is provided below.

1. *Permeability of the developing and mature blood-brain barriers to theophylline in rats. (Habgood MD. et al, May 1998)*

The uptake of theophylline and L-glucose into the adult and neonatal rat brain has been investigated in this study. 1) Steady state cerebrospinal fluid (CSF) and brain concentrations of theophylline were reached within 1 h following a single intraperitoneal injection, whereas steady state CSF and brain concentrations of L-glucose were not approached until after 5 h. 2) Steady state brain:plasma and CSF:plasma concentration ratios for theophylline and of L-glucose in

neonatal rats were significantly higher than ratios in adult rats. The article explains that the lower steady state ratios for theophylline in adult rats are likely to be due to a higher concentration of plasma proteins in adult blood compared with neonates, with a greater retention of protein-bound theophylline in adult blood, and are unlikely to be due to p-glycoprotein-mediated efflux of theophylline at the adult blood-brain barrier.

2 *Response to nimodipine in caffeine-induced neurotoxicity in cerebellar granular cell culture of rat pups. (Gepdiremen A. et al, Oct.1998)*

Methylxanthines are widely used as central nervous system stimulants, and caffeine is used in the treatment of apnea in newborns. Plasma therapeutic concentration of caffeine is around 110  $\mu$ M. Caffeine diffuses the blood brain barrier easily, increasing oxygen consumption in neurons and leading to cell death. In this study, cerebellar granular cell cultures were obtained from 4-7 day old rats and the voltage-dependent calcium channels in caffeine-induced neurotoxicity was tested with the doses of 100 and 200  $\mu$ M nimodipine 45 min before or after the 350  $\mu$ M caffeine. It was found that doses administered 45 min prior to caffeine reduced death cell score significantly. The article suggests that nimodipine may be used in the treatment of newborn apneas together with caffeine to prevent neurotoxic side effects of high or repeated doses of caffeine.

3 *Organ blood flow redistribution in response to hypoxemia in neonatal piglets. (Dyess DL et al, Nov 1998)*

This study was designed to determine the effects of severe hypoxemia on newborn piglet visceral blood flow. Cannulas were placed in the femoral vessels and left atrium of term (1-14 days old) and prematurely delivered (90% of term gestation) piglets. Animals were subjected to 1 h of ventilator-controlled hypoxia followed by 30 min of reoxygenations. Radiolabeled microspheres were injected in all animals at times 0 min (baseline), 5 and 60 min (hypoxia), and 90 min (reoxygenation), and blood flows to organs were determined. Throughout the experimental period, organ blood flows were almost uniformly lower in premature versus term animals. In term piglets, hypoxia produced an immediate and significant decline in small-intestinal blood flow followed by autoregulatory escape, an effect not readily observed in the premature piglets. However, mucosal blood flows measured in these younger animals declined throughout the experimental period to almost 50% of baseline, compared to complete restoration to baseline blood flow observed following reoxygenation of term piglets. Intestinal blood flow in premature infants is small when compared to term animals, and alterations in small intestinal blood mucosal flow induced by hypoxia appear less well tolerated by the premature animals. This may in part account for the increased risk of developing intestinal ischemic diseases in premature infants who are even temporarily exposed to a severe hypoxic challenge.

cc: NDA 20-793  
HFD-570/Div File  
HFD-570/ATrontell  
HFD-570/MChun

/S/

/S/

DEC 18 1997

C-2175

**DIVISION OF PULMONARY DRUG PRODUCTS**  
**REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA**  
**Original Submission, Review No. 1**

**NDA 20-793**

Reviewer: Misoon Y. Chun, Pharm.D., D.A.B.T.  
Date of Submissions: 8/25/97  
Date assigned: 9/1/97  
Date of Review: 11/5/97

**Information to be conveyed to Sponsor:** Yes ( x ), No ( )

**Sponsor:** O.P.R. Development, L.P.  
425 Volker Blvd.  
Kansas City, Missouri 64110

**Drug Name:** Caffeine Citrate Injection (CAFCIT™)

Established Name  
Caffeine, USP (USAN)

Chemical Name(s)  
3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione (Chemical Abstracts) 1,3,7-trimethylxanthine or 1,3,7-trimethyl-2,6-dioxopurine

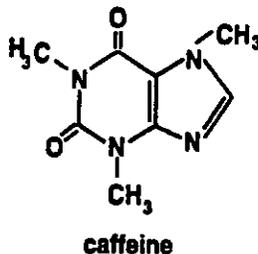
Other Name(s)  
caffeine (INN, BAN, JAN)  
coffeine  
thein  
guaranine  
methyltheobromine

CAS Registry Number  
58-08-2 (caffeine, anhydrous)

Molecular Weight  
194.19

Molecular Formula  
 $C_8H_{10}N_4O_2$

**Chemical Structure:**



**Related INDs/NDAs/DMFs:**

IND  DMF Nos  and

**Pharmacologic Class:** Central Nervous System Stimulant

**Indication:** Apnea of Prematurity

**Route of Administration:** Parenteral

**Dosage Form:** Sterile Aqueous Solution, 10 mg/mL

**Proposed Dose:** 10 mg/kg loading dose by I.V. followed by 2.5 mg/kg daily maintenance dose administered orally or intravenously

**Clinical Formulation:**

The quantitative composition of the dosage form is as follows:

	per mL	per vial*
Caffeine, USP, anhydrous	10 mg	
Citric acid, USP, monohydrate		
Sodium citrate, USP, dihydrate		
Water For Injection, USP	q.s.	q.s.

\* Based on a target fill volume of [ ] mL ([ ] g/vial)

**Background:**

Apnea of prematurity is defined as cessation of breathing for periods from 10 to 30 seconds, with or without bradycardia or cyanosis. The primary pharmacologic agents which have been used to treat apnea of prematurity since the early 1970s are caffeine and theophylline. Sterile sodium caffeine benzoate has been commercially available, but it is not indicated for the treatment of apnea of prematurity. The concentration and inert ingredients (benzyl alcohol) make this preparation unsuitable for use in infants.

In 1985, the FDA contracted with the [ ] to perform a literature search and to review and provide a summary of published data concerning the use of selected marketed drugs in newborn infants. The FDA Contract Report concluded that it would be in the interest of public health to encourage a NDA for caffeine for the indication of apnea of prematurity. Caffeine was designated as an Orphan Drug on September 20, 1988, and the development of Caffeine Citrate Injections was initiated under IND [ ] in September 1989.

Since there is extensive information on caffeine available in the published literature, only one double-blind, placebo-controlled study was conducted to support the safety and efficacy of caffeine citrate in the treatment of apnea of prematurity along with additional information obtained from review of the published literature. For the same reason, no animal studies were conducted for the development, and the data presented in the nonclinical section of the NDA were obtained from the published literature.

## Table of Contents

*Note: The following information was extracted and evaluated from the literature. The lists of references are provided under each section.*

	Page
<b>INTEGRATED NONCLINICAL DATA</b> .....	
<b>1. Pharmacology</b> .....	4
1.1. Sites of Action .....	
1.2. Spectrum of Pharmacological Activity: Caffeine vs. Theophylline .....	
<b>2. Absorption, Distribution, Metabolism, Elimination</b> .....	11
<b>3. General Toxicity</b> .....	20
3.1. Acute Toxicity .....	
3.2. Repeat-Dose Toxicity .....	
<b>4. Special Toxicity</b> .....	31
4.1. Central Nervous System .....	
4.2. Cardiovascular System .....	
4.3. Gastrointestinal System (NEC) .....	
4.4. Other Organ Systems .....	
<b>5. Carcinogenicity</b> .....	46
5.1. Mouse .....	
5.2. Rat .....	
<b>6. Reproduction Studies</b> .....	58
6.1. Fertility .....	
6.2. Developmental .....	
6.3. Pre- and Post-natal .....	
<b>7. Mutagenicity</b> .....	75
7.1. In Vitro .....	
7.2. In Vivo .....	
<b>8. Overall Summary and Evaluation</b> .....	80
<b>9. Conclusion and Recommendations</b> .....	88
<b>10. Labeling</b> .....	89

## REVIEW

*Note: Portions of this review were excerpted directly from the sponsor's submission.*

### 1. Pharmacology

#### 1.1. Sites of Action

Caffeine is one of the three commonly occurring methylxanthines; the other two are theophylline and theobromine. Methylxanthines act at multiple sites and stimulate all levels of the central nervous system (CNS) (Serafin, 1995); the cortex is most sensitive, and the spinal cord least sensitive (Arnaud, 1987). The medulla, which is the primary site of respiratory stimulation, exhibits intermediate sensitivity. In addition to respiratory stimulation, CNS-mediated effects include motor stimulation and behavioral stimulation.

Cardiovascular activity can be modified by the methylxanthines. Because cardiovascular homeostasis is maintained by a complex array of neural reflexes and hormonal balances, cardiovascular activity of the methylxanthines is influenced by the physiological state at the time of drug administration. Responses most frequently detected are alterations in cardiac rhythm (increases in heart rate) and relaxation of blood vessels in some vascular beds (Serafin, 1995). In addition, caffeine and congeners possess diuretic activity, relax bronchial smooth muscle, and improve skeletal muscle contractility (Tarka, 1982).

Mechanism of Action. Methylxanthines have been found to modify several cellular activities: phosphodiesterase inhibition at 0.5-1.0 mM concentration, interference with the uptake and storage of  $Ca^{++}$  by striated and cardiac muscle, and adenosine receptor antagonism at nM and  $\mu$ M range concentrations (Rall, 1990). Adenosine is naturally present in mammalian tissues, and is involved in many physiological processes, such as the respiratory, neural, and cardiovascular systems. Caffeine is equipotent for  $A_1$  and  $A_2$  receptors. The antagonism of endogenous adenosine may explain most, but not all responses to methylxanthines (Arnaud, 1987).

#### Sites Related to Proposed Therapeutic Use. Treatment of Apnea of Prematurity

As noted above, excitation of the medullary respiratory centers plays a primary role in the action of caffeine to stimulate respiration (Serafin, 1995). Experimentally, Trippenbach *et al.* (1980) showed that 10 mg caffeine/kg, intravenously, increased the frequency of breathing in 2-4 and 4-6 day old rabbits. The Herring-Breuer expiratory-promoting reflex also was increased in these animals. An increased frequency of breathing also occurred in awake adult rhesus monkeys following 3-30 mg caffeine/kg intravenously (Howell and Landrum, 1994). Other studies, using awake 10-12 week old lambs, showed that respiratory stimulation by caffeine may be mediated, in part, by a peripheral action of the drug, following a 3-5 minute infusion of 10 mg/kg (Blanchard *et al.*, 1986).

These studies provide experimental evidence to support the proposed loading dose of 10 mg caffeine/kg, administered intravenously, for the treatment of apnea of prematurity. Findings suggest that medullary centers are more sensitive to the stimulatory actions of caffeine under pathophysiologic conditions (Lopes *et al.*, 1994; Serafin, 1995), which is the basis for the proposed therapeutic use of caffeine citrate.

### 1.2. Spectrum of Pharmacological Activity: Caffeine vs. Theophylline

Although the methylxanthines (caffeine, theophylline and theobromine) have virtually identical sites of action, they are not equally potent at each site. The theobromine possesses modest activity at all sites, and its CNS activity is too weak for it to be considered a candidate for treating apnea of prematurity (Serafin, 1995). However, caffeine and theophylline both have been used to treat apnea of prematurity. When one compares the spectra of pharmacological activity for the two agents, it is clear that caffeine potentially has a greater margin of safety for treating apnea of prematurity than theophylline.

Table II-A-1. Sites of pharmacological activity for methylxanthines<sup>1</sup>

Site of Action	Caffeine	Theophylline	Theobromine
Brain	1 <sup>2</sup>	2	3
Skeletal muscles	1	2	3
Heart	3	1	2
Kidneys	3	1	2
Bronchioles	1	1	2

<sup>1</sup> Modified from Tarka, 1975.

<sup>2</sup> Potency based on a comparison of caffeine, theophylline, and theobromine: most potent = 1; least potent = 3.

The site of action most important for respiratory stimulation is the CNS; but stimulation of the diaphragm and intercostal muscles also may contribute to improved respiration observed following caffeine administration. As can be seen above in Table II-A-1, caffeine is more potent than theophylline at both sites of action for respiratory stimulation: the brain and skeletal muscle. Theophylline has much greater potency at cardiac and renal sites of action; thus making it less desirable for the indications.

Theophylline induces disturbances in cardiac rhythm frequently (Serafin, 1995); they can be as benign as moderate sinus tachycardia at lower doses, or as severe as life-threatening ventricular tachycardia at higher doses. Cardiac rhythm disturbances rarely are associated with caffeine administration (Serafin, 1995).

There was a difference in renal potency of caffeine and theophylline when 5 mg caffeine/kg and 4.9 mg theophylline/kg were administered intravenously to anesthetized 5-10 day old New Zealand rabbits. Caffeine had no effect on any of the renal parameters, but theophylline produced a significant diuresis (increased urine flow rate, V) and a significant increase in renal vascular resistance (RVR) with a concomitant decrease in renal blood flow (RBF) (Gouyon and Guignard, 1987). Premature infants with low birth weight are frequently present with low glomerular filtration rate and low renal blood flow, indicators of poor renal function (Guignard and John, 1986). Use of caffeine to control apnea of prematurity in such infants may be safer than the use of theophylline (Gouyon and Guignard, 1987).

Interconversion between caffeine and theophylline has been reported in preterm neonates. Caffeine levels were approximately 25% of theophylline content after theophylline administration and around 3-8% of caffeine administered was converted to theophylline. The N-7 demethylating isoenzyme necessary to convert caffeine to theophylline is less active in newborns than that in the adult (Berthou *et al.*, 1988), and very little or no theophylline is found in urine from infants. Therefore, caffeine may provide the benefit of respiratory stimulation without the risk of undesirable effects on cardiac and renal sites from theophylline in neonates (See metabolism of neonates on p. 16).

The summary table for selected pharmacology studies follows.

**APPEARS THIS WAY  
ON ORIGINAL**

**Summary Table: Results of Selected Pharmacology Studies**

Respiratory System					
Species (Strain)	No. of animals/group	Route of admin. (Vehicle)	Dose (mg/kg)	Observations	Reference
Rabbit-Sodium pentobarbital anesthesia (pre-vagotomy)	9 (2-4 days old) 10 (6-7 days old)	IP	10	Caffeine significantly increased the frequency of breathing and decreased tidal volume in the younger and older rabbits. There was no change in instantaneous minute ventilation in younger rabbits even after vagotomy but the response to caffeine was increased after vagotomy in older rabbits. Inspiratory and expiratory times were significantly decreased in younger animals but only inspiratory time was significantly decreased in the older rabbits. Caffeine increased the strength of the Hering-Breuer expiratory-promoting reflex in both groups.	Trippenbach <i>et al.</i> (1980)
(post-vagotomy)	8 <sup>1</sup> (2-4 days old) 7 <sup>1</sup> (6-7 days old)	IP	10 <sup>2</sup>		

1 From pre-vagotomy group of animals, respectively.

2 Second dose represents 20 mg cumulative dose.

APPEARS THIS WAY  
ON ORIGINAL

Summary Table: Results of Selected Pharmacology Studies, cont.

Respiratory System, cont.						
Species (Strain)	No. of animals/group	Route of admin. (Vehicle)	Dose (mg/kg)	Observations		Reference
Lamb-awake	6 (10-13 weeks old intact)	IV (0.9% saline)	10	Caffeine transiently increased minute ventilation 1 minute following administration. At 5 minutes, the response had dissipated. Minute ventilation remained unchanged from baseline at 15 and 120 minutes. CBD abolished initial transient response to caffeine.		Blanchard <i>et al.</i> (1986)
	6 (10-13 weeks old) carotid body denervated (CBD)		10			
Lamb (sodium pentobarbital anesthesia)	6 (3-10 days old) 7 (3-10 days old) - caffeine	IV (0.9% saline)	0 10	Under general anesthesia, total neuromuscular blockage and artificial ventilation with 100% O <sub>2</sub> , caffeine potentiated the effects of acetylcholine on respiratory conductance but not compliance.		Keklikian <i>et al.</i> (1992)
Monkey (Rhesus)-awake	3 M (adults) (same 3 animals for each dose treatment)	IV (0.9% saline)	0, 3, 10, 17, 30, (CD) <sup>1</sup>	In schedule-controlled behavior paradigms, caffeine increased respiratory rate above that observed during periods of extended time-outs.		Howell & Landrum (1994)
Pig - chloralose anesthesia	7 (1-4 days old) (same 7 pigs for 0 and 20 mg/kg)	IV (0.9 saline)	0, 20	Caffeine converted the biphasic ventilatory response to hypocapnic hypoxia (12% O <sub>2</sub> ), initial increased ventilation (2 min) followed by decrease to baseline (10 min), to a sustained increase in ventilation (10 min).		Lopes <i>et al.</i> (1994)

<sup>1</sup>CD = cumulative dosing.

APPEARS THIS WAY  
ON ORIGINAL

Summary Table: Results of Selected Pharmacology Studies, cont.

Cardiovascular				
Species (Strain)	No. of animals/group	Concentration	Observations	Reference
Rat ( <i>in vitro</i> )	7 ventricular papillary muscles <sup>1</sup>	0, 1, 2.5, 5 mM	Caffeine produced a concentration-dependent increase in the force of contraction of isolated papillary muscles in newborns in contrast to the negative inotropic response observed in adult heart tissue (adult rat heart is atypical)	Jourdon <i>et al.</i> (1981)
Rabbit (New Zealand White) ( <i>in vitro</i> )	7 adults (6-12 months old) (ventricular septum)  9 newborn (1 week old) (ventricle)	0, 20 mM	Age-related differences were observed in effects of caffeine on mechanical function e.g. significant increases in maximal rate of tension of 141% in adults, compared to 111% in newborns; significant increases in resting tension of 245% in adults, 132% in newborn. In newborns, caffeine perfusion decreased maximal rate of relaxation to 29% of baseline and increased half relaxation by 144%, both significantly greater than in adults.	George <i>et al.</i> (1984)
Rat (Holtzman) ( <i>in vitro</i> )	Newborn (3 days old) (ventricular cardiac myocyte) triplicate cultures	0, 0.2-10 mM	Caffeine inhibited both DNA and protein synthesis in cultured neonate cardiac muscle cells. The inhibition of DNA synthesis was reversed by adding zinc suggesting caffeine may inhibit zinc-dependant enzymes involved in DNA synthesis.	Kanemaru <i>et al.</i> (1992)

<sup>1</sup> Number of rats assumed to be 7; however, the number was not indicated in the paper.

APPEARS THIS WAY  
ON ORIGINAL

#### 1.4 List of References

- Arnaud MJ. The pharmacology of caffeine. *Prog Drug Res* 1987; 31:273-313.
- Blanchard PW, Cote A, Hobbs S, Foulon P, Aranda JV, Bureau MA. Abolition of ventilatory response to caffeine in chemodenervated lambs. *J Appl Physiol* 1986; 61:133-7.
- Clozel M, Branchaud CL, Tannenbaum GS, Dussault JH, Aranda JV. Effect of caffeine on thyroid and pituitary function in newborn rats. *Pediatr Res* 1983; 17:592-5.
- George BL, Shimizu T, Jarmakani JM. Caffeine effect on myocardial mechanical function in the neonatal rabbit heart. *Dev Pharmacol Ther* 1984; 7:398-408.
- Gouyon JB, Guignard JP. Renal effects of theophylline and caffeine in newborn rabbits. *Pediatr Res* 1987; 21:615-18.
- Howell LL, Landrum AM. Behavioral and pharmacological modulation of respiration in rhesus monkeys. *J Exp Anal Behav* 1994; 62:57-72.
- Jourdon P, Auclair MC, Lechat P. Caffeine effects on mechanical activity in newborn rat myocardium. *J Mol Cell Cardiol* 1981; 13:861-5.
- Kanemaru Y, Rossowska MJ, Narayanan CH, Nakamoto T. Effect of caffeine and zinc on DNA and protein synthesis of neonatal rat cardiac muscle cell in culture. *Res Exp Med* 1992; 192:115-22.
- Keklikian EN, Wolfson MR, Shaffer TH. Caffeine potentiates airway responsiveness in the neonatal lamb. *Pediatric Pulmonol* 1992; 12:17-22.
- Lopes JM, Davis GM, Mullahoo K, Aranda JV. Role of adenosine in the hypoxic ventilatory response of the newborn piglet. *Pediatric Pulmonol* 1994; 17:50-5.
- Rall TW. Drugs used in the treatment of asthma. The methylxanthines, cromolyn sodium, and other agents. In: Goodman AG, *et al.* editors. *Goodman & Gilman's. The pharmacological basis of therapeutics.* 8th ed. New York: Pergamon Press; 1990. p. 618-37.
- Serafin WE. Drugs used in the treatment of asthma. In: Hardman JG, *et al.*, editors. *Goodman & Gilman's. The pharmacological basis of therapeutics.* 9th ed. New York: McGraw Hill; 1995. p. 659-82.
- Tarka SM Jr. The toxicology of cocoa methylxanthines: A review of the literature. *Critical Reviews in Toxicology* 1982; 9:275-312.
- Toubas PL, Sekar KC, Sheldon RE, Seale TW. Fetal and newborn lambs differ in their cardiopulmonary responsiveness to adenosine agonists. *Dev Pharmacol Ther* 1990; 15:68-81.
- Trippenbach T, Zinman R, Milic-Emili J. Caffeine effect on breathing pattern and vagal reflexes in newborn rabbits. *Respir Physiol* 1980; 40:211-25.

## 2. Absorption, Distribution, Metabolism, Elimination

Similar to other nonclinical sections, scientific literature will be the source for documenting the absorption, distribution, metabolism, and elimination (ADME) of caffeine.

General information on ADME in adult animals will be presented first, followed by a discussion of the same parameters in neonatal animals under each heading. Most of the discussion will focus on data from *in vivo* studies in two species: the dog (Warszawski *et al.*, 1977) and the rabbit (McNamara *et al.*, 1992). However, information on metabolites is primarily from *in vitro* studies (Berthou *et al.*, 1988, 1989; Bienvenu *et al.*, 1993).

Pharmacokinetics data for adult and neonate animals are summarized in Table III-B-1, and are discussed below. Caffeine is distributed well into the tissues in young animals and is eliminated from the body much more slowly in young animals than in adult animals, because in general, the specific enzyme activity responsible for metabolism of caffeine may be attenuated relative to that of the adult. Therefore, the exposure to caffeine in the newborn is greater than in adult animals for a given dose.

**Table III-B-1.** Pharmacokinetic data following intravenous administration of caffeine to adult and neonate dogs (50.0 mg/kg) or lactating adult and neonate rabbit (5.0 mg/kg).

Parameter	Dogs <sup>1</sup>			Rabbits <sup>2</sup>	
	Adult	1 day old	1 week old	adult	19-21 days
	N=6	N=9	N=13	N=10	N=10
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
T <sub>1/2</sub> <sup>3</sup> (hours)	6.66 ± 0.85	47.6 ± 5.4	24.1 ± 2.0	2.6 ± 1.5	9.4 ± 3.9
V <sub>d</sub> <sup>4</sup> (l/kg)	0.78 ± 0.05	0.94 ± 0.03	0.84 ± 0.04	0.68 ± 0.06	0.83 ± 0.07
C <sup>5</sup> (ml/kg/min)	1.38 ± 0.15	0.28 ± 0.073	0.44 ± .047	3.83 ± 1.94	1.14 ± 0.80

<sup>1</sup>From Warszawski *et al.*, 1977

<sup>2</sup>From McNamara *et al.*, 1992.

<sup>3</sup>Elimination half-life.

<sup>4</sup>Apparent volume of distribution

<sup>5</sup>Clearance.

## 2.1. Absorption

Caffeine, like other methylxanthines (theobromine and theophylline), is absorbed readily after oral, rectal, or parenteral administration (Serafin, 1995). After an oral dose of 25 mg caffeine/kg, the maximum absorption occurred in less than an hour in both rat and rabbit: rat, 0.1 hr; rabbit, 0.7 hr (Burg, 1975). There was no significant first-pass effect in animals (or humans) following oral administration (Aldridge *et al.*, 1977). The absorption rate tends to increase with increasing doses (Garattini *et al.*, 1980).

No absorption studies in neonate animals were identified with the literature searches.

## 2.2. Distribution

Caffeine, like other methylxanthines, is distributed into all body compartments (Serafin, 1995). Tissue distribution is proportional to water content of the tissues (Warszawski *et al.*, 1977). The apparent volume of distribution for caffeine in adult dogs (N=6) following a bolus injection of 50.0 mg/kg was  $0.78 \pm 0.05$  L/kg (Warszawski *et al.*, 1977) and slightly lower ( $0.67 \pm 0.06$  l/kg) in lactating adult rabbits following an injection of 5.0 mg/kg (N=10) (McNamara *et al.*, 1992). Caffeine binds to plasma proteins (Serafin, 1995) in the range of 15% (Axelrod and Reichenenthal, 1953).

In young dogs and young rabbits as shown in Table III-B-1, the apparent volume of distribution is greater compared to that of adult animals. For example, in 1-day-old dogs, it is  $0.94 \pm 0.03$  l/kg vs.  $0.78 \pm 0.05$  l/kg in adult dogs (Warszawski *et al.*, 1977). The difference is statistically significant, ( $p < 0.005$ ), and this difference is thought to be due to the greater water content of tissues from the neonate than that in adult tissues. A similar difference in apparent volume of distribution for caffeine has been reported for premature infants compared to adult humans (Serafin, 1995).

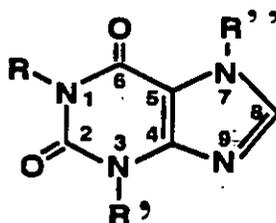
## 2.3. Elimination

Caffeine is eliminated primarily by metabolism in the liver in both the neonates and the adults (Serafin, 1995). Less than 5% of an administered dose of caffeine in rats, compared to less than 2% in human, is recovered unchanged in the urine (Serafin, 1995). The half-life for elimination of caffeine in the adult dog (50.0 mg/kg intravenously, N=6) was  $6.7 \pm 0.85$  hrs, and total body clearance was  $1.4 \pm 0.15$  ml/kg/min (Warszawski *et al.*, 1977). In lactating adult rabbits the half-life for elimination of caffeine (5.0 mg/kg intravenously, N=10) was  $2.6 \pm 1.5$  hr; total body clearance was  $3.80 \pm 1.94$  ml/min/kg (McNamara *et al.*, 1992).

Although the routes of elimination are similar in the neonate and the adult (Table III-B-1), the exposure to caffeine in the newborn is greater due to its reduced clearance (1-day old dog vs. adult dog:  $0.3 \pm 0.073$  ml/kg/min vs  $1.4 \pm 0.15$  ml/kg/min) and longer elimination half-life in the neonate (1-day old dog vs. adult dog:  $47.6 \pm 5.4$  hours vs  $6.7 \pm 0.85$  hours).

As the animal matures, elimination parameters become more like those of the adult as shown in Table III-B-1, 1-week old dog (Warszawski *et al.*, 1977).

## 2.4. Metabolism



**Xanthine**  
(R, R' & R'' = H)

Compound	R	R'	R''
Caffeine	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>
Theophylline	CH <sub>3</sub>	CH <sub>3</sub>	H
Theobromine	H	CH <sub>3</sub>	CH <sub>3</sub>

Caffeine metabolism has been studied in newborn and adult human hepatocytes and compared with adult rat hepatocytes (Berthou *et al.*, 1988), and also studied in liver slices, microsomes and hepatocytes from adult human liver (Berthou *et al.*, 1989; Serafin, 1995). Caffeine is extensively metabolized *in vivo* by cytochromes P-450 isoenzymes in the hepatic microsomal mixed function oxidase system. There are two major metabolic pathways: N-demethylation and oxidation at position C-8.

A total of fifteen primary and secondary metabolites from caffeine, have been identified by liver slices, microsomes and hepatocyte cultures from adult human liver as shown in in Table III-B-2, although only five primary metabolites can be identified following metabolism of caffeine *in vitro* using cultured hepatocytes from humans and rats (Berthou *et al.*, 1988, 1989; Bienvenu *et al.*, 1993).

The five primary metabolites are three dimethyl products of N-demethylation (1,7-dimethylxanthine or paraxanthine; 1,3-dimethylxanthine or theophylline; 3,7-dimethylxanthine or theobromine) and two products of oxidation at position C-8 (1,3,7-trimethyluric acid; 6-amino-5 (N-methylformylamino)-1,3-dimethyluracil).

**Table III-B-2. Caffeine metabolites identified *in vitro* models (slices, microsomes and hepatocyte cultures from adult human liver)<sup>1</sup>**

Primary	Secondary
1,7-dimethylxanthine (paraxanthine)	1,7-dimethyluric acid
1,3-dimethylxanthine (theophylline)	1,3-dimethyluric acid
3,7-dimethylxanthine (theobromine)	3,7-dimethyluric acid
1,3,7-trimethyluric acid	1-methylxanthine
6-amino-5(N-methylformylamino)-1,3-dimethyluracil	3-methylxanthine
	7-methylxanthine
	1-methyluric acid
	3-methyluric acid
	7-methyluric acid
	5-acetylamino-6-formyl-amino-3-methyluracil

<sup>1</sup>From Berthou *et al.*, 1989,

The level of biotransformation was very low in cultured hepatocytes (Berthou *et al.*, 1988). The concentrations of caffeine required to produce detectable metabolites *in vitro* was an order of magnitude greater than that required *in vivo* (0.1-1.0 mM and 0.02-0.1 mM, respectively). However, Berthou *et al.* (1988) made direct comparison between *in vitro* and *in vivo* systems based on total amount of drug, number of cells per system, and percentage drug metabolized. The calculations showed that the rate of caffeine metabolism (nmole/10<sup>6</sup> cells/24 hours) is approximately the same in all systems.

There were species differences in the extent and proportions of metabolites of caffeine in cultured adult rat hepatocytes and in adult human hepatocytes as summarized in Table III-B-3 (Berthou *et al.*, 1988).

APPEARS THIS WAY  
ON ORIGINAL

**Table III-B-3.** Comparison of metabolite profiles of caffeine produced by adult rat and adult human cultured hepatocytes.<sup>1</sup>

	Rat	Human
Ratio N-demethylated products to C-8 oxidation products	80:20 (pe (%))	96.5:3.5 (%)
Approximate percentage of N-demethylated products formed		
paraxanthine	33%	58%
theobromine	38%	23%
theophylline	29%	19% (97% <sup>2</sup> )

<sup>1</sup>From Berthou *et al.*, 1988.

<sup>2</sup>Hepatocytes from a minority of humans formed theophylline almost exclusively.

In cultured adult rat, the ratio of N-demethylated metabolites to those formed by C-8 oxidation is 80% to 20%, and the three dimethylxanthines were formed in approximately equal proportions, whereas in adult human hepatocytes, paraxanthine, theobromine, and theophylline account for 97% of the administered dose, 58, 23, and 19%, respectively. Also differently from rat hepatocytes, C-8 oxidation products represent only 3.5% (vs. 20%) of the total metabolites.

N-1 demethylation to theobromine is the major metabolic pathway in the rats and N-3 demethylation to paraxanthine is the major metabolites in adult human hepatocytes in addition to N-1 and N-7 demethylation. All three metabolites are formed in same quantities in young rats, although N-7 demethylation to theophylline is the only metabolite found in human neonates.

Berthou *et al.*, (1988) has found that there are genetic differences in the metabolism of caffeine. Theophylline was virtually the only dimethylxanthine formed (~97%) by human hepatocytes from a minority of individuals and the rate of caffeine metabolism in these cells was approximately 20 times greater than that in the cells of majority of individuals. Also, this data support the hypothesis that N-7 demethylation involves a cytochrome P-450 isoenzyme different from the one(s) involved in N-1 and N-3 demethylation reactions.

**Metabolism in Neonates:**

There is very little information on the metabolic profiles in newborn animals. However, development of the hepatic microsomal mixed function oxidase system has been studied using tissue slices of newborn rat liver (Bienvenu *et al.*, 1993) and cultured newborn human hepatocytes (Berthou *et al.*, 1988).

Bienvenu *et al.* (1993) compared the specific activity of the enzyme system in slices of liver from 1-, 7-, 14-, 21-, 28-, 35-, and 120-day old rats. Activity was low initially, peaked at 21 days, then decreased to a level in the adult that was greater than that in the neonate tissue. The data are summarized in Table III-B-4. In this study, N-1 demethylation (theobromine) was the main pathway, representing 70% of the total metabolism at 1, 35, and 120 days of age, and about 80% at 7, 14, 21, and 28 days.

**Table III-B-4.** Specific activity of the hepatic enzyme system for caffeine in liver slices from rats of increasing age.<sup>1</sup>

Age (days)	Specific Activity (nmol/g liver/hour)
1	24.9 ± 6.64
7	35.4 ± 14.5
21	98 <sup>2</sup>
28	86.8 ± 11.4
120	50 <sup>3</sup>

<sup>1</sup>From Bienvenu *et al.*, 1993.

<sup>2</sup>Read from chart found on p. 179 of reference; caffeine transformed.

<sup>3</sup>Read from chart found on p. 179 of reference; caffeine transformed.

Distribution of dimethyl products was qualitatively similar at two ages but slightly different quantitatively in neonatal and adult rats as shown in Table III-B-5.

**Table III-B-5.** Comparison of the distribution of dimethyl metabolites in neonatal and adult rat liver tissue slices.<sup>1</sup>

Dimethyl Metabolite	Neonate	Adult
Theobromine	36%	50%
Theophylline	30%	30%
Paraxanthine	33%	20%

<sup>1</sup>From Bienvenu *et al.*, 1993.

However, in neonatal and adult human cultured hepatocytes, there was a significant difference in the dimethyl metabolites, unlike in the rat. Theophylline was the only metabolite found in the neonatal tissue (Berthou *et al.*, 1988). It is suggested that, in the human neonate, the cytochrome P-450 isoenzyme for N-7 demethylation is the only one developed, and cytochrome P-450 isoenzyme(s) required for N-1 and N-3 demethylation are not active. Furthermore, the neonatal N-7 demethylating isoenzyme may be less active than that in the adult. Such altered metabolism appears to be the basis for the decreased clearance and increased elimination time observed in the human neonates. In the rat neonates however, decreased clearance appears to be due solely to decreased specific activity of the enzyme system overall.

Aldridge *et al.*, (1979) examined the concentrations of caffeine and metabolites in urine by [redacted] as a function of age to explore the remarkably slow elimination of caffeine by human infants. Urine samples were obtained from 3 adults and 10 infants aged 8 days to 8 months during therapeutic treatment with caffeine. During the first month of life, caffeine accounted for more than 85 % of the identifiable products in urine. Caffeine remained the predominant component for the first 3 months, but its percentage decreased gradually to the adult value of less than 2 % by the age of 7 to 9 months. This change reflected increasing metabolite production, not decreasing urinary caffeine concentration. The adult metabolite pattern of partially demethylated xanthines and urates was attained by 7 to 9 months. The data indicate that the 4-day plasma  $t_{1/2}$  of caffeine characteristic of the newborn depends in large part on slow urinary excretion of unchanged drug since there is little or no metabolism. Subsequent decrease in the  $t_{1/2}$  to about 4 hour by the age of 8 months correlates closely with the rise in metabolite production.

In neonates and infants, the interconversion between caffeine and theophylline has been reported, i.e. urinary or plasma caffeine levels were approximately 25% of theophylline content after theophylline administration and around 3-8% of caffeine administered was converted to theophylline.

The summary table of selected, absorption, distribution, metabolism and excretion studies follows.

APPEARS THIS WAY  
ON ORIGINAL

Summary Table: Results of Selected Absorption, Distribution, Metabolism, and Elimination Studies

Species and Strain	Study Group (number, age, weight, sex)	Route and Formulation	Dose mg/kg	Frequency and Duration	Serum Sampling Times (hrs)	Serum Half-life(hrs)*	Other Pharmacokinetic Parameters Assessed Vd(l/kg)* Cl(ml/min/kg)*	References
Rabbits New Zealand White	Two study groups n=10/group  1. Lactating females, 17-22 days post partum 4.49±0.54kg  2. 19-21 days old (1 or 2/litter) 0.273±0.06kg	I.V. Saline	5.0	One injection over 1-2 min.	Adults: 0.125, 0.25, 0.5, 1, 2, 3, 4, 6, 8  Kits: 0, 1, 6, 12, 24, 48, 80, 120	Adults: 2.6±1.5  Kits: 9.4±3.9	Adults: Vd=0.679±0.06 Cl=3.83±1.94  Kits: Vd=0.825±0.07 Cl=1.14±0.80	McNamara, <i>et al.</i> 1992
Mongrel Dogs (both sexes)	1. Adult 11-15 kg n=6  2. 1-day old 260-275g n=9  3. 1-wk old <sup>1</sup> n=13  4. 30-45 days <sup>2</sup> n=5	I.V. water	50.0	Single bolus injection	Sampling times not indicated	Group 1: 6.66±0.85  Group 2: 47.58±5.53  Group 3: 24.09±1.95  Group 4: 3.70±0.53	Vd=0.78±0.05 Cl=1.38±0.15  Vd=0.94±0.03 Cl=0.28±0.07  Vd=0.84±0.04 Cl=0.44±0.05  Vd=0.60±0.03 Cl=2.12±0.07	Warzawski, <i>et al.</i> 1977

\* Mean ± S.D. <sup>1</sup> Weight not provided in paper. <sup>2</sup> Weight not provided in paper.

2.7 List of References

- Aldridge A, Parsons WD, Neims AH. Stimulation of caffeine metabolism in the rat by 3-methylcholanthrene. *Life Sci* 1977; 21:967-74.
- Aldridge A, Aranda J.V. and Neims A.H. Caffeine metabolism in the newborn. *Clin. Pharmacol. Ther.* April 1979; 447-453
- Axelrod J, Reichenenthal J. The fate of caffeine in man and a method for its estimation in biological material. *J Pharmacol Exp Ther* 1953; 107:519-23.
- Bienvenu T, Pons G, Rey E, Thiroux G, Olive G. Caffeine metabolism in liver slices during postnatal development in rats. *Drug Metab Dispos* 1993; 21:178-80.
- Berthou F, Ratanasavanh D, Alix D, Carlhant D, Riche C, Guillouzo A. Caffeine and theophylline metabolism in newborn and adult human hepatocytes: comparison with adult rat hepatocytes. *Biochem Pharmacol* 1988; 37:3691-700.
- Berthou F, Ratanasavanh D, Riche C, Picart D, Voirin T, Guillouzo A. Comparison of caffeine metabolism by slices, microsomes and hepatocyte cultures from adult human liver. *Xenobiotica* 1989; 19:401-17.
- Burg AW. Physiological disposition of caffeine. *Drug Metab Rev* 1975; 4:199-228.
- Garattini S, Bonati M, Latini R, Galetti F. Caffeine kinetics and metabolism. Proceedings of Third International Caffeine Committee Workshop; 1980 October 27-28; Hunt Valley, Maryland.
- McNamara PJ, Burgio D, Yoo SD. Pharmacokinetics of caffeine and its demethylated metabolites in lactating adult rabbits and neonatal offspring. *Drug Metab Dispos* 1992; 20:302-8.
- Serafin, WE. Drugs used in the treatment of asthma. In: Hardman JG, *et al.* editors. Goodman & Gilman's. The pharmacological basis of therapeutics. 9th ed. New York: McGraw-Hill; 1995. p. 659-82.
- Warszawski D, Gorodischer R, Moses SW, Bark H. Caffeine pharmacokinetics in young and adult dogs. *Biol Neonate* 1977; 32:138-42.

APPEARS THIS WAY  
ON ORIGINAL

### 3. General Toxicity

The toxicity studies of caffeine have not been conducted as part of a prospective development program. There are, however, numerous published data to gain insight on toxic dose ranges of caffeine relative to doses required to produce the proposed therapeutic effect, respiratory stimulation.

Acute toxicity studies and repeat-dose toxicity studies will be summarized in a tabular format from the sponsor's submission. These studies appear to have been individually designed as independent research projects. Such projects usually were not carried out according to 21CFR Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

#### 3.1. Acute Toxicity

##### Rat

##### Methods

To compare the acute toxicity of aminophylline and caffeine in newborn and adult rats, three doses of caffeine were administered subcutaneously to each age group of male Charles River rats: adults--250, 275, and 300 mg/kg; 2-3 day olds--100, 125, and 175 mg/kg. The LD<sub>50</sub> values were derived by probit analysis on a log-dose scale. Animals were observed for seven days following drug administration.

##### Findings

LD<sub>50</sub> values are presented in Table IV-AT-1. Neonate animals were more sensitive to caffeine than adult animals during the 24 hrs immediately following drug administration. When followed for one week, neonatal animals showed an even greater sensitivity to caffeine. No additional adults died after 24 hrs.

In adults, the cause of death most likely was respiratory failure secondary to tonic-clonic convulsions. Surviving animals exhibited tremors, lethargy, and licking of lips. In neonate animals, the cause of death during the first 24 hrs is not reported (only the LD<sub>50</sub> is reported). Failure to thrive, (probably failure to nurse) as exhibited by decreased weight gain, appears to be the cause of death after 24 hrs.

APPEARS THIS WAY  
ON ORIGINAL

**Table IV-AT-1.** Acute toxicity of caffeine administered subcutaneously to young and adult rats.

<u>Observation Time</u>	<u>Acute LD50 (mg/kg)</u>	
	2-3 day old rats	adult rats
24 hours	220	265
1 week	155	265

<sup>1</sup> From Warszawski *et al.*, 1978.

It was also noted here that the acute toxicity of caffeine is less than that of theophylline. In newborn, as well as adult rats, the LD<sub>50</sub> for caffeine administered subcutaneously is approximately 30% greater than the LD<sub>50</sub> for theophylline administered by the same route, meaning caffeine is slightly less toxic than theophylline (Warszawski *et al.*, 1978).

	<u>LD<sub>50</sub></u>	
	<u>Theophylline</u>	<u>Caffeine</u>
Adult rat	202 mg/kg	265 mg/kg
2-Day old rat	169 mg/kg	220 mg/kg

Conclusions

The toxicity of caffeine is greater in 2-3 day old rats compared to adult rats. This can probably be explained by the finding that caffeine is eliminated more slowly in neonate rats because the hepatic microsomal enzyme system is not fully developed in neonate animals.

**Mouse**

Singh *et al.* (1984) noted the oral LD50 for female mice as 297.5 mg/kg.

**Dog**

No formal acute toxicity studies in the dog were identified by the literature searches. Dimitrov *et al.* (1969) administered 150 mg caffeine sodium benzoate/kg (ca. 75 mg caffeine/kg) to two dogs in order to determine the effects of caffeine on the hexose monophosphate shunt in leukocytes. The investigators noted that this dose of caffeine produced marked salivation, incontinence, tachycardia, tremor, restlessness, convulsions, and death. The effect of sodium benzoate alone was not determined.

APPEARS THIS WAY  
ON ORIGINAL

### 3.2. Repeat-Dose Toxicity Studies

#### Rat

##### a) Fourteen Day Exposure

*Peters JM, Boyd EM. The influence of sex and age in albino rats given a daily oral dose of caffeine at a high dose level. Can J Physiol Pharmacol 1967;45:305-311.*

##### Methods

To study the significance of sex and age of albino rats on chronic oral toxicity of caffeine, male and female albino Wistar rats of varying age (males: 1.5, 4.5, and 12 months; females: 1.5, 2.5, 4.5, and 12 months) were exposed to only one dose of caffeine 185 mg/kg by intragastric administration for 14 days. Caffeine was dissolved in distilled water, and a volume of 2.0 ml/100 g body weight was administered. Animals in control groups received the same volume of vehicle only for 14 days. Body weight, food and water intake, and urine output were measured daily. Gross and microscopic observations were recorded.

##### Findings

Mortality rates at 14 days are summarized in Table IV-RT-1. The death rate is higher in old rats than in young rats given daily oral administrations of 185 mg/kg caffeine. Death occurred mainly during the first 2-3 days. Psychotoxic (automutilation plus hemorrhage) deaths were most common in young rats, and hypokinetic-convulsive deaths in older animals. The incidence of mortality was similar for both sexes, in the adult age group.

In survivors, the incidence of glycosuria and hydration of the kidneys was greater, and dehydration of the adrenal glands and gonads less, in older than in younger rats. Sublethal signs of toxicity in male survivors were greater than in female survivors. Anorexia and loss of body weight were greater in male than in female survivors, diuresis was less evident, loss of muscle weight was greater, and the relative weight of the adrenal glands and gastrointestinal tract were greater in males.

**Table IV-RT-1** Mortality following 14-day intragastric administration of 185 mg caffeine/kg/day to rats.

Age (months)	Mortality Rates at 14 Days			
	Males		Females	
	control	treated	control	treated
1.5	0/10		0/19	0/10
2.5	NT <sup>2</sup>	NT <sup>2</sup>	0/7	6/18
4.5	0/9	2/13	0/10	1/17
12.0	0/8	10/13	0/10	9/13

<sup>1</sup>From Peters and Boyd, 1966.

<sup>2</sup>NT=None treated

Conclusion

Fourteen-day intragastric administration of 185 mg caffeine/kg/day to 1.5-, 2.5-, 4.5-, and 12-month old male and female rats significantly increased mortality (77 and 69% in male and females, respectively) only in 12-month old animals (compared to control groups). Morbidity appeared to be greater in the oldest group. In younger animals, caffeine inhibited growth with greater effects in males than in females. Young animals are found to be more sensitive to the diuretic effect of caffeine than older ones and females more than male rats. Caffeine was found to be more toxic in older than in younger rats and in males than in females.

b) 100-Day Exposure

Boyd EM, Dolman M, Knight LM, Sheppard EP. The chronic oral toxicity of caffeine. *Can J Physiol Pharmacol* 1965;43:995-1007.

Methods

The acute intragastric LD<sub>50</sub> was determined in 50 Female CBL Wistar albino rats given doses of 200 to 350 mg/kg of Caffeine (dissolved in distilled water and given in a volume of 20 ml/kg).

For repeat-dose administration, 5 days a week, in a series of decreasing fractions of the acute oral LD<sub>50</sub>, each daily doses (264, 238, 181, 165, 158, 142, and 136 mg/kg) were given until 60% of the animals had died or for 100 days (1/10 the life span of a rat, or 0.1L), whichever occurred first. Controls were given daily the same volume of distilled water. Clinical measurements were obtained at weekly intervals and autopsies were performed upon survivors at 100 days or after 60% of the animals had died, whichever occurred first.

Findings

*Mortality.* The endpoints for intragastric dosing (mg/kg) were (mean ± SE):

acute LD <sub>50</sub>	264±10	(50% death on single dose)
LD <sub>50</sub>	150±3.1	(50% death on repeat-dose)
minimal LD <sub>100</sub>	191±5.7	(100% death at 100 days)
maximum LD <sub>0</sub>	110±2.2	(maximum non-lethal dose in 100 days)

Respiratory failure secondary to convulsions was the cause of death in animals dying within a week from the larger daily doses of caffeine. When death occurred after the first week, premortal clinical signs were anorexia, a relative oligodipsia and oliguria, loss of body weight, a fall in colonic temperature, proteinuria, aciduria, and often glycosuria.

*Clinical Signs.* Growth was inhibited by daily doses greater than the 150 mg/kg in a dose-dependent manner. Polydipsia occurred in all caffeine-treated animals, except in a few given the lowest daily dose (136 mg/kg). The response was dose-dependent (see Table IV-RT-2). For a given dose, the polydipsic water intake did not vary significantly from week to week.

**Table IV-RT-2.** Polydipsic and diuretic responses in female rats during 100 days of daily intragastric caffeine administration.<sup>1</sup>

Polydipsic and Diuretic Responses to Caffeine <sup>2</sup>		
dose	polydipsic response	diuretic response
LD50 <sub>(0.1L)</sub> (150 mg/kg)	water intake increased 40-50%	urinary volume increased 400% (4-fold)
LD100 <sub>(0.1L)</sub> (191 mg/kg)	water intake increased 100%	urinary volume increased 1600% (16-fold)

<sup>1</sup>From Boyd *et al.*, 1965.

<sup>2</sup>Compared to respective control groups.

Diuresis was observed in all dose group, although diuresis declined premortally. In addition, blepharitis at high doses, psychotic-like reactions in a few rats (animal biting and mutilating its own feet or tail) and alopecia and dermatitis in a few rats were observed.

*Gross findings.* Lower doses (between 110 mg/kg and 150 mg/kg) produced a significant increase in weights of lungs, adrenal glands, salivary glands, all parts of the gastrointestinal tract, liver, kidneys, and heart, although the thymus gland weight was reduced. At doses greater than 150 mg/kg, changes in organ weights were variable, with some organs losing weight at doses greater than 191 mg/kg. There were no changes in the weight of brain and thyroid gland.

*Histopathology.* Histopathologic findings are summarized in Table IV-RT-3. At doses less than 150 mg/kg, bronchopneumonia in the lung and mucosal hypertrophy in the gastrointestinal tract were the main findings. At higher doses ( $\geq 150$  mg/kg) in the lungs, congestion, edema, thrombosis and hemorrhage were noted, and mild hyperemia and mild to moderate inflammation were noted in the gastrointestinal tract. Increased organ weights were found to correlate with cortical hypertrophy in the adrenal glands and mucosal hypertrophy in the gut, and associated with hyperemia in the heart and mild vascular congestion in the kidneys. There was some inhibition of oogenesis in the ovary.

*Gastric change:*

As shown in the following table, increased organ weights correlated with mucosal hypertrophy and increased water content in the gut. There were small ulcers extending about halfway down the glands of pyloric stomach, but no necrosis was observed. Gastric ulcers were found in rats given daily doses of 110 mg/kg in this study, although smaller doses of caffeine have been found to produce gastric ulcers in cats and rats from previous studies. (See the discussion of effects on the gastrointestinal tract under Special Toxicity.)

Table IV-RT-3. The histopathologic effects of caffeine on rats after 100 days of dosing.

Organ	Daily dose of caffeine (mg/kg)		
	136 and 142	158 and 165	181 and 238
Adrenal glands	Cortical hypertrophy	Cortical hypertrophy	Sinusoids congested
Brain	Mild hyperemia	Mild hyperemia	Meningeal congestion
Gastrointestinal tract			
(a) Cardiac stomach	Mucosal hypertrophy	Normal	Normal
(b) Pyloric stomach	Mucosal hypertrophy Occasional small ulcer	Capillary congestion Occasional small ulcer	Mild inflammation
(c) Small bowel	Mucosal hypertrophy	Mild hyperemia	Moderate inflammation
(d) Cecum	Mucosal hypertrophy	Mild hyperemia	Moderate inflammation
(e) Colon	Mucosal hypertrophy	Normal	Mild inflammation
Heart	Mild hyperemia	Mild capillary congestion	Marked capillary congestion
Kidneys	Mild edema and congestion	Edema and congestion, occasional venous thrombosis and thrombophlebitis	Marked edema and congestion
Liver	Normal	Mild sinusoidal congestion	Cloudy swelling and congestion
Lungs	Bronchopneumonia	Congestion, edema, thrombosis	Congestion, edema, hemorrhage
Muscle (abd. wall)	Normal	Weak cross striation	Weak cross striation
Ovaries	Deficiency primary follicles	Mild congestion	Mild congestion
Salivary gland (submax.)	Normal	Normal	Mild hyperemia
Skin	Normal	Occasional dermatitis	Normal
Spleen	Mild loss red pulp	Moderate loss red pulp	Marked loss red pulp
Thymus gland	Moderate atrophy	Marked atrophy	Marked atrophy
Thyroid gland	Colloid deficiency	Mild congestion	

APPEARS THIS WAY  
ON ORIGINAL

### Conclusion

In female CBL Wistar albino rats, 191 mg caffeine/kg/day was lethal when administered intragastrically for 100 days. Approximately 50% of that dose, 110 mg/kg/day, was calculated as that dose which would produce no mortality. Between those two doses, caffeine produced many dose-dependent responses including growth inhibition, polydipsia, and diuresis. Several organ weights were increased. Corresponding histopathological observations included cortical hypertrophy of adrenal glands (a response to stress), hyperemia of the heart and the gut, and mild vascular congestion in the kidneys. Of the particular interest, the mucosal hypertrophy, mild hyperemia and moderate inflammation were observed as a dose-dependent phenomena in the stomach, small bowel and cecum, since methylxanthines are implicated in the development of necrotizing enterocolitis in neonates. (See discussion, *Grosfeld et al.*, 1983 in Special Toxicity 4.3 section.)

There was some inhibition of oogenesis in the ovary. Although there were no changes in weight of brain and thyroid gland, histopathological changes were noted such as mild hyperemia in the brain and meningeal congestion and colloid deficiency and mild congestion in the thyroid gland. Caffeine toxicity was apparent in this study at all dose levels. The LD<sub>50</sub> was 150 ± 3.1 mg caffeine/kg, which produces an approximate therapeutic index of 15, based on the nonclinical dose that stimulates respiration (10 mg/kg). Although the findings at the lowest dose of caffeine were not severe, the reversibility of these lesions was not determined.

### Other Studies

Gans (1984) studied effects of dietary caffeine (0.5% of a pulverized rat chow) on rat testes and thymus gland. Control or treated male CD Sprague-Dawley derived rats were exposed to diet alone or 0.5% caffeine, respectively, for 7 weeks or 8 weeks (n=6/group).

There were no deaths and no signs of self-mutilation. Weight gain was significantly decreased by caffeine (approximately 50% less than controls), accompanied by significantly lower cumulative food intake. Weights of thymus glands and of testes were decreased in treated animals compared to respective control animals. However, relative organ/body weights in treated and control groups were comparable for both organs.

Histological examination of testes showed scattered areas of spermatogenic-cell degeneration following both treatment regimens, with more prominent response following 8-week treatment than following 7-week treatment. There were no well-defined microscopic structural changes in the thymus gland. It was concluded that repeat-dose exposure to high oral doses of caffeine (3.0g of total dose over 7 weeks; 3.6g over 8 weeks) are toxic to the testes, as shown by spermatogenic-cell degeneration.

The summary table of selected toxicity studies follows.

**Summary Table: Results of Selected Toxicity Studies****Acute Toxicity**

Species	No. of animals /group	Route of Admin.	LD <sub>50</sub> (mg/kg) Male	Remarks	Reference
Rat	2 experiments; 3 groups/exp't.  Neonate (2 days old) 10-25/group  Adult M 5-15/group	Subc.	220(1) 155(2)  265(3)	Acute deaths probably due to respiratory failure. After 24 hours, failure to thrive (probably did not nurse) appears to be cause of death.  Cause of death, respiratory failure secondary to tonic-clonic convulsions.	Warszawski <i>et al.</i> (1978)
Dog	2	IV.	-	Both dogs died after dosing with 150 mg/kg of caffeine sodium benzoate (ca. 75 mg caffeine/kg). Symptoms were marked salivation, incontinence, tachycardia, tremors, restlessness and convulsions	Dimitrov <i>et al.</i> (1969)

(1) 2-3 day old rats after 24 hours

(2) 2-3 day old rats after 1 week

(3) adult rats after 24 hours and one week

APPEARS THIS WAY  
ON ORIGINAL

## Repeat-Dose Toxicity

Species (Strain) Duration	No. of animals/ group	Route of admin. (vehicle)	Dose(s) caffeine (mg/kg/day)	Pharmacotoxic signs	Mortality	Body Weight	Organ Weights & Histopath	Reference
Rat (Wistar) 14 days	Control 8-10 M (1) 7, 10 F (2)  Caffeine 13, 19 M (1) 10-18 F (2)	Gavage (water)	0 and 185	Young rats: automutilation and extensive blood loss; Older rats: prolonged morbidity preceding mild tonic- clonic convulsions and respiratory failure.	1.5 mo.-2/19 M, 0/10 F; 2.5 mo.- 6/18 F, 4.5 mo.- 2/13 M & 1/17 F; 12 mo. 10/13 M, 9/13 F; deaths occurred mainly 2-3 days post-dose, no control deaths	Weight loss greater in males and especially in younger rats	Males: greater increase in relative wt. of adrenals, liver and salivary glands; muscle wt. loss greater than in female.	Peters and Boyd (1967)
Rat (Wistar) 100 days	Controls: 8-12 F Caffeine: 20 F	Gavage (water)	0, 136, 142, 158, 165, 181, 238, & 264 (5 doses/ week)	Polydipsia, diuresis, occasional psychotic- like responses in a few animals at two lowest doses, occasional dermatitis & alopecia at intermediate doses, blepharitis at highest doses	LD0 = ~110 mg/kg LD50 = ~150 mg/kg LD100 = ~191 mg/kg	Decreased body wt. at higher doses	Weight of several organs increased. Histopathology: adrenal glands: cortical hypertrophy, heart: hyperemia, kidneys: mild vascular congestion, ovary: some oogenesis, brain: mild hyperemia & meningeal congestion, thyroid: colloid deficiency & congestion.	Boyd (1965)

(1) 3 age groups, 1.5, 4.5, and 12 months.

(2) 4 age groups, 1.5, 2.5, 4.5, and 12 months.

**Repeat-Dose Toxicity (continued)**

Species (Strain) Duration	No. of animals/ group	Route of admin. (vehicle)	Dose	Observations	Reference
Rat (Sprague Dawley) 7 and 8 weeks	6 M	Oral-diet (pulverized rat chow) Caffeine-0.5%	7 weeks=3.0 g 8 weeks=3.6 g	No deaths, food intake & weight gain significantly decreased (50% less than controls), thymus & testes weight (absolute) less than controls, scattered areas of spermatogenic-cell degeneration.	Gans (1984)

APPEARS THIS WAY  
ON ORIGINAL

### 3.3 List of References

#### *Acute Toxicity*

Dimitrov NV, Miller J, Ziegra SR. The effects of caffeine on glucose metabolism of polymorphonuclear leukocytes. *J Pharmacol Exp Ther* 1969; 168:240-43.

Singh KP, Saxena AK, Srivastava SN, Shanker R. Effect of caffeine (1, 3, 7-trimethylxanthine) on bone marrow cells of mice. *Indian J Exp Biol* 1984; 22:608-11.

Warszawski D, Gorodischer R, Kaplanski J. Comparative toxicity of caffeine and aminophylline (theophylline ethylenediamine) in young and adult rats. *Biol Neonate* 1978; 34: 68-71.

#### *Repeat-Dose Toxicity*

Boyd EM, Dolman M, Knight LM, Sheppard EP. The chronic oral toxicity of caffeine. *Can J Physiol Pharmacol* 1965; 43:995-1007.

Croxton FE. Elementary statistics with applications in medicine and the biological sciences. New York: Dover Publications, Inc., 1959.

Gans JH. Comparative toxicities of dietary caffeine and theobromine in the rat. *Food Chem Toxicol* 1984; 22:365-9.

Peters JM, Boyd EM. The influence of sex and age in albino rats given a daily oral dose of caffeine at a high dose level. *Can J Physiol Pharmacol* 1967; 45:305-11.

APPEARS THIS WAY  
ON ORIGINAL

#### 4. Special Toxicity

The special toxicity section will include the investigations that focus on the behavioral toxicity of caffeine, especially when exposure occurs during the neonatal period. The cardiovascular toxicity and the effects on the gastrointestinal tract are also discussed in the section.

##### 4.1. Central Nervous System

Two types of CNS toxicity have been associated with caffeine, both are related to motor activity and to behavior. Convulsions are observed only with high (toxic) doses of caffeine, and have been studied in relation to general toxicity of the drug. When convulsions occur, it usually is within 24-48 hours of exposure to a high dose of caffeine as an acute response.

Behavioral effects of caffeine can be observed in the lower dosage range for caffeine at 10-20 mg/kg in neonate, juvenile, and adult rats. In addition to these CNS effects, the relationship between neurochemical and behavioral effects of caffeine, and the putative role of the adenosine receptor system in CNS activity is discussed.

##### Postnatal Exposure

a) Zimmerberg *et al.* (1991) followed a protocol that simulates the doses and schedule of drug administration proposed for caffeine in the treatment of apnea of prematurity. Rat pups were exposed to caffeine at either 1 or 9 mg/kg daily by intragastric injection during postnatal (PN) days 1-6. Measurements to evaluate the behavior were developmental (righting reflex, eye-opening), locomotor activity, and operant learning.

Table IV-ST-1. Six-day postnatal (PN) caffeine exposure in Long-Evans rat pups: Protocol summary.<sup>1</sup>

Groups (n=8/sex <sup>2</sup> )	Non-injected control; Vehicle control; 1 mg caffeine/kg; 9 mg caffeine/kg
Exposure time	PN1-PN6
Parameters	Weight gain, PN1-PN17 Righting reflex, PN10+ Eye-opening, PN10+ Locomotor activity (open field), PN13-17 Operant behavior (learning), PN69-97

<sup>1</sup> From Zimmerberg *et al.*, 1991.

<sup>2</sup> One male and one female/group from each of 8 litters.

Although caffeine was administered for only six days, effects were observed after completion of drug administration. There were no differences between the two control groups for any of the parameters monitored.

**Table IV-ST-2. Six-day postnatal (PN1-6) caffeine exposure in Long-Evans rat pups: Summary of findings.<sup>1</sup>**

Parameter	Non-injected Control	Vehicle Control	1 mg caffeine/kg	9 mg caffeine/kg
Weight gain PN1-17	Baseline	Baseline <sup>2</sup>	Decrease <sup>3</sup>	Decrease <sup>3</sup>
Adult weight (g)				
male	467.2±12.4	451.2±27.0	460.1±11.9	452.6±10.9
female	281.1±11.0	298.8±14.6	289.6±11.1	291.6±13.5
Righting reflex PN10+	Baseline	Baseline <sup>2</sup>	-- <sup>4</sup>	--
Eye-opening PN10+	Baseline	Baseline <sup>2</sup>	--	--
Locomotor activity (PN13-17)	Baseline	Baseline <sup>2</sup>	Decrease <sup>3</sup>	Decrease <sup>3</sup>
Operant learning (adult rat)	Baseline	Baseline <sup>2</sup>	Decrease <sup>5</sup>	Decrease <sup>3</sup>

<sup>1</sup> From Zimmerberg *et al.*, 1991. Mean±S.D.

<sup>2</sup> No effect of vehicle on parameter.

<sup>3</sup> Decreased compared to baseline,  $p < 0.05$ ; no difference between the two doses of caffeine.

<sup>4</sup> No difference from control groups.

<sup>5</sup> Decreased compared to baseline,  $p < 0.01$ ; no difference between the two doses of caffeine.

Zimmerberg *et al.* (1991) found that intragastric administration of 1 mg caffeine/kg or 9 mg caffeine/kg on PN1-6 had significant effects later in life: locomotor activity was decreased in juvenile rats shortly after eye-opening, and there was a deficit in spatial learning of adult treated rats. Although weight gain was decreased PN1-17, adult treated rats weighed the same as control rats of the same sex. Neither of the two developmental measures of behavior (eye-opening and righting reflex) was altered by caffeine treatment.

b) Gullberg *et al.* (1986) exposed Sprague-Dawley rat pups to caffeine for 21 days via dam's milk. Dams received caffeine either 23-30 mg/kg/day via drinking water for three weeks, or 94-135 mg/kg/day for three weeks. Rate of weight gain was not assessed. After approximately four weeks (PN24-30), there was no significant difference between weights of treated and untreated animals of the same sex. Three developmental measurements of behavior were auditory startle reflex, eye-opening, and righting reflex.

There were no dose-related effects and no difference in eye-opening between treated and control groups. Unlike Zimmerberg *et al.*, (1991), Gullberg *et al.* (1986) found that animals in caffeine-treated groups developed the righting reflex and the auditory startle reflex sooner than control animals. However, Gullberg *et al.* (1986) started testing the animals later (PN14 vs PN10) and held them approximately an inch closer to the pad.

When effects of caffeine on locomotor activity in animals exposed to caffeine via dam's milk (Gullberg *et al.*, 1986) were evaluated on PN18, no difference was found between treated and control groups. Whereas, activity was decreased PN13-17 in animals exposed to intragastrically administered caffeine PN1-6 (Zimmerberg *et al.*, 1991). Thus, the behavioral responses were slightly different between the two studies on early postnatal exposure to caffeine, depending on the route (intragastric injection or via dam's milk), duration of caffeine exposure and presence or absence of caffeine during testing (Gullberg *et al.*, 1986; Zimmerberg *et al.*, 1991).

c) Holloway and his collatorators have studied behavioral effects of postnatally administered caffeine. Holloway *et al.*, (1980) have studied the effects of acute and chronic exposure on the behavior of neonatal rats. When 1- and 10-day old rats were exposed to caffeine (0, 5, 20, 40 and 80 mg/kg subcutaneously), spontaneous activity was increased with dose in pups of both ages, as well as latency to attach to the nipple in on-nipple and on-mother suckling tests, while weight gain and attachment frequencies were decreased. In addition, the home orientatation of 10-day old rats was impaired at by high doses of caffeine.

The effects of chronic caffeine administration on pup behavior were similar at the two ages studied. In both 1- and 10-day old rats there was a moderate increase in the spontaneous activity of pups chronically exposed to caffeine (days 1-9). Thus, long term exposure to caffeine during gestation (1-day old pups) or on days 1-9 of lactation (10-day old pups) increased the pups' activity levels and altered the activity increase observed following an acute caffeine challenge.

Caffeine was found to alter the expression of 3 behaviors in the juvenile rat (Holloway and Thor, 1982). After caffeine administration (0, 10 and 40 mg/kg S.C.), locomotor activity was increased in rats of all ages studied (24-84 days old). Pinning, a behavior characteristic of the juvenile period and used as an index of play-fighting, was suppressed following caffeine exposure, but only in rats 24-54 days old. Social investigation was not affected by caffeine in 24- and 34-day old rats. However, in animals 44-84 days of age caffeine increased both the frequency and duration of investigation of a novel juvenile.

Effects of acute and chronic exposure to caffeine on the behavior of juvenile rats were assessed in three experiements. In older animals, 32-34 days and 44-84 days, acute dose of caffeine to naive animals (10 and 40 mg/kg) increased locomotor activity; increased "social investigation" (44-84 days only); but, decreased play-fighting (Holloway and Thor, 1983). When caffeine was administered to 28-32 day old rats for 11 days, a biphasic response in play fighting was observed (Holloway and Thor, 1984). The activity was decreased during days 2-4, but increased during days 9-11.

APPEARS THIS WAY  
ON ORIGINAL

Gestational/Lactational Exposure

a) Sobotka *et al.* (1979) exposed Sprague-Dawley dams to caffeine via drinking water from the seventh gestational day to PN22, at which time pups were weaned. As outlined below, there were three caffeine-treated groups. The concentrations in drinking water as well as average doses administered are indicated below. Animals in the control group (fourth group) received the vehicle, tap water.

Caffeine Concentration (per cent)	Gestation Dose (mg/kg/day)	Lactation Dose (mg/kg/day)
0.0125	23±3	37±4
0.025	49±6	76±7
0.05	92±10	138±11

Maternal body weights were not altered by caffeine at any time during the study.

Caffeine treatment during gestation and lactation had minimal effects on behavior in neonatal animals. Eye-opening was delayed on one day (PN13), but in neither a sex-dependent nor a dose-dependent manner. There were no changes in motility or righting reflex. Significant effects were observed postweaning in juvenile (adolescent) animals. The responses were: increased exploratory behavior, increased number of rearings, and facilitated operant performance. Other parameters of juvenile behavior were not altered: stress responsiveness, acquisition and extinction of avoidance responses.

b) Hughes and Beveridge (1991) also investigated the effects of gestational (26 or 45 mg/kg/day) and lactational (25 or 35 mg/kg/day) exposure (to PN25) to caffeine. They administered two dose levels via drinking (tap) water to Wistar albino females. In an attempt to maintain the same exposure level during both gestation and lactation, the concentration of caffeine during lactation was decreased by half. At the larger dose, dams drank less, and therefore exposure of the high-dose group was less than planned during lactation. The control group received tap water only. Only eye-opening was monitored in neonatal animals.

Five juvenile (1-2 mo old animals) and adult (4 and 6 mos.) behaviors were monitored. One or both of the gestational or lactational doses reduced locomotor activity and increased defecation in the open field at all ages for males only. Rearing was decreased for both sexes by 25 mg/kg/day lactational caffeine. Numbers of rats that failed to or took longer than 1 min to emerge into the brightly lit area were increased by 26 mg/kg/day gestational caffeine.

The results of both studies provide evidence that combined gestational and lactational exposure to caffeine appears to produce changes (not consistent) in first generation juvenile rat behavior when the behavior is evaluated after exposure to caffeine has ceased.

Mechanism of Action for Effects on Behavior

**Brain sites of action.** In a preliminary study, Nakamoto *et al.* (1988) investigated the effects of caffeine on brains of neonate rats. Caffeine was administered orally to neonate rats via dam's milk, PN1-15. Dams were fed 20% protein diets supplemented with caffeine at one of two doses: 10 mg caffeine/kg (n=5); 20 mg caffeine/kg (n=5). The control group of dams (n=3) was fed the 20% protein diet. Six parameters were evaluated, as shown in Table IV-ST-3.

**Table IV-ST-3** Effect of caffeine supplemented to the maternal diet on the brains of newborn rats.<sup>1</sup>

Group (n=10)	Brain weight (g)	DNA (mg/g)	Protein (mg/g)	Cholesterol (mg/g)	Zinc (µg/g)	Alk. phos/protein <sup>2</sup>
Control	1.273± 0.013	2.41± 0.04	95.3± 1.3	9.82± 0.09	10.66± 0.93	88.41± 6.64
10 mg caffeine/kg	1.306± 0.018	2.26± 0.07	75.2± 1.0 <sup>3</sup>	9.19± 0.14 <sup>3</sup>	13.74± 2.22	102.58± 3.14 <sup>3</sup>
20 mg caffeine/kg	1.342± 0.013 <sup>3</sup>	2.25± 0.09	97.5± 1.6	9.72± 0.11	9.14± 0.35	76.46± 2.24

<sup>1</sup>After Nakamoto *et al.*, 1988. Mean±S.D.

<sup>2</sup>Alkaline phosphatase/protein (IU/mg)/1000.

<sup>3</sup>Significantly different from the control group. p<0.05.

As shown in Table IV-ST-3, caffeine only at 10 mg/kg, the amount of protein, cholesterol, zinc and alkaline phosphate was different (low or higher) than either groups and not dose related. There was, however, a trend toward a dose-dependent increase in brain weight.

The same group (Yazdani *et al.*, 1988) investigated further the effects of caffeine on the brain following administration to dams during gestation and lactation (PN22), and to pups from PN23-43, at which time animals were sacrificed. Only male pups were continued after PN22. One dose of caffeine was administered throughout, 10 mg/kg, to dams and to pups.

Caffeine decreased total brain weight, and weights in the following brain areas: cerebellum, medulla oblongata, hypothalamus, striatum, cortex-midbrain, and hippocampus. Total brain DNA and RNA, but not protein, were decreased. All differences were significant, p<0.05. The investigators analyzed selected brain areas for DNA, RNA, protein, and cholesterol content. Significant differences in content were found in several areas, but no consistent pattern was easily discernible.

Nakamoto and his associates (1991) continued their investigation of caffeine effects on rat brain in a more complex experimental design that included assessment of markers for behavior. Brains of female F<sub>1</sub> rats were analyzed in this study, and a larger dose of caffeine was administered, 20 mg/kg/day.

Caffeine had no effect on body weight gain. The different cumulative locomotor activity scores were found in three different subgroups in both control and caffeine-treated groups at PN93: high activity, medium activity, and low activity. During caffeine administration, differences between control and treated animals did not occur with the same pattern in the three subgroups. In the high activity group, differences were noted on PN51, 58, and 93; the medium activity group, PN72-93; and the low activity group showed no difference.

From PN94-388 there was a striking difference between the activity subgroups. In the medium activity group only, activity was decreased consistently in animals that had been, but were no longer exposed to caffeine.

Unlike the findings in younger male animals exposed to caffeine for a shorter period of time (Yazdani *et al.*, 1988), weights of various brain regions showed no difference between control and caffeine-treated groups. Again, there was no consistent pattern of caffeine effect on brain content, whether one compared all caffeine-treated brain areas with all control brain areas, or compared control and caffeine-treated brain area by activity-level subgroup. Thus, one cannot correlate effects of caffeine on behavior with effects of caffeine on content of specific brain components, much less specific components in specific brain areas.

**Adenosine receptor.** As noted in the introduction to the Pharmacology section of this document, antagonism of endogenous adenosine at the adenosine receptor appears to be the mechanism of action for most, but not all, of the pharmacological actions attributed to methylxanthines in general, and to caffeine in particular. There is strong evidence that CNS adenosine receptor antagonism is the mechanism of action underlying the proposed therapeutic action of caffeine, respiratory stimulation. It has been proposed that adenosine receptor antagonism also is involved in the effects of caffeine on behavior.

Guillet and Kellogg (1991a) studied specific binding of adenosine at the adenosine receptor in five distinct brain regions of rats aged 2 weeks to 3 months, after early neonatal exposure to caffeine. All analyses were carried out at times when circulating caffeine was no longer measurable. Caffeine was administered by gavage to Long-Evans pups daily PN2-6, to maintain caffeine levels in serum of 5-15  $\mu\text{g/ml}$ ; 20 mg caffeine/kg on PN2, 15 mg caffeine/kg on PN3-6. Control animals received water by gavage on PN2-6. There was no additional handling of animals until time of sacrifice. Ages at which animals were sacrificed are: PN14, 18, 21, 28, 35, 42, 60, and 90 days. Brain areas studied were: cortex, cerebellum, hippocampus, brain stem, and hypothalamus.

Body weight ( $p < 0.0001$ ) and regional brain weights ( $p < 0.01$ ) increased with age; but, there was no difference between control and treated animals of a given age. After neonatal exposure to caffeine, there was a significant increase in specific binding in cortex ( $p = 0.029$ ), cerebellum ( $p = 0.015$ ), and hippocampus ( $p = 0.025$ ). Specific binding in brain stem and hypothalamus was not altered by caffeine.

In a subsequent study using the same protocol for caffeine administration, Guillet and Kellogg (1991b) attempted to correlate neurochemical and behavioral activities of caffeine. Effects of acute doses of caffeine [15, 30, or 60 mg/kg, intraperitoneally (i.p.)] on locomotor activity were assessed at 12, 15, 18, or 28 days of age. Specific adenosine binding in three brain areas was determined (cortex, cerebellum, and hippocampus) on tissues from 14-, 18-, 21-, and 28-day old rats. D-phenylisopropyladenosine (D-PIA), an adenosine receptor agonist, also was administered acutely to some animals.

Baseline locomotor activity varied as a function of age, but not neonatal caffeine exposure. Responses to acutely administered caffeine were age-, treatment-, and dose-related as shown in the following table.

Age of animals	Dose (i.p.)	Observations
12-day	60 mg/kg 15 and 30 mg/kg.	Decreased activity, from baseline, in control and caffeine- treated animals No effect
15-day	30 and 60 mg/kg. 15 mg/kg.	Decreased activity in both groups Inactive
18-day	15 and 30 mg/kg 60 mg/kg	Increased activity in control animals, but had no effect caffeine-treated animals No effect on activity in either group
28-day	15 and 30 mg/kg 60 mg/kg	Increased activity in control and caffeine-treated animals No effect in control animals and decreased in caffeine-treated animals

At 15 days, caffeine-treated animals showed increased sensitivity to depressant activity of the adenosine receptor agonist D-PIA. Results of the binding studies indicated that changes in the adenosine receptor occur as a function of age in different brain regions. This development process can be influenced by neonatal caffeine exposure.

In spite of the apparent correlation of certain behavioral and neurochemical findings, the investigators caution against making simplistic conclusions from these observations. Rather than a one-to-one correspondence between neurochemical and behavioral changes, the authors interpret the data to suggest that the entire adenosine receptor system must be intact for normal, age-appropriate function. Guillet and Kellogg (1991b) note that early developmental exposure to caffeine over a limited period of time appears to affect normal adenosine receptor development, and such activity can be demonstrated on both a neurochemical level and a behavioral level long after the exposure period.

#### 4.2. Cardiovascular System

Johansson (1981) reported on cardiovascular lesions in Sprague-Dawley rats induced by long-term treatment with caffeine. The main objective of the study was to investigate the effects of long-term administration (117 weeks) of phenacetin, phenazone, and caffeine singly and in various combinations. The study was designed as a carcinogenicity study, but during analysis it became apparent that cardiovascular lesions occurred disproportionately in animals that received caffeine.

All test agents were administered in the diet. There were seven groups of 30 males each. The doses of each test agent were: caffeine, 0.102% (approximately 50 mg/kg); phenacetin, 0.535%; and phenazone, 0.535%. The seven groups were: 1-phenacetin; 2-phenazone; 3-caffeine; 4-all three agents; 5-phenacetin and phenazone; 6-phenacetin and caffeine; 7-control. The findings are summarized in Table IV-ST-6.

Mean survival was decreased in the group treated with caffeine alone, 78 weeks vs 94 weeks for the control group. Cardiac insufficiency was the primary cause of death in animals treated with caffeine alone (Group 3), and those treated with caffeine and phenacetin (Group 6). Rats with cardiac insufficiency exhibited enlarged dilated hearts and signs of acute and chronic congestion of lungs, liver, and spleen. There was histological evidence of acute myocardial infarction in 4 rats treated with caffeine only, and in 4 others the extent of scarring was consistent with old myocardial infarction. Prominent abdominal vascular changes (mesenteric arteries and arteries near the pancreas) were detected in 12 animals treated with caffeine only, and 12 treated with caffeine and phenacetin.

Table IV-ST-6. Mortality and cardiovascular changes following long-term administration of caffeine, phenacetin, and/or phenazone to male Sprague-Dawley rats.<sup>1</sup>

	Control n=29	Group 1 n=29 ph'acetin	Group 2 n=29 ph'zone	Group 3 n=28 caffeine	Group 4 n=30 all three	Group 5 n=29 p'tin/p'zn	Group 6 n=29 caf/p'tin
Mean survival (weeks)	94	101	92	78 <sup>2</sup>	101	104	92
Cardiac insuf death (%)	17.2	13.8	24.0	64.3 <sup>3</sup>	12.3	14.0	58.6 <sup>3</sup>
Myocard. fibrosis <sup>4</sup>							
mild	13	12	11	3	14	9	10
moderate	4	3	4	9	4	5	9
severe	1	2	2	11	1	1	3

<sup>1</sup> From Johansson, 1981.

<sup>2</sup> Significantly different from all other groups,  $p < 0.05$ .

<sup>3</sup> Significantly different from control group,  $p < 0.05$ .

<sup>4</sup> Frequency of myocardial fibrosis.

Johansson (1981) concluded that long-term administration of caffeine to Sprague-Dawley rats is associated with markedly reduced life-span due to cardiovascular toxicity.

Temples *et al.* (1985, 1987) carried out a series of studies where caffeine 10 mg/kg/day, was administered in diet to dams and to pups on various schedules. Following sacrifice, cardiac function was assessed using the isolated perfused hearts of the young rats. The result (Table IV-ST-7) shows that caffeine had no deleterious effects on cardiac function when exposure was limited to gestation and lactation periods.

Table IV-ST-7 Effects of caffeine exposure during gestation and lactation on cardiac function in young male rats.

Caffeine-treatment Period (10 mg/kg/day)	Dry Heart Weight	Cardiac Output	Peak Systolic Pressure	Myocardial Work	Coronary Flow
Gestation & Lactation (PN50 hearts studied) <sup>1</sup>	no difference <sup>2</sup>	no difference	increased slightly	increased slightly	increased slightly
Gestation, lactation, & pups to PN50 <sup>1</sup>	no difference	decreased	decreased	decreased	decreased
Gestation, lactation, & pups to PN88 <sup>3</sup>	increased	decreased	decreased	decreased	no difference

<sup>1</sup> From Temples, *et al.*, 1987; N=5 or 6/group

<sup>2</sup> Compared to respective control group

<sup>3</sup> Temples, *et al.*, 1985; N=8/group

APPEARS THIS WAY  
ON ORIGINAL

### 4.3. Gastrointestinal Tract

Grosfeld JL, Dalsing MC, Hull M and Weber TR: Neonatal apnea, xanthines, and necrotizing enterocolitis. *J Pediatric Surgery*. Vol.18, No.1 February, 1983

#### Methods

To study the relationship of xanthine treatment of premature apnea and NEC in a bowel ischemia model, the superior mesenteric artery was occluded for one minute in 82 weanling rats. Group I (n=41) were untreated controls (normal saline I.P.) and Group II (n=21) received aminophylline 40 mg/kg I.P. at 4 hours and immediately prior to clamping. Animals were evaluated for bowel infarction, perforation, and mortality at 7 days. In 20 additional rats (10/group) bowel was evaluated by scanning electron microscopy (EM) at timed intervals (5 and 30 minutes) after unclamping following reperfusion.

#### Results

Mortality occurred 25/41 (60%) in controls and 19/21 (90.5%) in aminophylline treated animals. Bowel necrosis occurred more commonly in aminophylline treated rats 15/21 (71.4%) compared to 18/41 (43%) in control animals. Bowel perforation occurred in 7/41 (17%) in control group and 4/19 (21%) in the aminophylline treated group as shown in the following table.

Observations	Controls	Aminophylline 40 mg/kg, I.P.
Mortality*	25/41 (60%)	19/21 (90.5%)
Bowel Necrosis	18/41 (43%)	15/21 (71.4%)
Bowel Perforation	7/41 (17%)	4/19 (21%)

\* Aminophylline treated animals expired within the first two days of observation, while control animals lived longer ( $3.56 \pm 2.28$  days).

#### Histologic evaluation of bowel sections by EM

	Observations	
	Control animals (n=10)	Aminophylline treated animals (n=10)
<b>5 minutes post reperfusion</b>	Mucosal damage (loss of micro villi and mucosal ulceration)	Significant bacterial overgrowth on the mucosa (rod shaped white objects)
<b>30 minutes post reperfusion</b>	More ulceration and mucosal loss	Overgrowth of bacteria which extend into the mucosal cell surface; mucosal damage was worse

### Summary

Ischemic bowel occurred in 25 of 41 (60%) controls of which 18 (43%) showed necrosis and 7 (17%) with perforations. Rats treated with aminophylline had 19 of 21 (90%) ischemic bowel of which 15 (70%) had necrosis and 4 (19%) with perforations. Mortality was 60% (controls) and 90% (aminophylline) respectively ( $p < 0.05$ ).

On EM, aminophylline enhanced bacterial overgrowth; however, actual mucosal damage appeared similar. Following ischemia, aminophylline has an adverse effect on the bowel. Use of aminophylline in prematures may be at risk for NEC.

### Discussion on NEC

There have been several reports of NEC following xanthine treatment in apneic infants, and the association of methylxanthine treatment for the apnea and the necrotizing enterocolitis (NEC) has been suggested by several authors.

Aminophylline is a mild gastric irritant when administered either orally or intraperitoneally and was often implicated for the onset of NEC. This study suggests that the aminophylline has an adverse effect on both length of survival and overall survival in animals challenged with an ischemic bowel insult. Apneic episodes may result in hypoxemia which could potentially cause a transient ischemic bowel insult and subsequent necrotizing enterocolitis.

Several possible etiologic causes of NEC were discussed by the author. Aminophylline enhances bowel injury following a short term ischemic insult. This may be related to decreased gastrointestinal motility resulting in bacterial proliferation especially at a potentially ischemic site and increases the risk of bowel necrosis. Additional observations by others, suggest that release of toxic free radical anions leading to cellular destruction may be influenced by aminophylline acting as a substrate for xanthine oxidase production. The author concludes that these data raise some concern and suggest avoidance of methylxanthine treatment in infants with apneic episodes who are at high risk for NEC.

When caffeine dissolved in water (Boyd *et al.*, 1965) was administered to female rats for 100 days, there were increased organ weights correlated with mucosal hypertrophy and increased water content in the gut. There were small ulcers extending about halfway down the glands of pyloric stomach at daily doses of 110 mg/kg and above in this study, but no necrosis was observed. Caffeine being one of the methylxanthines are irritating to the gut like theophylline. However, the acute toxicity of caffeine seems to be slightly (30%) less (Warszawski *et al.*, 1978) than theophylline ( $LD_{50}$ : 220 vs 165 mg/kg in 2-day old rat), and the amount of caffeine being converted to theophylline by N-7 demethylation may be low since the isoenzyme required for N-7 demethylation is less active in neonates than adults and isoenzymes required for N-1 and N-3 demethylation are not active in neonates (Berthou *et al.*, 1988). Therefore, caffeine should be used with caution in apneic neonates, since caffeine has the potential to cause bowel injury as theophylline following a ischemic insult.

#### 4.4. Other system

##### Bone

Effects of caffeine on bone have been studied *in vivo* and *in vitro*. Batirbaygil *et al.* (1985) administered 10 mg caffeine/kg by gavage to 3-, 5-, 7-, 9-, 11-, and 13-day old rat pups. Animals were sacrificed on PN15. Caffeine had no effect on growth rate during this period, nor was there a difference in mandible or long-bone weights. With respect to components of bone, caffeine did not alter total DNA, total protein, or total calcium content. Bergman *et al.*, (1988) studied release of calcium from mouse calvaria *in vitro*. Caffeine had no effect on calcium release in concentrations from  $2.6 \times 10^{-5}$  M to  $2.6 \times 10^{-3}$  M.

##### Teeth

Nakamoto and his colleagues investigated the effects of caffeine administered during lactation on molar development in rats. Plasma concentrations of caffeine in the pups were  $0.21 \pm 0.04$   $\mu\text{g/ml}$  following daily exposure of dams to 20 mg caffeine/kg during lactation (Hashimoto *et al.*, 1992). This exposure resulted in an alteration in enamel content of the first, but not the second molar of PN22 pups. A subsequent study (Nakamoto *et al.*, 1993) showed that the altered first molar developed caries more easily, compared to control rats, when animals were fed a cariogenic diet PN22-50.

The summary table of selected special toxicity study is provided.

APPEARS THIS WAY  
ON ORIGINAL

**Summary Table: Results of Selected Toxicology Studies****Special Toxicity**

Species (Strain) Duration	No. of animals/ group	Route of Admin. (vehicle)	Doses	Observations	Reference
Rat (Sprague Dawley) 117 weeks <sup>a</sup>	30 M	Oral-diet (pellets)  Caffeine 0, 0.102%	(50 mg/kg) <sup>b</sup>	In the caffeine-treated animals, mean survival time was less (78 weeks vs 94 weeks in controls); the primary cause of death being cardiac insufficiency. At necropsy, the hearts were enlarged with signs of chronic congestion of the lungs, liver, & spleen; histologically evidence of acute myocardial infarctions in four rats and scarring that is consistent with old infarctions in four other rats. Prominent abdominal vascular changes (periarteritis nodosa-like lesions in the mesenteric vessels) were detected in 12 animals (40%) treated with caffeine and in 12 animals treated with caffeine and phenacetin.	Johannson (1981)

<sup>a</sup> This study was designed as a carcinogenicity study but only chronic effects on the cardiovascular system were reported.

<sup>b</sup> Other groups received caffeine + phenacetin and phenacetin or phenazone alone or in combination.

APPEARS THIS WAY  
ON ORIGINAL

#### 4.5. List of References

- Batirbaygil Y, Quinby GE Jr, Nakamoto T. Effects of oral caffeine intubation on bones in protein-energy malnourished newborn rats. *Biol Neonate* 1985; 48:29-35.
- Bergman EA, Newbrey JW, Massey LK. Caffeine does not cause *in vitro* calcium loss from neonatal mouse calvaria. *Calcif Tissue Int* 1988; 43:281-83.
- Grosfeld JL, Dalsing MC, Hull M and Weber TR. Neonatal apnea, xanthines, and necrotizing enterocolitis. *J Pediatric Surgery*. Vol.18, No.1 February, 1983
- Guillet R, Kellogg C. Neonatal exposure to therapeutic caffeine alters the ontogeny of adenosine A1 receptors in brain of rats. *Neuropharmacology* 1991a; 30:489-96.
- Guillet R, Kellogg C. Neonatal caffeine exposure alters developmental sensitivity to adenosine receptor ligands. *Pharmacol Biochem Behav* 1991b; 40:811-17.
- Gullberg EI, Ferrell F, Christensen HD. Effects of postnatal caffeine exposure through dam's milk upon weanling rats. *Pharmacol Biochem Behav* 1986; 24:1695-1701.
- Hashimoto K, Joseph F Jr, Falster AU, Simmons WB, Nakamoto T. Effects of maternal caffeine intake during lactation on molar enamel surfaces in new-born rats. *Arch Oral Biol* 1992; 37:105-9.
- Holloway WR Jr. Caffeine: Effects of acute and chronic exposure on the behavior of neonatal rats. *Neurobehav Toxicol Teratol* 1982; 4:21-32.
- Holloway WR Jr, Thor DH. Caffeine: Effects on the behaviors of juvenile rats. *Neurobehav Toxicol Teratol* 1983; 5:127-134.
- Holloway WR Jr, Thor DH. Acute and chronic caffeine exposure effects on play fighting in the juvenile rat. *Neurobehav Toxicol Teratol* 1984; 6:85-91.
- Hughes RN, Beveridge IJ. Behavioral effects of exposure to caffeine during gestation, lactation or both. *Neurotoxicology Teratol* 1991; 13:641-7.
- Johansson S. Cardiovascular lesions in Sprague-Dawley rats induced by long-term treatment with caffeine. *Acta Pathol Microbiol Scand* 1981; Sect. A 89:185-91.
- Nakamoto T, Cheuk SL, Yoshino S, Falster AU, Simmons WB. Cariogenic effect of caffeine intake during lactation on first molars of newborn rats. *Arch Oral Biol* 1993; 38:919-22.
- Nakamoto T, Joseph F Jr, Yazdani M, Hartman AD. Effects of different levels of caffeine supplemented to the maternal diet on the brains of newborn rats and their dams. *Toxicol Lett* 1988; 44:167-75.
- Nakamoto T, Roy G, Gotschalk SB, Yazdani M, Rossowska M. Lasting effects of early chronic caffeine feeding on rats' behavior and brain in later life. *Physiol Behav* 1991; 49:721-27.

Seale TW, Johnson P, Carney JM, Rennert OM. Interstrain variation in acute toxic response to caffeine among inbred mice. *Pharmacol Biochem Behav* 1984; 20:567-73.

Sobotka TJ, Spaid SL, Brodie RE. Neurobehavioral teratology of caffeine exposure in rats. *Neurotoxicology* 1979; 1:403-16.

Temples TE, Geoffray DJ, Nakamoto T, Hartman AD, Miller HI. Effects of chronic caffeine ingestion on growth and myocardial function. *Proc Soc Exp Biol Med* 1985; 388-95.

Temples TE, Geoffray DJ, Nakamoto T, Hartman AD, Miller HI. Effect of chronic caffeine intake on myocardial function during early growth. *Pediatr Res* 1987; 21:391-5.

Yazdani, M, Hartman AD, Miller HI, Temples TE, Nakamoto T. Chronic caffeine intake alters the composition of various parts of the brain in young growing rats. *Dev Pharmacol Ther* 1988; 11:102-8.

Zimmerberg B, Carr KL, Scott A, Lee HH, Weider JM. The effects of postnatal caffeine exposure on growth, activity and learning in rats. *Pharmacol Biochem Behav* 1991; 39:883-8.

APPEARS THIS WAY  
ON ORIGINAL

## 5. Carcinogenicity Studies

Two repeat-dose carcinogenicity studies will be presented from the literature. In the study by Macklin and Szot (1980), caffeine was administered orally in the feed for 18 months to C57BL/6 mice. Caffeine was one of the three agents (aspirin, phenacetin and caffeine) studied, and only one dose-level of caffeine (55 mg/kg/day) was evaluated.

Mohr, *et al.* (1984) studied four dose-levels of caffeine, administered in drinking water to Sprague-Dawley rats for 24 months. The mean overall treatment levels for males were 15, 26, 49, and 102 mg caffeine/kg/day. Corresponding levels for females were 15, 37, 80, and 170 mg caffeine/kg/day.

In another study by Johansson (1981a), there was no increase in the incidence of tumors in rats fed a diet containing 0.102% caffeine (21.4g caffeine/rat) for an average of 78 weeks.

### 5.1. Mice

Macklin AW, Szot RJ. Eighteen month oral study of aspirin, phenacetin and caffeine in C57BL/6 mice, *Drug Chem Toxicol* 1980; 3:135-63.<sup>1</sup>

*Study Objective.* The study was undertaken to evaluate the potential carcinogenic and nephrotoxic effects of maximum tolerated doses of aspirin, phenacetin and caffeine alone or in combination.<sup>2</sup>

#### Study Description

*Test agent.* Caffeine was purchased from [redacted] Drug-diet mixes were prepared fresh weekly and stored at  $41^{\circ} \pm 5^{\circ}\text{F}$ . The mixes had been found to be stable over the 7-day period of use. Concentration was adjusted weekly so that the appropriate dose level would be provided.

*Animals.* A total of 360 male and 360 female 4-week old C57BL/6 mice were randomized to nine groups. The two groups under discussion here are indicated in Table V-C-7. Animals were housed in suspended stainless steel mesh bottomed cages in a temperature ( $72^{\circ} \pm 2^{\circ}\text{F}$ ), humidity ( $50\% \pm 10\%$ ) and photo-period controlled (12 hours on/off) room with 12-15 air changes per hour. Feed and tap water were supplied *ad libitum*.

<sup>1</sup> Phenacetin was the primary focus of this investigation. Most of the data are not important for the focus of this NDA for Caffeine Citrate. The paper was chosen because it provides data on long-term oral caffeine administration in a second species (besides the rat). Only data for caffeine and control groups will be presented.

<sup>2</sup> Although this is the stated objective, the maximum tolerated dose was determined only for phenacetin. The doses of aspirin and caffeine were chosen based on their content in APC formulated for OTC use.

**Table IV-C-1.** Treatment groups; C57BL/6 mice given 55 mg caffeine/kg/day for up to 18 months<sup>1</sup>

Group Number <sup>2</sup>	Treatment	Animals/Group	
		male	female
9	Control	40	40
8	55 mg caffeine/kg/day	40	40

<sup>1</sup> From Macklin and Szot, 1980

<sup>2</sup> Group number in the study.

*Treatment.* Although there were nine treatment groups, only two groups are described here, control and caffeine, 55 mg/kg/day.<sup>3</sup> These groups are listed in Table IV-C-1. As shown in Table IV-C-2, 10 males and 10 females were sacrificed during the course of the study for interim analyses. Thirty animals/sex/group were treated for 75-80 weeks.

**Table IV-C-2.** Exposure subgroups within each group; C57BL/6 mice given 55 mg caffeine/kg/day for up to 18 months<sup>1</sup>

Exposure Time	Animals/Group	
	male	female
6 weeks	2	2
18 weeks	2	2
33 weeks	2	2
45 weeks	2	2
58 weeks	2	2
75-80 weeks	30	30

<sup>1</sup> From Macklin and Szot, 1980

<sup>3</sup> The other groups were: aspirin/phenacetin/caffeine (696 mg/kg/day); aspirin/phenacetin (639 mg/kg/day); phenacetin/caffeine (321 mg/kg/day); aspirin/caffeine (429 mg/kg/day); phenacetin (75 mg/kg/day); phenacetin (268 mg/kg/day); aspirin (382 mg/kg/day)

*Observations and parameters evaluated.* General appearance, behavior and activity of the mice were noted daily. Group body weights and feed consumption were determined and recorded weekly. Blood samples were collected by orbital puncture for hemoglobin, methemoglobin, and sulfhemoglobin determinations, and for serum urea nitrogen, creatinine, and lactic dehydrogenase determinations. Pooled urine samples were collected from 2-4 animals. Specific gravity, pH, and the presence or absence of protein, glucose, ketone, and blood were determined.

Complete gross external and internal examinations were conducted. Liver, kidneys, spleen, heart, brain, and urinary bladder or representative sections thereof were fixed in 10% neutral buffered formalin. Additional tissues were fixed when they appeared abnormal. Only sections of bladder and kidney were routinely processed, stained with hematoxylin and eosin, and examined by light microscopy.

**Table IV-C-3** Schedule for observations (A) and obtaining samples (B); C57BL/6 mice given 55 mg caffeine/kg/day for up to 18 months<sup>1</sup>

A. Observation	Daily	Weekly				
Cage check	X					
Feed consumption		X				
Body weight recorded		X				
B. Sample	Weeks 5-6	Weeks 17-18	Weeks 33-34	Week 46	Week 57	Week 76-77
Orbital blood sample N=4 N=8		X		X	X	X
Orbital serum sample N=4 N=8		X		X		X
Pooled urine sample N=4 N=8	X	X	X	X		X

<sup>1</sup>From Macklin and Szot, 1980

*Statistical methods.* Student's t-test, Fisher's Exact test.

### Results

*Mortality.* There was no difference between the control group and the caffeine-treated group with respect to survival. The data are confounded by the fact that "a watering system failure occurred during the 68th week of study in the rack of cages [housing these two and aspirin groups of animals]." As shown in Table IV-C-4, there were several deaths attributed to dehydration due to the failure in watering system compounded by caffeine administration which has the diuretic effects.

Table IV-C-4. Mortality; C57BL/6 mice given analgesics in the Diet for up to 18 months<sup>1</sup>.

Group No.	Treatment Group	Group Size		Dehydration Deaths*		Other Deaths <sup>2</sup>	
		male	female	male	female	male	female
9	Control	40	40	6	0	7	4
8	55 mg caffeine/kg/day	40	40	2	12	2	3
7	aspirin (382)	40	40	0	7	13	2
6	phenacetin (268)	40	40	-	-	1	4
5	phenacetin (754)	40	40	-	-	7	2
4	aspirin/caffeine	40	40	-	-	6	6
3	phenacetin/caffeine	40	40	-	-	2	8
2	aspirin/phenacetin	40	40	-	-	1	1
1	aspirin/phenacetin/caffeine	40	40	-	-	2	16

<sup>1</sup> From Macklin and Szot, 1980- \* In groups (7, 8, and 9), a watering system failure occurred.

<sup>2</sup> "Includes mice sacrificed in poor condition. Deaths were scattered during the dosing period and were related to fighting and entanglement in the cage opening provided for the automatic watering tube."

*Body weight gain.* There was no difference in body weight gain between control and caffeine-treated animals.

*Clinical/laboratory findings.* There were no remarkable clinical or laboratory findings in either the control group or the caffeine-treated group.

*Tumor incidence.* There were no remarkable gross pathologic findings.

Only three tissues were examined microscopically: liver, kidney, urinary bladder. All tissues were negative for tumors in the caffeine-treated group as well as the control group.

Conclusion

Oral administration of 55 mg caffeine/kg/day in the diet to C57BL/6 mice, for up to 80 weeks, did not modify survival, body weight gain, or any of the clinical and laboratory parameters monitored. Gross autopsy findings were unremarkable. Liver, kidney, and urinary bladder were the only tissues examined microscopically. No evidence of either benign or malignant tumors was found in either caffeine-treated or control animals. (NOTE: Identification of phenacetin toxicity was the primary objective of this study. Thus, histologic examination was limited to liver, kidney, and urinary bladder, which are known sites of phenacetin toxicity.)

## 5.2. Rats

Mohr U, Althoff J, Ketkar MB, Conrād P, Morgareidge K. *The influence of caffeine on tumor incidence in Sprague-Dawley rats, Food Chem Toxicol 1984; 32:377-82.*

**Study Objective.** The study was undertaken to provide further information on the carcinogenic potential of caffeine when administered in the drinking water to rats.

### Study Description

**Test agent.** The test agent was food-grade natural caffeine. It contained less than 0.01% theobromine, and no other xanthine alkaloids were detectable by high-pressure liquid chromatography. A sample was reanalyzed at the conclusion of the study; it had retained fully its identity and purity.

**Animals.** There were six (6) treatment groups, as summarized in Table IV-C-5. Groups 1 and 2 served as controls and received plain water *ad libitum*. Caffeine was administered orally to Groups 3-6 via drinking water, which was available *ad libitum*. As shown in Table IV-C-5, the concentrations ranged from 200 mg caffeine/liter to 2000 mg caffeine/liter. The gender-related difference in exposure is due to the fact that the females drank about 1.6 times more fluid in proportion to body weight than did males.

**Table IV-C-5.** Treatment groups; Sprague-Dawley rats given 0-2000 mg caffeine/liter drinking water for up to two (2) years<sup>1</sup>

Group Number	Treatment mg caffeine/l	Animals/Group		Caffeine Mean Overall Dose-Level mg/kg/day	
		Male	Female	Male	Female
1	Plain water	80	80	0	0
2	Plain water	80	80	0	0
3	200	80	80	15	15
4	430	80	80	26	37
5	930	80	80	49	80
6	2000	80	80	102	170

<sup>1</sup> From Mohr *et al.*, 1984.

As shown in Table IV-C-6 on the next page, 30 males and 30 females were pre-designated for interim-sacrifice. Fifty animals/sex/group were treated for 24 months.

**Table IV-C-6.** Exposure subgroups within each group; Sprague-Dawley rats given 0-2000 mg caffeine/liter drinking water for up to two (2) years<sup>1</sup>

Exposure Time	Animals/Group	
	Male	Female
3 months	10	10
6 months	10	10
12 months	10	10
24 months	50	50

<sup>1</sup> From Mohr *et al.*, 1984.

*Observations and parameters evaluated.*

**Table IV-C-7** Schedule for observations; Sprague-Dawley rats given 0-2000 mg caffeine/liter drinking water for up to two (2) years<sup>1</sup>

Observation	Frequency
Cage checks	Twice/day <sup>2</sup>
Feed consumption measured	Twice/week <sup>3</sup>
Fluid consumpt'n measured	Twice/week <sup>3</sup>
Clinical examination	Weekly
Body weights recorded	Weekly

<sup>1</sup> From Mohr *et al.*, 1984.

<sup>2</sup> Seven days/week.

<sup>3</sup> Measured as weight differences on a per cage basis.

Blood samples were collected from all animals killed, except when animals were found moribund. Standard hematological and biochemical parameters were measured. Urinalysis was not carried out. Complete necropsy was performed on all rats found dead or moribund, and on animals sacrificed during or at the end of the study period. Tissues and organs were fixed in 10% neutral buffered formalin and processed for routine histology.

Results

**Mortality.** Cumulative mortality in treated groups was not statistically different from that in the control groups. Survival times are summarized in Table IV-C-8.

**Body weight gain.** Body weight gain of animals in Groups 5 and 6 was significantly less than that in control groups in both males and females. Terminal body weights of treated animals were 11% lower than concurrent controls in Group 5 and by approximately 25% in Group 6.

There was no difference between the sexes. Although food consumption was somewhat lower at the highest caffeine dose, there was no effect on the efficiency of food utilization.

*Clinical/laboratory findings.* There were no differences between control and treated animals with respect to clinical findings and no treatment-related trends in either hematology or clinical chemistry, as monitored with interim sacrifices.

**Table IV-C-8.** Survival time, tumor incidence, and tumor latency and multiplicity; Sprague-Dawley rats given 0-2000 mg caffeine/liter drinking water for up to two (2) years<sup>1,2</sup>

Group Number	Caffeine Dose		Group Size	Survival Time (week)	Tumor-bearing Animals		Tumor Latency (week)	Tumor Multiplicity
	(mg/l)	(mg/kg/d)			number	(%)		
MALES								
1	0	0	50	97.5± 15.5	37	74	97.7± 15.2	1.41±0.60
2	0	0	50	102.1± 5.5	32	64	103.1± 4.3	1.56±0.76
3	200	15	50	100.2± 10.7	35	70	100.0± 10.8	1.37±0.65
4	430	26	50	96.3± 17.6	29	58	99.8± 12.3	1.24±0.51
5	930	49	50	99.5± 12.6	27	54	99.2± 12.8	1.48±0.80
6	2000	102	50	96.2± 14.5	22	44	98.8± 11.6	1.05±0.21
FEMALES								
1	0	0	50	97.8± 10.3	46	92	97.4± 10.5	1.46±0.78
2	0	0	50	95.0± 19.2	38	76	92.7± 20.9	1.63±0.82
3	200	15	50	91.2± 22.4	40	80	92.3± 17.9	1.68±0.97
4	430	37	50	93.6± 21.2	40	80	93.0± 18.8	1.60±0.74
5	930	80	50	92.7± 22.0	36	72	92.9± 18.4	1.61±0.80
6	2000	170	50	91.6± 21.5	31	61	93.3± 15.9	1.23±0.50

<sup>1</sup> From Mohr *et al.*, 1984; p.379

<sup>2</sup> Values for survival time, tumor latency, and tumor multiplicity are expressed as mean±SD.

*Tumor incidence.* The data summarizing tumor incidence are presented in Tables IV-C-8 and IV-C-9. There was no dose-response relationship for tumor incidence; rather the number of benign tumors in the highest-dose group was significantly decreased in both sexes compared with controls.

At the same time, the highest incidence of malignant tumors occurred in the two lower-dose groups (Group 3 and 4). High numbers of neoplasms (more than 10%) occurred in endocrine organs, namely in the pituitary and thyroid glands of both sexes, leydig cell tumors in males and in the mammary glands of female rats including control animals.

**Table IV-C-9.** Distribution of tumors originating in multiple organs, and of benign and malignant tumors; Sprague-Dawley rats given 0-2000 mg caffeine/liter drinking water for up to two (2) years<sup>1,2</sup>

Group Number	Caffeine Dose		Number of Rats with Tumors					Number of Tumors		
	(mg/liter)	(mg/kg/day)	number of tumors					benign	malignant	all
			1	2	3	4	5			
MALE										
1	0	0	24	11	2	0	0	33	19	52
2	0	0	18	11	2	1	0	34	16	50
3	200	15	25	7	3	0	0	24	24	48
4	430	26	23	5	1	0	0	18	18	36
5	930	49	18	6	2	1	0	27	13	40
6	2000	102	21	1	0	0	0	12*	11	23**
FEMALE										
1	0	0	32	8	5	1	0	55	12	67
2	0	0	21	11	5	1	0	45	17	62
3	200	15	23	10	5	1	1	43	24*	67
4	430	37	21	15	3	1	0	41	23*	64
5	930	80	20	11	4	1	0	40	18	58
6	2000	170	25	5	1	0	0	26*	12	38

<sup>1</sup> From Mohr *et al.*, 1984; p.380

<sup>2</sup> Numbers marked with asterisks are significantly different from control numbers (\*P < 0.05, \*\*P < 0.01; Mann-Whitney U-test).

As shown in Table IV-C-10, there were no statistically significant differences between control and treated animals for tumors of any type except for mammary fibroadenomas, the incidence of which was 50% in the controls compared with 26% in the highest-dose group. The incidence of mammary fibroadenomas showed a significant inverse dose-response relationship (chi-square test:  $p < 0.001$ ). The frequency of C-cell (medullary) carcinomas of the thyroid was about 20% in both sexes and was evenly distributed across all treatment groups. Histological examinations of tissues from animals sacrificed at 3, 6, and 12 months were unremarkable.

**Comment:** The fact that the highest incidence of malignant tumors occurred in the two lower-dose groups (Group 3 and 4) and the benign tumor incidence showed the inverse dose-response relationship suggests that the reduction of terminal body weights and lower incidence of tumor findings in the two highest-dose groups may be the result of restricted diet because of poor

palatability, contrary to what the author said. Then the increased incidence in the low and mid dose groups of adenocarcinomas in the thyroid and mammary gland and liposarcoma in the mesentary could be significant findings, and it may explain the absence of dose-response relationship.

**Table IV-C-10.** Sites where the number of malignant tumors in Groups 3 and 4 females exceeded the number of malignant tumors in Groups 1 and 2 females; Sprague-Dawley rats given 0-2000 mg caffeine/liter drinking water for up to two (2) years<sup>1</sup>

Site	Description	mg/liter mg/kg/day	Number of Rats with Tumor <sup>2</sup> caffeine-dose				
			0	0	200	430	930
Thyroid	C-cell carcinoma	7	12	10	12	8	8
	Adenocarcinoma	0	0	2	2	0	0
Liver	Cholangiocellular adenoma <sup>3</sup>	1	0	2	0	1	1
	Cholangiocellular carcinoma	1	0	0	2	0	0
Mesentary	Liposarcoma	0	0	1	1	0	0
Mammary gland	Fibroadenoma <sup>3</sup>	27	26	24	20	20	13
	Adenocarcinoma	0	1	4	3	4	1

<sup>1</sup> After Mohr *et al.*, 1984; p.381

<sup>2</sup> Fifty (50) rats examined/group; does not include interim sacrifice.

<sup>3</sup> Included for purpose of comparison.

### Conclusion

The author stated that MTD dose of caffeine in drinking water when given for up to two years in Sprague-Dawley rats was considered to be 26 mg/kg/day for males and 37 mg/kg/day for females based on body weight changes. Levels of two high doses led to dose-dependent reductions of 11 and 25%, respectively, in terminal body weights, associated with reduced food and fluid consumption. The effects on weight gain was considered to be partially to poor palatability and less to toxic effects by the author, since longevity of the animals and their efficiency of food utilization was not affected.

In this study, tumors in treated animals reaching a frequency of 10% or more were pituitary adenomas, thyroid C-cell carcinomas, testicular Leydig-cell tumors and mammary fibroadenomas. Incidences were similar to those observed in historical controls (Ketkar, Althoff & Mohr, 1982). Although mammary glands and the thyroid in females appear to be the sites of potential carcinogenic activity, no dose-response relationship was present for either neoplasm.

Mohr *et al.* (1984) concluded that there was no statistically significant increase in tumor incidence in treated animals as compared to controls even at doses exceeding the maximum tolerated dose and given to animals over a major portion of their life span.

**Summary Table of Carcinogenicity Studies**

**Carcinogenicity**

Species and Strain	Study Group (number, age, weight, sex)	Route and For- mulation	Dose Level	Frequency and Duration	Parameters Assessed and Results	Comments	Reference
Rats, Sprague-Dawley	Six study groups n=50/sex/group <sup>4</sup> 28 days old; specified pathogen free; no weights given  1. Control 2. Control 3. Caffeine 4. Caffeine 5. Caffeine 6. Caffeine	Oral; drinking water	(mg/kg/day) female male	Daily, <i>ad libitum</i> ; 24 months	<u>Mortality</u> : No difference between control and treated groups. <u>Body weight gain</u> : decreased in dose-dependent manner--Group 5, -11%; Group 6, -25%. <u>Tumor incidence</u> : isolated decreases/increases, treated vs controls; no consistent statistically significant trend; <i>no evidence for enhancement or induction of neoplasia</i> . <u>Clinical/laboratory findings</u> : no differences between control and treated groups for any of standard parameters assessed.	Serum concentration of caffeine was not assessed.	Mohr <i>et al.</i> (1984)
			0 0				
			0 0				
			15 15				
			26 37				
			49 80				
			170 102				

APPEARS THIS WAY  
ON ORIGINAL

<sup>4</sup> Main study; an additional 30 animals/sex/group were sacrificed at intervals during the course of the study.

Summary Table of Selected Carcinogenicity Studies

Carcinogenicity (continued)

Species and Strain	Study Group (number, age, weight, sex)	Route and For- mulation	Dose Level	Frequency and Duration	Parameters Assessed and Results	Comments	Reference
Mice, C57BL/6	Two study groups <sup>5</sup> n=30/sex/ group <sup>6</sup> ; 4 weeks old; no weights given  8. Caffeine 9. Control	Oral; mixed in diet	(mg/ kg)  55 0	Daily, <i>ad libitum</i> ; 75-80 weeks	<u>Mortality</u> : No difference between control and treated groups. (see comments) <u>Body weight gain</u> : No difference between control and treated groups. <u>Tumor incidence</u> : No difference between control and treated groups. (see comments) <u>Clinical/laboratory findings</u> : No difference between control and treated groups.	<ul style="list-style-type: none"> <li>● Phenacetin was primary focus of study; most groups related to it.</li> <li>● Confounded somewhat because several deaths due to dehydration, &amp; mechanical problems.</li> <li>● Incomplete histological examination.</li> <li>● Serum concentration of caffeine was not assessed.</li> </ul>	Macklin and Szot (1980)

APPEARS THIS WAY  
ON ORIGINAL

<sup>5</sup>Please see "comments."

<sup>6</sup>Main study; an additional 10 animals/sex/group were sacrificed at intervals during the course of the study.

### 5.3. List of References

Eaton DL, Klaassen CD. Principles of toxicology. In. Klassen CD, editor. Casarett & Doull's Toxicology. The basic science of poisons. 5th ed. New York. McGraw-Hill; 1996; p.13-33.

Johansson SL. Carcinogenicity of analgesics: long-term treatment of Sprague-Dawley rats with phenacetin, phenazone, caffeine and paracetamol (acetamidophen). Int J Cancer 1981a; 27:521-9.

Macklin AW, Szot RJ. Eighteen month oral study of aspirin, phenacetin and caffeine in C57BL/6 mice. Drug Chem Toxicol 1980; 3:135-63.

Mohr U, Althoff J, Ketkar MB, Conradt P, Morgareidge K. The influence of caffeine on tumor incidence in Sprague-Dawley rats. Food Chem Toxicol 1984; 22:377-82.

Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH. SPSS-Statistical package for the social sciences. 3rd ed. New York: McGraw-Hill Inc; 1980.

APPEARS THIS WAY  
ON ORIGINAL

## 6. Reproductive Studies

### 6.1. Fertility and Reproductive Function in Male and Female Rodents

#### Male Rats

Soyka and Joffe (1980) discussed caffeine in a review of male mediated drug effects on offspring. Male rats were injected subcutaneously twice daily with caffeine at 5 and 50 mg/kg/day or distilled water. Animals were injected for 4 consecutive days, followed by overnight mating with a drug-naive-virgin-female. Observations were made of birth weight, sex and number, neonatal mortality, and weaning weight.

It was found that the higher dose of caffeine decreased fertility of the males, decreased litter size and birth weights, and increased neonatal mortality. Most deaths occurred from 8-14 days of life. Three males treated with the high dose failed to mate, and five females with a positive vaginal smear for sperm failed to deliver. Of 12 high-dose sired litters delivered, three were small (2-4 pups) and none of the pups survived, and only three of the larger litters (13-16 pups) had no dead pups. The neonatal mortality rates for offspring of males treated with caffeine were:

Control	10%
5 mg caffeine/kg/day	20%
50 mg caffeine/kg/day	37%

#### Female Mice

Nagasawa and Sakurai (1986) examined the effects of caffeine on reproduction in female mice in addition to the effects on normal mammary gland growth, but only the findings regarding the effects on reproduction will be discussed here.

After weaning on Day 20, half the female litter were given caffeine in drinking water, 0.05%; the other half received tap water as the control for 6 weeks. On Day 63, female mice were mated with drug-naive males. Average caffeine exposure was approximately 67 mg/kg/day. The reproductivity parameters observed were: percentage of pregnancy during 28 days of mating; still-birth rate; rate of still-born pups; litter size; average pup's weights on Days 0 (delivery), 12, and 20 (lactation); rearing rate; pup's growth rate on Days 12 and 20.

There were significant differences in caffeine-treated animals from controls in average pups weight and rearing rate. Five out of 12 caffeine-treated dams (42%) lost all pups before Day 12, compared to one out of 13 control dams (8%); and the average pup's weight on Day 20 was significantly lower in the caffeine-treated group (caffeine treated,  $10.53 \pm 0.27$  g vs control,  $11.53 \pm 0.23$  g). There was no apparent effect on parameters associated with female reproductive function (pregnancy rate, delivery interval, still-birth rate, still-born pup's rate, or litter size).

The results indicate that the chronic injection of caffeine would induce high mortality of pups during early stage of lactation.

Summary

Caffeine was reported to cause embryotoxicity when administered to male rats at a dose of 5 mg/kg/day for four days prior to mating with untreated females. At 50 mg/kg/day for four days, caffeine was reported to decrease male reproductive performance in addition to causing embryotoxicity. Daily administration of 67 mg caffeine/kg/day to female mice from weaning through lactation had no effect on reproductive parameters, but was toxic to their offspring.

**6.3. Development Toxicology and Teratology**

a) *Elmazar MMA, McElhatton PR, Sullivan FM. Studies on the teratogenic effects of different oral preparations of caffeine in mice. Toxicology 1982; 23:57-71.*

The study was conducted in two parts, using different mode of administration for caffeine: Part A in drinking water and Part B in sustained release pellet.

Materials and Methods

**Table IV-RE-1.** Study plan for evaluating teratogenic potential of caffeine administered orally to the Albino CD1 mice<sup>1</sup>

	Part A	Part B	Blood level study
Groups	n=15	n=17	n=4 or 5
1	control (water)	control (no treatment)	50 mg caffeine/kg/day pellet
2	0.8 g caffeine/liter water ( ~140-178 mg/kg/day)	0.8 g caffeine/liter water (= 150 mg/kg/day)	150 mg caffeine/kg/day drinking water
3	1.6 g caffeine/liter (~207-242 mg/kg/day)	lactose control pellet	50 mg caffeine/kg/day water/gavage
4	N/A <sup>2</sup>	50 mg caffeine/kg/day (sustained release pellet)	150 mg caffeine/kg/day pellet
5	N/A <sup>2</sup>	150 mg caffeine/kg/day (sustained release pellet)	
Treatment Days	5-18 of pregnancy	6-16 of pregnancy	6-14 of pregnancy
Fetal delivery <sup>3</sup>	day 18	day 19	

<sup>1</sup> From Elmazar *et al.*, 1982.

<sup>2</sup> Does not apply.

<sup>3</sup> Fetuses delivered by Cesarean section following cervical dislocation in dams.

Observations

Maternal body weights, and food and water intakes were measured at regular intervals during the treatment period to calculate daily intake of caffeine from the drinking water. Additional mice were used to study blood levels of caffeine. Four groups of pregnant mice were administered caffeine from days 6 to 14 of pregnancy.

Findings

## Part A (in drinking water)

*Effects on dams* The body weight gain in the low-dose group was similar to that in the control group, but weight gain was decreased in the high-dose group. However, no data on body weights from the control group was provided to make the comparisons. There was a significant decrease in food and water intake in the dams exposed to the higher dose of caffeine (207-242 mg/kg/day).

*Effects on pregnancy* The data are summarized in Tables IV-RE-2 and IV-RE-3. There were no significant differences in the number of live fetuses/litter, and the slight apparent increase in the resorption rate was not significant.

Table IV-RE-2. Effects of caffeine in drinking water given to mice on days 5-18 of pregnancy<sup>1</sup>.

Dose (g/l) (approx. mg/kg)	No. pregnant No. plugged	No. live fetuses	No. resorptions (%)	Mean No. live fetuses/litter	Mean fetal wt. (g ± S.E.)	No. fetuses with major abnormalities (%)	No. fetuses with visceral anomalies (%)
Control (Water)	13 15	134	21 (13.5)	10.3 ±0.88	1.10 ±0.005	0	3 <sup>2</sup> (2.2)
Caffeine 0.8 (140-178)	12 15	135	29 (17.6)	11.3 ±0.38	0.96 ±0.03	0	18 <sup>2</sup> 1 <sup>3</sup> (13.9)
Caffeine 1.6 (207-242)	9 15	108	33 (23.4)	12.0 ±0.65	0.85 ±0.05 (p < 0.005)	1 <sup>4</sup> (0.93)	18 <sup>2</sup>

<sup>1</sup> Table I from Elmazar *et al.*, 1982.

<sup>2</sup> Subcutaneous hemorrhages.

<sup>3</sup> Bent tail.

<sup>4</sup> Open eye.

**Effects on fetus** Mean fetal weight was significantly decreased ( $p < 0.005$ ) in the high-dose group. No gross malformations were observed with the exception of 1 fetus with one open eye due to failure of fusion of the eyelids. An increased number of fetuses with subcutaneous hemorrhages was observed in the treated groups.

On skeletal examination, none of the fetuses had cleft palate but bowing and apparent separation of the palatal bones indicative of retarded ossification was observed with high incidence (31%) at the high dose level only. In addition incomplete ossification of the supraoccipital bones were observed in both treated groups with 97% and 93% compared to 43% in controls. In the high dose caffeine group 40% of these were completely unossified. The incidence of fetuses with 14 pairs of ribs was also increased.

Table IV-RE-3.

Skeletal anomalies in the offspring of CD1 mice treated with caffeine in the drinking water from days 5 to 18 of pregnancy.<sup>1</sup>

Dose (g/l) (approx. mg/kg)	No. fetus examined	Extra ribs (%)	Retarded ossification		
			Sternebral (%)	Supraocci- pital (%)	Palatal (%)
Control (water)	134	7 (5.2)	25 (18.7)	57 (42.5)	0
Caffeine 0.8 (140-178)	135	17 (12.5)	64 (47.1)	131 (97.0)	2 (1.8)
Caffeine 1.6 (207-242)	108	22 <sup>2</sup> (20.3)	59 (53.4)	101 (93.4)	34 (31.4)

<sup>1</sup> Table II from Elmazar *et al.*, 1982.

<sup>2</sup> 3F also had fused ribs.

#### Part B (with caffeine pellets)

**Effects on dams.** Maternal weight gain was decreased in all treated groups compared to untreated controls, but was greatest in the 150 mg caffeine/kg/day (sustained release pellet) and lactose-control pellet groups. Food consumption was markedly decreased in these groups. Water consumption was more or less consistently decreased in two groups: 150 mg caffeine/kg/day in drinking water and lactose-pellet control.

**Effects on pregnancy.** The data are summarized in Tables IV-RE-4 and IV-RE-5. The number of pregnancies and the number of live fetuses/litter were lower in both 150 mg caffeine groups in pellets and drinking water compared to control group but without statistical significance. The resorption rates were significantly higher in the 150 mg/kg/day caffeine-pellet group compared with controls ( $p < 0.05$ ), and 2 dams in this group completely resorped their litters.

Resorptions were significantly higher also in the lactose-pellet group ( $p < 0.01$ ), and one dam had a runt litter in which all the fetuses weighed less than 0.9 g. Fetal weights were significantly reduced in the 150 mg/kg/day caffeine-pellet group ( $p < 0.05$ ) and 150 mg caffeine/kg/day in drinking water ( $p < 0.01$ ).

Table IV-RE-4. Effects of caffeine orally on CD1 mice from days 6 to 16 of pregnancy.<sup>1</sup>

Treatment	No. pregnant No. plugged	No. live fetuses	No. resorptions (%)	Mean No. live fetuses/litter ( $\pm$ S.E.)	Mean fetal wt. (g) $\pm$ S.E.
Absolute control	$\frac{17}{17}$	216	9 (4.0)	12.7 $\pm$ 0.47	1.32 $\pm$ 0.02
Caffeine drinking water 150mg/kg	$\frac{14 + 1^2}{17}$	175	9 (4.8)	12.5 $\pm$ 0.54	1.23** $\pm$ 0.02
Lactose pellets	$\frac{17}{17}$	194	21 (8.7)**	11.4 $\pm$ 0.50	1.32 <sup>3</sup> $\pm$ 0.02
Caffeine pellets 50mg/kg	$\frac{17}{17}$	209	14 (6.4)	12.3 $\pm$ 0.44	1.32 $\pm$ 0.02
Caffeine pellets 150 mg/kg	$\frac{14 + 1^4}{17}$	137	27 (23.6)*	11.4 <sup>5</sup> $\pm$ 0.70	1.23* $\pm$ 0.05

<sup>1</sup> Table III from Elmazar *et al.*, 1982.

<sup>2</sup> 1 litter contained only 1 fetus and was omitted from the figures on this table

<sup>3</sup> 1 litter average weight 0.9 g excluded

<sup>4</sup> 1 dam died day 16

<sup>5</sup> Excludes 2 litters resorbed completely

\*  $P < 0.05$

\*\*  $P < 0.01$

The incidence of major malformations in the 150 mg/kg/day caffeine-pellet group was significantly greater compared to the untreated group. However, there was no difference in the incidence of major malformations in the high-dose pellet group when compared to the lactose-pellet group, although both the 50 mg/kg and 150 mg/kg caffeine pellet groups had a low incidence of cleft palate/lip which was not seen with lactose pellets.

Table IV-RE-5. External and visceral defects in fetuses exposed to caffeine from days 6 to 16 of pregnancy<sup>7</sup>

Treatment	Gross external defects (litters)				Visceral anomalies Wilson's Slicing		
	No. examined	Major		Minor	No. examined	(litters)	
Absolute control	216	0		2(2) 1 sc 1 cf <sup>2</sup>	70	3(3)	2 drp 1 drp <sup>2</sup>
Caffeine drinking water 150 mg/kg	175	0		4(4) 1 cf 1 cf 1 cf 1 sc	58	0	
Lactose pellets	194	4(2)	3 ex 1 ex	7 (5) 2 cf 1 oe <sup>3</sup> 1 sc 1 cf 2 cf	65	1 (1)	1 drp <sup>3</sup>
Caffeine pellets 50 mg/kg	209	2 (1)	2 cp	2(2) 1 cf 1 sc	71	2(2)	1 drp 1 cyst
Caffeine pellets 150 mg/kg	137	8** (4*)	5 ex 1 cp 1 cp 1 cl	3(3) 1 cf 1 cf 1 sc	45	1 (1)	1 drp

1 Table IV from Elmazar *et al.*, 1982

2 Same Fetus

3 Same Fetus

ex, exencephaly; cp, cleft palate; cl, cleft lip; oe, open eye; sc, subcutaneous haemorrhage; drp, dilated renal pelvis; cf, club foot

\*\* P = 0.001 vs. absolute control; P = 0.12 vs. lactose pellets

\* P = 0.04 vs. absolute control; P = 0.34 vs. lactose pellets

On skeletal examination of the fetuses (2/3 of each litter), the highest incidence of retarded ossification occurred in the group treated with 150 mg/kg caffeine in the drinking water, with supraoccipital bones, sternbrae and xiphisternum being affected. Abnormal fusion of the sternbrae occurred in all groups but was only significantly increased in the 150 mg/kg caffeine drinking water group compared with the controls.

*Caffeine blood levels.* Animals dosed with the 150 mg caffeine/kg/day pellet showed the greatest exposure to caffeine, although the highest peak plasma concentration occurred following 50 mg caffeine/kg/day administered in water by gavage.

	AUC ( $\mu\text{g/ml}\cdot\text{hr}$ )	Peak ( $\mu\text{g/ml}$ )
50 mg caffeine/kg/day pellet	51	12 <sup>1</sup>
150 mg caffeine/kg/day drinking water	59	5.4 $\pm$ 0.59
50 mg caffeine/kg/day water/gavage	127	36.2 $\pm$ 2.97
150 mg caffeine/kg/day pellet	200	16.4 $\pm$ 0.91

<sup>1</sup>Value read from Figure 1, p. 62.

### Summary

When caffeine was administered to pregnant mice in the drinking water (207-242 mg/kg/d) on Days 5-18 of gestation, maternal weight gain was decreased in high dose group, associated with a reduced food and water consumption prior to day 12 day of gestation, which may be partly related to the unpalatability. Administration of caffeine during gestational period to mice did not produce increase in gross malformations in the fetuses, but decreased weight and incomplete ossification were observed.

Administration of caffeine via intragastric pellet to pregnant mice at a dose of 50 or 150 mg/kg/day, on Days 6-16 of pregnancy resulted in a significant increase in resorption in the pellet groups and a low level of teratogenicity (cleft palate 2/209 for 50 mg/kg and 3/137 for 150 mg/kg). A dose of 150 mg caffeine/kg/day via intragastric pellet reduced food and water intake and decreased body weight in the dams, which might have resulted in embryofetal toxicity (increased resorption, decrease in fetal weight and retarded ossification). Embryotoxicity, seen in the 150 mg caffeine pellet group was also observed with lactose pellets, although no cleft palate was seen in the latter.

Analysis of caffeine blood level data showed that the total exposure from the pellets was greater than the exposure from the drinking water.

APPEARS THIS WAY  
ON ORIGINAL

b) Collins TFX, Welsh JJ, Blöck TN, Ruggles DI. A study of the teratogenic potential of caffeine ingested in drinking-water. *Food Chem Toxicol* 1983; 21:763-77.

### Study Objective

The purpose of this study was to measure maternal and fetal effects after *ad lib* sipping of caffeine and to compare these results with those found after similar doses given by oral intubation to rats.

### Materials and Methods

Osborne-Mendel rats were used in this study. Mating was allowed to occur overnight on specified schedules. Mating was continued until 61 females had been assigned to each group. Caffeine solution or distilled water was provided, as drinking water, from day 0 until animals were sacrificed (carbon dioxide asphyxiation) on day 20, at which time Cesarean sections were performed. Parameters monitored included: food consumption and water consumption; maternal weight gain; resorption sites, fetuses, and implantation sites; fetal weight, gross external malformations; skeletal variations (approximately one-half of the fetuses in a group); internal variations of soft tissues (other half of fetuses).

There were eight groups of animals evaluated with each group with calculated amount of caffeine (mg/kg/day) intake from the drinking water: Groups 1 (0), 2 (10.1), 3 (27.4), 4 (50.7), 5 (86.6), 6 (115.8), 7 (160.9), & 8 (204.5)

### Findings

*Effects on dams.* No overt behavioral differences were noted in any of the treated animals, and no dose-related gross effects were seen. Animals receiving the three lowest doses of caffeine (Groups 2, 3, and 4) drank significantly more fluid than did controls. At the three highest doses (Groups 6, 7, and 8) fluid consumption was significantly lower than controls, in a dose-dependent manner. Food consumption was decreased initially (Day 0-7) in Groups 5, 6, 7, and 8; the animals in the Groups 7 and 8 ate significantly less than the controls during the entire gestation period. Maternal body weight gains in Groups 5, 6, 7, and 8 were significantly lower than the controls from day 0 to day 20, which were dose-related and corresponded to the amount of food consumed.

*Effects on pregnancy.* Pregnancy rates in treated and control groups were similar, greater than 90%. Significant effects on fetuses were observed at the two highest doses (Groups 7 and 8): decreased mean number of implants; decreased mean number of viable fetuses per litter; increased average percentage of total resorptions per litter; increased mean number of early deaths, and increased mean number of late deaths. The percentages of females with at least one resorption and of those with at least two resorptions showed a significant linear trend with increased dosage.

*Effects on fetus.* Fetal body weight and length were decreased and edematous fetuses were increased at dosages  $\geq 86.6$  mg/kg/day (Group 5). No dose-related differences in any external variation was observed in fetuses from the three lowest dose-groups (2, 3, and 4). In the three highest dose groups (6, 7, and 8), there were significant increases in edematous fetuses and in fetuses with hemorrhages. Caffeine available *ad lib* in drinking-water produced no dose-related gross anomalies. Only two animals with missing or hypoplastic nails were produced, both in the 160.9 mg/kg groups. Sternbral ossification deficiencies were increased at all dose levels except 10.1 mg/kg/day (Group 2). Skeletal ossification deficiencies were increased in a dose-related manner at the four highest dose levels ( $\geq 86.6$  mg/kg).

With the exception of edema, the incidence of soft-tissue variations was low, variable, and not significantly different from control. However, two significant differences were noted when soft-tissue edema was included in the incidence-analysis as shown below:

Group	Caffeine Dosage (% w/v) in drinking water	Caffeine Intake (mg/kg/day)	Average number of variations per fetus	Percent of litters having fetuses with one or more variations
1	0	0	0.46	35.1
5	0.07	86.6	0.36	27.6
6	0.19	115.6	1.45*	48.2
7	0.15	160.9	4.18*	92.7*
8	0.20	204.5	3.81*	100.0*

\* = significantly different from control, Group 1

### Summary

Daily administration of caffeine via drinking water to pregnant rats during day 1-20 of gestation produced significant toxic effects on several pregnancy and fetal parameters. However, water and food consumption were significantly decreased in dams receiving the three highest doses of caffeine (Group 6, 115.8 mg/kg/day; Group 7, 160.9 mg/kg/day; Group 8, 204.5 mg/kg/day). Dosages of 160.9 and 204.5 mg/kg were associated with decreased implantation, increased resorptions and decreased mean numbers of viable fetuses. Numbers of runts were significantly increased after dosages  $\geq 115.8$  mg/kg/day. Fetal body weight and length were decreased and edematous fetuses were increased at dosages  $\geq 86.6$  mg/kg/day. Caffeine available in drinking-water produced no dose-related gross anomalies. However, dose-related ossification deficiencies and skeletal variation were observed consistently in Groups 5-8 in a dose-related manner starting from 86.6 mg/kg/day. Therefore, caffeine produced maternal toxicity and fetotoxicity at doses  $\geq 86.6$  mg/kg and embryotoxicity at dose  $\geq 160.9$  mg/kg.

Discussion of Teratogenicity:

Oral intubation of 125 caffeine/kg led to a significant increase in maternal deaths (Collins et al. 1981), whereas when ingested in small increments via drinking-water, caffeine at up to 204.5 mg/kg/day produced no similar maternal toxicity in rats. (Because average peak level of caffeine in blood after gavage was approximately ten times that achieved by *ad lib* sipping.)

In this study, maternal body weight gain with corresponding food and fluid consumption was significantly decreased in rats at doses  $\geq 115.8$  mg/kg/day. The pups from these groups were significantly lighter, smaller and less well developed, but they showed no teratogenic response.

In the caffeine gavage study, the number of runts significantly increased at  $\geq 80$  mg/kg. When given in drinking water, the numbers of runts were significantly increased at  $\geq 115.8$  mg/kg, correlated with the caffeine-induced reduction in fetal weights.

Caffeine dosage at approximately 100 mg/kg/day affected fetal osmotic balance, the effects were similar regardless of the method of dosing. In the gavage study, the number of edematous animals was significantly increased only at the 125 mg/kg/day, in drinking water at 115.8 mg/kg/day.

Fetotoxicity was less severe when approximately 80 mg caffeine/kg was ingested in drinking water, due to possibly lower fetal exposures compared to the gavage administration. Several developmental parameters (fetal weights and crown-rump length) were significantly decreased at 40 mg/kg in the gavage study, whereas in the drinking-water study, 86.6 mg/kg, although the incidence of hemorrhages was significantly increased approximately the same daily dosage of 80 mg/kg by gavage or in the drinking water (86.6 mg/kg).

In the previous gavage study, significant reductions in ossification of the central arch, pubis, metacarpals, metatarsal and nasal bones were seen at  $\geq 80$  mg/kg. Comparable dose levels in this study,  $\geq 86.6$  mg/kg reduced ossification in the same bones. Numerous bones showed decreased development after the 115.8 mg/kg dose in rats, unlike the findings of Elmzar et al. (1982), in which ossification of the supraoccipital bone was delayed in mice after 150 mg/kg caffeine in drinking water.

The effects on maternal mortality, fetal weight, and fetal ossification development were less severe after *ad lib* sipping than after oral intubation. No teratogenic effects were related to the dose of caffeine ingested via sipping.

At very high doses of caffeine, teratogenic effect was observed with cleft palate in mice.

### 5.3. Perinatal and Postnatal Toxicity

#### Rat

Aeshbacher *et al.* (1980) investigated possible effects of caffeine on mortality, birth weight, and development of the young when caffeine was administered during pregnancy and/or lactation. Caffeine was administered orally with the feed, at three dose levels:

g caffeine/kg feed	n	average mg caffeine/kg body wt./day
0	80	0
0.25	10	15.9
0.50	10	35.3
1.00	10	62.3

At birth, half of the dams from each group had their pups removed and exchanged either for pups from treated mothers in the case of control dams, or pups from untreated animals in the case of treated dams. The remaining animals per group were maintained with their own pups.

At the two lower dose-levels, exposure throughout gestation and lactation had no significant effect on birth weight, litter size, or development. There was also no effect at those doses following treatment during either gestation alone, or lactation alone. At the highest dose-level, there was a slight reduction of birth weight, and a trend towards lower weight gain in litters from dams fed the test diet throughout gestation and lactation.

Glavin and Kreuger (1985) studied the effects of prenatal caffeine exposure on offspring mortality, open-field behavior and adult gastric ulcer susceptibility by oral administration of caffeine via drinking water to pregnant rats throughout gestation. Offspring were cross-fostered to non-caffeine-treated mothers at birth. There were three caffeine groups:

mg caffeine/ml water	n	average mg caffeine/kg body wt./day
0	10	0
0.17	10	12.5
0.34	10	25.0
0.50	10	35.0

A dose-related increase in offspring mortality (2/87, 1/96, 4/98 12/98 for control, LD, MD & HD, respectively at 24 hr) was observed at 24 hr and at 10 days post partum. No significant differences in birth weights or growth rates between groups, in average litter size at parturition, or gross malformations were observed. Prenatal caffeine exposure did not significantly influence open-field ambulation or defecation when tested at 48, 68, or 196 days of age.

A significant dose-related increase was observed in restraint-stress gastric ulcers susceptibility at 200 days of age (adult). Offspring from rats treated with 0.05% caffeine during pregnancy, developed significantly more frequent and more severe gastric lesions than did offspring from control rats or from rats prenatally exposed to 0.017% and 0.034% caffeine. Prenatal caffeine exposure may: (1) predispose organisms to increased gastric disease susceptibility as adults and (2) interfere with neonatal feeding ability and thereby increase infant mortality.

*Summary:* Moderate doses of caffeine administered prenatally produced two striking effects detected in this study: (a) a marked increase in neonatal mortality and (b) a sensitization to stress-induced gastrointestinal disease as adults. No consistent behavioral effects of caffeine were observed in repeated open-field testing at 48, 68 or 196 days of age. The increased neonatal mortality may be speculated by impaired sucking/feeding behavior in neonates caused by blocking the role of putative neurotransmitter adenosine in feeding behavior by high dose of caffeine.

### Monkey

Gilbert and his associates (Gilbert *et al.*, 1988; Gilbert and Rice, 1991) exposed adult female monkeys (*Macaca fascicularis*) to caffeine in drinking water for 25 weeks before and during pregnancy. There were two caffeine groups (0.15 mg caffeine/ml, ~10-15 mg/kg/day, n=14; 0.35 mg caffeine/ml, ~25-35 mg/kg/day, n=14) and one control group (n=12). Reproductive failure in the form of stillbirths and miscarriages was observed in treated groups. In addition, decreased maternal weight gain and decreased infant birth-weight was observed

#### Pregnancy Outcome\*

	Control (n=12)	Low Dose (n=14)	High Dose (n=14)
live birth	19	14	9
still birth	0	5	6
miscarriage	0	2	5

\* Results after rebreeding the monkey

*Summary:* Chronic exposure of female monkeys to caffeine in drinking water (10-15 mg/kg/day; 25-35 mg/kg/day) before and during pregnancy resulted in increased stillbirths and miscarriages. In addition, decreased maternal weight gain and decreased infant birth-weight was observed indicating that exposure to methylxanthines was detrimental to fetal development in these monkeys. Treated male infants displayed decreased mean body weight and smaller somatic measurements days 1-5; however, after Day 31 there was no difference between treated and control groups. No significant differences were observed between treated and control groups of female offspring.

The summary table of selected reproductive toxicity studies follows.

APPEARS THIS WAY  
ON ORIGINAL

### Summary Table: Results of Selected Toxicology Studies

#### Reproductive Toxicity

Species (Strain)	No. of animals /group	Route of admin. (vehicle)	Doses (mg/kg)	Observations	Reference
Mouse (C3H/HEM1)  Fertility and early embryonic development	Controls: 13 F Caffeine: 12 F	Oral-drinking water	0, 67 (daily from weaning thru lactation)	No effects were seen on reproductive parameters but embryofetal mortality was increased in treated mice (42% vs 8%) and average pup weight on day 20 was significantly lower.	Nagasawa and Sakurai (1986)
Rat  Fertility and early embryonic development	20 Males	IP -BID (Water)	0, 5, 50/day (4 days dosing)	At 50 mg/kg, males had decreased fertility (3 failed to mate, 5 females with positive smear failed to deliver). At both doses, litter sizes were smaller and neonatal mortality was increased (0-10%, 5 mg/kg-20%, 50 mg/kg-37%).	Soyka and Joffe (1990)

APPEARS THIS WAY  
ON ORIGINAL

Species (Strain)	No. of animals /group	Route of admin. (vehicle)	Doses (mg/kg)	Observations	Reference
Mouse (CD1)  Embryo-fetal development	A. 15F  B. 17F	A. Oral-drinking water  B. Oral - drinking water or gavage of sustained-release pellets	A. 0, 140-178, 207-242  B. 0, 50, 150 <sup>(1)</sup>	A. Weight gain in dams was decreased in high-dose group (no statistics); food & water intake were significantly lower in the high-dose group; 1 resorption rate; fetal weight was significantly decreased in high-dose group; incomplete ossification of the supraoccipital (SO) bones was greater in the caffeine -treated groups (97% & 93% vs 43% in controls), and in the high-dose 40% of SO bones completely unossified; in both caffeine groups there was an increased incidence of supernumary ribs. B. Maternal weight gain was decreased in all treated groups but was greater in the pelleted groups (lactose and 150 mg/kg); Also in these two groups there was a marked decrease in food consumption, a decrease in water consumption, a significant increase in resorption rates, and a significant decrease in fetal weight. Cleft palate (2 of 209) in low dose pellet group; cleft palate/cleft lip (3/137) in high dose pellet group.	Elmazar <i>et. al.</i> (1982)

<sup>(1)</sup> Absolute control (no treatment), lactose control pellets (size = 150 mg/kg dose pellet), 150 mg/kg in drinking water, 50 mg/kg in sustained release pellet, 150 mg/kg in sustained release pellet.

APPEARS THIS WAY  
ON ORIGINAL

## Reproductive Toxicity

Species (Strain)	No. of animals /group	Route of admin. (vehicle)	Doses Caffeine (mg/kg)	Observations	Reference
Rat (Osborne Mendel) Embryo-fetal development	61 F	Oral-drinking water Days 0-20	0, 10.1, 27.4, 50.7, 86.6, 115.8, 160.9, 204.5	No overt behavioral differences or dose-related gross effects were seen; in Groups 6-8 fluid consumption was significantly decreased & food intake was consistently decreased; body weight was decreased in Groups 5-8; Groups 7 & 8 had significant decreases in the mean no. of implants & mean no. of viable fetus/litter, and significant increases in mean % of total abortions/litter, and mean no. of early & late deaths. Groups 5-8 had dose-dependant decreases in mean fetal body wt., ossification deficiencies, & significant decreases in crown rump length; Groups 6-8 had significant increases in total no. of runts, in edematous fetuses, fetuses with hemorrhage, & soft tissue edema; Groups 7-8 had significant increases in the % of litters with one or more fetal variation.	Collins <i>et.al.</i> (1983)
Rat Prenatal and postnatal development	Control: 80 F Caffeine: 10 F	Oral-diet	0, 15.9, 35.3, 62.3 gestation &/or lactation	No significant effects	Aeshbacker <i>et.al.</i> (1980)
Rat (Wistar) Effect on offspring	10 F	Oral-drinking water	0, 12.5, 25, & 35 throughout gestation	Significant increase in mortality after 24 hours in the highest dose group. In 196 day-old rats three hour restraint-cold stress produced a significant increase in ulcer frequency in the high -dose group and an increase in cumulative ulcer length in the mid- and high-dose group animals.	Glavin and Kreuger (1985)
Monkey Effect on pregnancy	Control: 12 F Low Dose:14 F High Dose:14 F	Oral-drinking water	0, 10-15, 25-35 >25 week before mating	Stillbirths & miscarriages were observed in both treated groups; treated male infants had significantly less body weights days 1-5 but the difference from control animals decreased with maturity. Significantly smaller somatic measurements were observed initially in treated males, but this difference also disappeared during the first year. Treated female infants showed no significant differences from control female infants.	Gilbert <i>et.al.</i> (1988) Gilbert and Rice (1991)

6.4. List of References

- Aeschbacher HU, Milon H, Poot A, Wurzner HP. Effect of caffeine on rat offspring from treated dams. *Toxicol Lett* 1980; 7:71-7.
- Collins TFX, Welsh JJ, Black TN, Ruggles DI. A study of the teratogenic potential of caffeine ingested in drinking-water. *Food Chem Toxicol* 1983; 21:763-77.
- Elmazar MMA, McElhatton PR, Sullivan FM. Studies on the teratogenic effects of different oral preparations of caffeine in mice. *Toxicol* 1982; 23:57-71.
- Gilbert SG, Rice DC. Somatic development of the infant monkey following *in utero* exposure to caffeine. *Fundam Appl Toxicol* 1991; 17:454-65.
- Gilbert SG, Rice DC, Reuhl KR, Stavric B. Adverse pregnancy outcome in the monkey (*Macaca fascicularis*) after chronic caffeine exposure. *J Pharmacol Exp Ther* 1988; 245:1048-53.
- Glavin GB, Krueger H. Effects of prenatal caffeine administration on offspring mortality open-field behavior and adult gastric ulcer susceptibility. *Neurobehav Toxicol Teratol* 1985; 7:29-32.
- Nagasawa H, Sakurai N. Effects of chronic ingestion of caffeine on mammary growth and reproduction in mice. *Life Sc* 1986; 39:351-7.
- Soyka LF, Joffe, JM. Male mediated drug effects on offspring. In: Schwartz RH, Yaffa SJ, editors. *Drug and chemical risks to the fetus and newborn*. New York: Alan R. Liss, Inc., 1980. p. 49-66.

APPEARS THIS WAY  
ON ORIGINAL

## 7. Mutagenicity Studies

*Mutagenicity Testing of Caffeine.* Because of widespread human exposure to caffeine, and the structural similarity to nucleic acid bases, potential mutagenic activity of caffeine has been studied widely. There are numerous mutagenicity studies in the literature where caffeine has been tested. However, in general, the exposure levels in mutagenicity studies ( $10^3\text{M}$ -range) greatly exceed human-exposure levels ( $10^6$ - $10^5\text{M}$ ), and most of the literature deals with interaction studies where the effect of caffeine on DNA damaged by known genotoxins was assessed. Even at these high levels, caffeine appears to have virtually no effect on normal DNA. Rather, caffeine appears to interact with damaged DNA to hinder repair (D'Ambrosio, 1994; Legator and Zimmering, 1979; Roberts, 1984). Consequently, there are a few hundred reports and reviews concerned with the interaction of caffeine with known genotoxic agents but virtually no dose-response studies on caffeine alone. Therefore, only brief summaries are presented.

### 7.1. Review of Selected Studies

#### *In Vitro Studies*

*Amacher DE, Zelljadt I. Mutagenic activity of some clastogenic chemicals at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. Mutat Res 1984; 136:137-45.*

Caffeine (one of the four presumptive clastogens) was tested in the CHO/HGPRT gene mutation assay for the induction of 6-thioguanine (6TG)-resistant mutants. Ethyl methanesulfonate (EMS) was used as the positive control dissolved in DMSO, which was the negative control. Caffeine was dissolved directly into the culture medium and tested in CHO cells at the concentration of 2000-8000  $\mu\text{g/ml}$  ( $10^{-3}$ - $10^{-2}\text{M}$ ) without exogenous metabolic activation.

Caffeine at very high concentrations (6667-8000  $\mu\text{g/ml}$ ) caused a slight elevation in mutant frequency over background, probably due to a selective effect of caffeine against the HGPRT<sup>+</sup> phenotype, for 2 different HGPRT cell lines which were refractory to the toxic effects of caffeine at the highest level (8000  $\mu\text{g/ml}$ ). Caffeine was concluded to be not mutagenic.

*Palitti F, Tanzarella C, DeGrassi F, De Salvia R, Fiore M. Enhancement of induced sister chromatid exchange and chromosomal aberrations by inhibitors of DNA repair processes. Toxicol Pathol 1984; 12:269-73.*

The effects of post-treatment with inhibitors of DNA synthesis (hydroxyurea, aphidicolin) and repair (caffeine, 3-aminobenzamide) have been studied on the frequencies of chromosomal aberrations and sister chromatid exchange (SCE) induced by mitomycin C (MMC) and decarbamoyl mitomycin D (DCMMC) both in Chinese hamster cells (CHO) and in human lymphocytes *in vitro*.

Caffeine alone (5 mM) had no mutagenic activity in either assay. However, the same concentration of caffeine potentiated the genotoxicity of mitomycin C (0.1  $\mu\text{g/ml}$ ) in CHO cells and in Human lymphocytes. Caffeine potentiated the SCE and chromosomal aberrations in the  $G_2$  phase, observed in MMC-treated cells.

**Table IV-M-1** Effect of 5mM caffeine to induce SCE and chromosomal aberrations in CHO cells and isolated human lymphocytes.<sup>1</sup>

Assay	Percent Aberrations $\pm$ S.E.		SCE/cell $\pm$ S.E.	
	control	caffeine	control	caffeine
CHO Cells	2 $\pm$ 1.4	5 $\pm$ 2.2	5.35 $\pm$ 0.40	5.25 $\pm$ 0.35
Human lymphocytes		1 $\pm$ 1.0	4.4 $\pm$ 0.3	6.3 $\pm$ 0.5

<sup>1</sup> From Palitti *et al.*, 1984.

When the Chinese hamster and human lymphocytes were treated with mitomycin C, there is an increased frequency of both chromosomal aberrations and SCE after  $G_2$  post-treatment with the inhibitors, while no increase is observed for DCMMC-treated cells. Since SCE are DNA synthesis-dependent phenomenon, an increase in the frequency of SCE also in the  $G_2$  phase might suggest that after mitomycin C-treatment there is a residual DNA synthesis still going on very late in the cell cycle.

#### *In Vivo Studies*

MacGregor JT, Schlegel R, Wehr CM, Alperin P, Ames BN. Cytogenetic damage induced by folate deficiency in mice is enhanced by caffeine. *Proc Natl Acad Sci. USA* 1990; 87:9962-5.

Caffeine, 75 mg/kg or 150 mg/kg dissolved in isotonic saline, was administered intraperitoneally to male weanling Swiss mice (10-12g, 8/group) for four consecutive days. Isotonic saline was administered intraperitoneally to control animals on the same schedule. On the fifth day, blood samples as well as femur marrow smears were obtained.

Neither dose of caffeine exhibited clastogenic activity. The approximate number of micronucleated erythrocytes per 1000 cells read was: control, 2.2; 75 mg caffeine/kg, 2.5; 150 mg caffeine/kg, 2.3.<sup>9</sup> There was, however, an increase of up to 5 times these rates in mice reared on a folate deficient diet. Thus, folate deficiency causes cytogenetic damage in mice and that caffeine can act synergistically with folate deficiency to induce cytogenetic damage *in vivo*.

<sup>9</sup> Numbers read from Figure 1-B, p. 9963.

In another paper by MacGregor JT, (1990), it was suggested that dietary deficiencies of nutrients required for nucleotide synthesis, such as folate, vitamin B<sub>12</sub> and magnesium, may increase spontaneous chromosomal damage, and may strongly influence the genotoxic response to other dietary factors in human.

Panigrahi GB, Rao AR. *Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells in vivo. Mutat Res 1983; 122:347-53.*

Inbred Swiss albino mice of both sexes (4-6 months of age) were exposed to caffeine via the drinking water, 0.5% solution (5 mg/ml) *ad libitum*, for 5, 10, or 15 days. Number of SCE/cell metaphases was evaluated from seventy-five metaphases scored/group (25/animal). The paper gave no information on average doses to which animals were exposed, sex distribution within groups (N=3), or clinical condition of animals.

Caffeine enhance the SCE frequency in the bone marrow cells of mice at the concentration used, and the frequency of SCEs increased as the days of exposure of caffeine increased. (See Table IV-M-1.) In addition, caffeine increased additively the SCEs frequencies induced by various doses of arecoline (a known clastogen) given for different intervals of time. This may be a significant finding, although the average doses to which animals were exposed were not provided, and potential confounding issues (i.e., significant differences between control and treatment groups and average daily doses) were not addressed by the investigators.

Table IV-M-1. Effect of exposure of albino Swiss mice to 5 mg caffeine/ml of drinking water *ad libitum* (0.5% solution) to induce SCE.<sup>1</sup>

Exposure Time (days)	Treatment	Number of Animals	SCE/cell (mean ± S.E.)
5	Control	3	3.01 ± 0.18
	Caffeine	3	3.88 ± 0.14 <sup>2</sup>
10	Control	3	3.21 ± 0.18
	Caffeine	3	5.34 ± 0.15 <sup>2</sup>
15	Control	3	4.10 ± 0.32
	Caffeine	3	7.30 ± 0.21 <sup>2</sup>

<sup>1</sup>From Panigrahi and Rao, 1983.

<sup>2</sup>P < 0.001 on comparison with respective control.

The summary table of selected mutagenicity studies follows.

**7.3. Summary Table: Results of Selected Toxicology Studies**

**Mutagenicity**

Test System	Caffeine Concentration/Dose	Results	Reference
<i>In Vivo</i> Mouse micronucleus	75, 150 mg/kg , IP.	No clastogenic activity; Enhanced formation of micronuclei with folate deficiencies	MacGregor <i>et.al.</i> (1990)
Mouse metaphase analysis	0.5% solution (5 mg/mL); 5, 10, 15 day exposure	Increased SCE/cell metaphases at all exposures	Panigrahi and Rao (1983)
<i>In Vitro</i> CHO/HGPRT	2000-8000 ug/mL	No mutagenic activity; Positive at high concentrations	Amacher and Zelljadt (1984)
CHO/chromosomal aberrations	5 mM	No clastogenic activity; Potentiated genotoxicity	Palitti <i>et.al.</i> (1984)
Human lymphocyte/chromosomal aberrations	5 mM	No clastogenic activity; Potentiated genotoxicity	Palitti <i>et.al.</i> (1984)

**7.4. List of References**

- Amacher DE, Zelljadt I. Mutagenic activity of some clastogenic chemicals at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. *Mutat Res* 1984; 136:137-45.
- D'Ambrosio SM. Evaluation of the genotoxicity data on caffeine. *Regulatory Toxicol Pharmacol* 1994; 19:243-81.
- Hoffman GR. Genetic toxicology. In: Klassen CD, editor. *Casarett & Doull's Toxicology. The basic science of poisons. 5th ed.* New York: McGraw-Hill; 1996. p. 269-300.
- Legator MS, Zimmering S. Review of the genetic effects of caffeine. *J Environ Sci Health* 1979; C13:135-88.
- MacGregor JT, Schlegel R, Wehr CM, Alperin P, Ames BN. Cytogenetic damage induced by folate deficiency in mice is enhanced by caffeine. *Proc Natl Acad Sci USA* 1990; 87:9962-5.
- MacGregor, JT. Dietary factors affecting spontaneous chromosomal damage in man. *Prog Clin Biol Res.* 1990; 347: 139-53
- Palitti F, Tanzarella C, Degrassi F, De Salvia R, Fiore M. Enhancement of induced sister chromatid exchange and chromosomal aberrations by inhibitors of DNA repair processes. *Toxicol Pathol* 1984; 12:269-73.
- Panigrahi GB, Rao AR. Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells in vivo. *Mutat Res* 1983; 122:347-53.
- Roberts JJ. Mechanism of potentiation by caffeine of genotoxic damage induced by physical and chemical agents. *Nucleic Acids Symp Ser* 1984; 13:193-215.

**APPEARS THIS WAY  
ON ORIGINAL**

## 8. Overall Summary and Evaluation

### Pharmacology

Caffeine seems to stimulate respiration by acting either centrally or peripherally. Caffeine increases the frequency of breathing but has no effect on the respiratory minute volume. The primary mechanism of action appears to be blockade of adenosine receptors, thereby preventing activity of endogenous adenosine. Caffeine has been shown to increase responsiveness of bronchial smooth muscle to acetylcholine in neonatal lambs when skeletal muscle and centrally mediated respiratory activities have been blocked. In addition, caffeine effects motor activity and behavior.

Caffeine has direct activity on cardiac tissue and increases heart rate and force of contraction at millimolar ( ) mM) concentrations, which are much higher than concentrations achieved therapeutically ( )  $\mu$ M). The mechanisms underlying direct cardiac activity have been identified and involve intracellular calcium ion translocation and /or phosphodiesterase inhibition (Rall, 1990). The small cardiovascular responses observed *in vivo* in the therapeutic dose-range probably represent indirectly mediated responses.

Caffeine at doses similar to those used in treatment of apnea of prematurity have little effect on renal function in the newborn New Zealand rabbit. Two hours following 10 mg caffeine/kg, renal vascular resistance was increased. In spite of this increase, glomerular filtration rate and renal blood flow were unchanged.

Caffeine and theophylline have similar pharmacological activities at the identical sites of action, although the potency for caffeine is different from that of theophylline at each site.

Table II-A-1. Sites of pharmacological activity for methylxanthines<sup>1</sup>

Site of Action	Caffeine	Theophylline	Theobromine
Brain	1 <sup>2</sup>	2	3
Skeletal muscles	1	2	3
Heart	3	1	2
Kidneys	3	1	2
Bronchioles	1	1	2

<sup>1</sup> Modified from Tarka, 1975.

<sup>2</sup> Potency based on a comparison of caffeine, theophylline, and theobromine: most potent=1; least potent=3.

### Absorption, Distribution, Metabolism and Elimination (ADME)

Caffeine is absorbed readily after oral, rectal, or parenteral administration, and is distributed into all body compartments in proportion to water content of the tissues in animals (Warszawski *et al.*, 1977; Serafin, 1995). The apparent volume of distribution for neonates has been shown to be greater than that for adults of the same species, including humans (Warszawski *et al.*, 1977; Serafin, 1995) due perhaps to the higher water content of tissues from the neonate compared to the adult.

Caffeine in adults is extensively metabolized in the liver (Serafin, 1995), by cytochrome P-450 isoenzymes in the hepatic microsomal mixed function oxidase system, and less than 2% is excreted unchanged. There are two major metabolic pathways: N-demethylation and oxidation at position C-8. In humans and other species, there is evidence that more than one cytochrome P-450 isoenzyme is involved. Experimental data suggest that the P-450 isoenzyme(s) required for N-1 and N-3 demethylations are inactive in the human newborns, and only the isoenzyme required for N-7 demethylation is present at birth (Berthou *et al.*, 1988) but much less active than in adults. In the rat neonates however, all three dimethyl metabolites are found but with decreased specific activity of the enzyme system.

Comparative metabolic profile of caffeine

Dimethyl Metabolite	Rat		Human		
	Neonate	Adult		Neonate	Adult
Theobromine	36% <sup>1</sup>	50% <sup>1</sup>	38% <sup>2</sup>	NF <sup>2</sup>	23% <sup>2</sup>
Theophylline	30%	30%	29%	3-8%	19%
Paraxanthine	33%	20%	33%	NF	58%

<sup>1</sup> From Bienvenu *et al.*, 1993      NF: not found

<sup>2</sup> From Berthou *et al.*, 1988

N-1 demethylation to theobromine is the major metabolic pathway in the rats and N-3 demethylation to paraxanthine is the major metabolites in adult human hepatocytes in addition to N-1 and N-7 demethylation. All three metabolites are formed in young rats in approximately equal proportion, although N-7 demethylation to theophylline is the only metabolite found in human neonates.

In human neonates and infants, the interconversion between caffeine and theophylline has been reported, i.e. urinary or plasma caffeine levels were approximately 25% of theophylline content after theophylline administration and around 3-8% of caffeine administered was converted to theophylline.

In the neonatal dog (Warszawski *et al.*, 1977) and rabbit (McNamara *et al.*, 1992), clearance of caffeine is reduced and half-life for elimination is increased compared to the adults. Thus, the neonate differs from the adult in all species studied by having greater apparent volume of distribution, longer elimination half-life, slower clearance and a different metabolic profile (in human but not in rat). Therefore, the exposure in the neonate for any given dose of caffeine (mg/m<sup>2</sup> body surface area) would be significantly greater than that in the adult.

Comparative pharmacokinetic data for caffeine in adults and neonates are presented in the following table from the literature.

Species	Age	Parameters (mean $\pm$ SD)			
		T <sub>1/2</sub> (hours)	Vd (l/kg)	Clearance (ml/kg/min)	Ae (unchanged in urine %)
Dog	1 day old	47.6 $\pm$ 5.4	0.94 $\pm$ 0.03	0.28 $\pm$ 0.07	
	1 week old	24.1 $\pm$ 2.0	0.84 $\pm$ 0.04	0.44 $\pm$ 0.05	
	Adult	6.66 $\pm$ 0.85	0.78 $\pm$ 0.05	1.38 $\pm$ 0.15	
Rabbit	19-21 days	9.4 $\pm$ 3.9	0.83 $\pm$ 0.07	1.14 $\pm$ 0.80	
	Adult	2.6 $\pm$ 1.5	0.68 $\pm$ 0.06	3.83 $\pm$ 1.94	
Human	Neonates:				
	0-1 month	97.6 $\pm$ 32.7	0.8-0.9		86%
	1.25-2	75.2 $\pm$ 28.8			
	2.25-3	71.1 $\pm$ 32.3			
	3.25-4	42.8 $\pm$ 25.2			
	4.25-5	28.8 $\pm$ 20.9			
	5.25-6	11.7 $\pm$ 9.9			
7-9	5.2 $\pm$ 5			< 2%	
Human	Adults	4.9 $\pm$ 1.8	0.61 $\pm$ 0.02	1.4 $\pm$ 0.5	1.1 $\pm$ 0.5

The mean fraction of unchanged caffeine excreted in urine (Ae) and the mean T<sub>1/2</sub> are reported to be inversely related to gestational/postconceptual age. It was reported that mean unchanged caffeine accounted for around 86% (within 6 days) in 0-1 month neonates with very small fractions of metabolites found in urine and remained the predominant component for the first 3 months, but its percentage decreased gradually to the adult value of less than 2% by the age of 7 to 9 months.

The data indicate that the 4-day plasma t<sub>1/2</sub> of caffeine characteristic of the newborn depends in large part on slow urinary excretion of unchanged drug since there is little or no metabolism. Subsequent decrease in the t<sub>1/2</sub> to about 4 hours by the age of 8 months correlates closely with the rise in metabolite (Aldridge *et al.*, 1979).

### General Toxicity

Following subcutaneous administration, the  $LD_{50}$  in neonate rats were 220 mg caffeine/kg for 2-3 day old rats and 155 mg caffeine/kg for one week old rats, compared to 265 mg/kg in adult rats (Warszawski *et al.*, 1978). Acute toxicity of caffeine is greater in 2-3 day old rats compared to adult rats and is 30% less than that of theophylline of which  $LD_{50}$  in 2-day old rats was 169 mg/kg and 202 mg/kg for adult rats.

Fourteen-day intragastric administration of 185 mg caffeine/kg/day to 1.5-, 2.5-, 4.5-, and 12-month old male and female rats significantly increased mortality (77 and 69% in male and females, respectively) only in the 12-month old animals (compared to control groups). Morbidity appeared to be greater in the oldest group. In younger animals, caffeine inhibited growth with greater effects in males than in females. Young animals are found to be more sensitive to the diuretic effect of caffeine than older ones and females more than male rats. Caffeine was found to be more toxic in older than in younger rats and in males than in females (Peters and Boyd, 1967).

In female CBL Wistar albino rats, 110 mg caffeine/kg/day was non-lethal in adult rats when administered intragastrically for 100 days, 150 mg/kg/day produced 50% mortality and 191 mg/kg/day was lethal (Boyd *et al.*, 1965). Between 110 and 191 mg/kg/day (dosed up to 100 days), caffeine produced many dose-dependent responses including growth inhibition, polydipsia, and diuresis. Several organ weights were increased. Corresponding histopathological observations included cortical hypertrophy of adrenal glands (a response to stress), hyperemia of the heart and the gut, and mild vascular congestion in the kidneys. Of particular interest, the mucosal hypertrophy, mild hyperemia and moderate inflammation were observed as a dose-dependent phenomena in the stomach, small bowel and cecum, since methylxanthines are implicated in the development of necrotizing enterocolitis in neonates. The  $LD_{50}$  was  $150 \pm 3.1$  mg caffeine/kg, which produces an approximate therapeutic index of 15, based on the nonclinical dose that stimulates respiration (10 mg/kg).

Gans (1984) studied effects of dietary caffeine (0.5% of a pulverized rat chow) on rat testes and thymus gland. In male CD Sprague-Dawley, scattered areas of spermatogenic-cell degeneration was found, with a more prominent response noted following 8-weeks of treatment than following 7-weeks of treatment. Thus, the repeat-dose exposure to high oral doses of caffeine (3.0 g over 7 weeks; 3.6 g over 8 weeks) are toxic to the testes, as shown by spermatogenic-cell degeneration.

When caffeine was given in drinking water for up to two years to Sprague-Dawley rats, there were dose-dependent reductions of more than 10% in terminal body weights at doses  $\geq 50$  mg/kg/day, associated with reduced food and fluid consumption but no changes in survival. In C57BL/6 mice, 55 mg caffeine/kg/day in the diet for up to 80 weeks did not modify survival, body weight gain, or any of the clinical and laboratory parameters monitored or pathological change.

## Special Toxicity

Central Nervous system: Acute as well as chronic administration of caffeine during gestation and/or lactation can produce behavioral responses in the rat. When a single dose of caffeine (20, 40, and 80 mg/kg, S.C.) was administered to 1-day old and 10-day old rat pups (Holloway, 1980), significant decrease in suckling was observed at  $\geq 20$  mg/kg with decrease in weight gain from  $\geq 40$  mg/kg and increased spontaneous activity from  $\geq 40$  mg/kg.

On repeat-dose administration of caffeine (1 and 9 mg/kg, intragastric injection) during postnatal days (PN1-6), transient decreases in weight gain during the first weeks of life were observed but no long-term effects were noted (Zimmerberg *et al.*, 1991). Development of the righting reflex and eye-opening were not altered by either dose of caffeine, but locomotor activity at two weeks (PN14-17) and operant learning in the adult rat were decreased. The result suggests that short-term administration of low doses of caffeine (1 and 9 mg/kg) can have behavioral sequelae later in adult animals following the termination of caffeine exposure.

Effects of transient early caffeine exposure on specific binding of adenosine at the adenosine receptor in the brain regions has been studied in the rat (Guillet and Kellogg, 1991a and 1991b). Caffeine had no effect on body weight or regional brain weight, but there was an increase in specific binding of adenosine in cortex, cerebellum, and hippocampus and no change in either brain stem or hypothalamus. The studies show that a loading dose of 20 mg caffeine/kg (gavage) followed by four daily doses of 15 mg caffeine/kg (gavage) produce changes in specific binding of adenosine in some brain regions and alter locomotor responses to caffeine administered acutely, several weeks after initial neonatal exposure. The plasma concentrations in the study were 5-15  $\mu\text{g/ml}$ .

Therefore, the results from the studies in the rat described above suggest that transient postnatal exposure to caffeine citrate (PN1-6) at doses that are within the proposed therapeutic range and somewhat higher, alters weight gain initially; modifies locomotor activity variably, depending on the age at the time of testing and the type of test; decreases adult operant learning and increases specific binding of adenosine in some brain regions. Guillet and Kellogg (1991b) note that early developmental exposure to caffeine over a limited period of time appears to affect normal adenosine receptor development, and such activity can be demonstrated on both a neurochemical level and a behavioral level long after the exposure period.

Longer term exposure to caffeine has been studied during lactation and/or gestation. Gullberg *et al.* (1986) exposed rat pups to caffeine via dam's milk PN1-21 and found no effects of the pups development. The plasma concentrations of caffeine in the pups was 0.1-0.4  $\mu\text{g/ml}$ . However, exposure to caffeine during gestation and lactation has been found to modify the behavior in juvenile and adult rats (Sobotka *et al.*, 1979; Hughes and Beveridge, 1991). Such exposure also has been shown to modify, brain content of some regions in adult animals (Nakamoto *et al.*, 1988; Yazdani *et al.*, 1988; Nakamoto, 1991).

Cardiovascular Toxicity: Long-term oral administration of 50 mg caffeine/kg/day in the diet to male Sprague-Dawley rats for 117 weeks resulted in major cardiovascular sequelae which shortened life span (caffeine, 78 weeks; control, 94 weeks). Cardiac insufficiency (64% treated vs 17% controls) was the primary cause of death in these animals treated with caffeine. Severe myocardial fibrosis was observed in 11/28 caffeine-treated animals, and 1/29 control animals (Johansson, 1981).

There was no evidence of deterioration in cardiac function when perfused isolated hearts were studied from PN50 animals that had been exposed to 10 mg caffeine/kg/day during gestation and lactation only (weaned PN22). When caffeine was administered directly to pups PN23-50 following earlier exposure, heart weight was unchanged but a significant deterioration in cardiac function was observed. When animals were exposed to 10 mg/kg/day during lactation and to PN88 postweaning, further deterioration occurred as shown by increased heart weight (Temples et al., 1985, 1987). Therefore, cardiotoxicity may be of minimal concern from the short term exposure to caffeine during the neonatal period.

Necrotizing Enterocolitis (NEC): There have been several reports of NEC following xanthine treatment in apneic infants, and the association of methylxanthine treatment for apnea with NEC has been suggested by several authors. When the relationship of xanthine treatment of premature apnea and NEC (Grosfeld et al., 1983) was studied by a bowel ischemia model, aminophylline (theophylline) was found to enhance the bacterial growths and decreased motility causing mucosal necrosis and perforation in rats.

Glavin and Kreuger (1985) studied the effects of prenatal caffeine exposure on offspring - mortality, open-field behavior and adult gastric ulcer susceptibility by oral administration of caffeine via drinking water to pregnant rats throughout gestation. Moderate doses of caffeine (0.017 - 0.034%) administered prenatally produced two striking effects in the study: (a) a marked increase in neonatal mortality and (b) a sensitization to stress-induced gastrointestinal disease as adults (200 days of age).

When caffeine in water (Boyd et al., 1965) was administered to adult female rats for 100 days, there were increased organ weights correlated with mucosal hypertrophy and increased water content in the gut. There were small ulcers in the pyloric stomach region at daily doses of 110 mg/kg and above, but no necrosis was observed.

Caffeine being one of the methylxanthines may be irritating and may have similar effects on the gut as theophylline, although caffeine seems to be slightly (30%) less toxic (Warszawski et al., 1978) than theophylline, and the conversion to theophylline by N-7 demethylation may be low due to undeveloped hepatic microsomal enzymes at birth (Berthou et al., 1988). Therefore, caffeine should be used with caution in apneic neonates, since caffeine has the potential to cause bowel injury as theophylline following the ischemic insults.

## **Carcinogenicity**

In the C57BL/6 mouse, oral administration of 55 mg caffeine/kg/day in the feed for up to 80 weeks did not modify survival, body weight gain, or any of the clinical and laboratory parameters monitored. Gross or histological examination revealed no tumors in the liver, kidney, or urinary bladder from caffeine-treated animals (Macklin and Szot, 1980). Other tissues were not examined microscopically, since identification of phenacetin toxicity was the primary objective of this study.

Sprague-Dawley rats were exposed to caffeine in drinking water for up to 102 weeks (Mohr *et al.*, 1984). Males received 15, 26, 49, and 102 mg/kg/day; females received 15, 37, 80, and 170 mg/kg/day. In both males (49 and 102 mg/kg/day) and females (80 and 170 mg/kg/day), high doses of caffeine compromised weight gain in a dose-dependent manner.

There were increased thyroid adenocarcinomas and mammary adenocarcinomas in females only; however, there was no dose-response relationship with the incidence occurring only in the low and mid dose groups. Therefore, tumor incidence in Sprague-Dawley rats was considered not modified by daily administration of caffeine in the drinking water at levels up to 2000 mg/l (average dose in males 102 mg/kg/day, and in females 170 mg/kg/day), respectively, which are 10 and 17 times, respectively based on mg/kg or 2 and 4 times based on mg/m<sup>2</sup> the dose recommended for treating apnea of prematurity (10 mg/kg).

Thus, no carcinogenic activity was observed following oral administration of caffeine in either C57BL/6 mice (55 mg/kg/day in the feed) for 80 weeks or Sprague-Dawley rats (102 and 170 mg/kg/day in the drinking water, males and females, respectively) for 24 months. There was no evidence of carcinogenicity following long-term daily oral administration of caffeine in doses 5 (mouse) to 10 (rat) times the proposed loading dose of caffeine (10 mg/kg) for treating apnea of prematurity, based on mg/kg dose comparison. These caffeine doses are approximately equal (mouse) to 2 times (rat) the maximum recommended loading IV dose for children on a mg/m<sup>2</sup> basis. No causal relationship could be established between caffeine intake and cancer finding from the additional literature search.

## **Reproductive Toxicity**

*Fertility and reproductive function in male and female.* Caffeine was reported to cause embryotoxicity when administered to male rats at a dose of 5 mg/kg/day, S.C. for four days prior to mating with untreated females. At 50 mg/kg/day, S.C. for four days, caffeine was reported to decrease male reproductive performance in addition to causing embryotoxicity (Soyka and Joffe, 1980). In addition, repeat-dose exposure to high oral doses of caffeine (3.0g over 7 weeks) was toxic to rat testes with spermatogenic-cell degeneration. Daily administration of 67 mg/kg/day to female mice had no effect on reproductive parameters, but was toxic to the offspring (Nagasawa, 1986).

*Developmental toxicology and teratology.* When caffeine was administered to pregnant mice in doses up to 242 mg/kg/day in drinking water or up to 150 mg/kg/day in sustained release pellets (Elmazar *et al.*, 1982), caffeine was not teratogenic apart from a low incidence of cleft palate (2/209 for 50 mg/kg and 3/137 for 150 mg/kg) in the pellet groups and retarded ossification particularly of the supraoccipital bones in the fetuses from the group of 150 mg caffeine in drinking water. In another study by Collins *et al.*, 1983, daily administration of caffeine via drinking water to pregnant rats during day 1-20 of gestation, caffeine produced maternal toxicity and fetotoxicity at doses  $\geq$  86.6 mg/kg and embryotoxicity at dose  $\geq$  160.9 mg/kg, but caffeine was not teratogenic at doses of up to 204.5 mg/kg/day in drinking water.

*Perinatal and postnatal toxicity.* In rats, gestational exposure at  $\leq$ 35.0 mg/kg/day had no effect on perinatal parameters. Birth weight was decreased slightly at 62.3 mg caffeine/kg/day, and gastric ulcer size and incidence in response to fasting-restraint-cold stress was increased in adult rats following gestational exposures at 25 and 35 mg caffeine/kg/day, respectively. Reproductive failure was also observed in female monkeys following daily exposure to caffeine before and during pregnancy (10-15 mg/kg/day; 25-35 mg/kg/day).

However, there was no association between caffeine and male fertility or teratogenicity in humans from the literature, and the reproductive toxicity is not a relevant concern for the patient population (infant) for whom the caffeine is indicated.

### Mutagenicity

*In vitro* studies with millimolar concentrations of caffeine had no mutagenic activity in CHO/HGPRT or chromosomal aberrations in CHO cells and cultured human lymphocytes, in the absence of exogenous metabolic activation. However, caffeine potentiated the mitomycin C-induced chromosomal aberrations and sister chromatid exchange.

In two *in vivo* studies, caffeine and metabolites were negative in the mouse bone marrow micronucleus assay at doses of 75 and 150 mg/kg by IP for four days. However, in folate-deficient mice, there was a 5-fold increase in micronuclei formation by caffeine. Caffeine was found to increase SCE/cell metaphase (proportional to duration) in Swiss albino mice when administered orally in drinking water for 5, 10, or 15 days. Thus, with the exception of one *in vivo* study where the effect of caffeine/metabolites to induce SCE was positive, there is no evidence presented for mutagenic activity of caffeine, even at the high concentrations/doses studied.

There are numerous mutagenicity studies in the literature where caffeine has been tested. In general, the exposure levels in mutagenicity studies ( $10^3$ M-range) greatly exceed human-exposure levels ( $10^{-6}$ - $10^{-5}$ M), and most of the literature deals with interaction studies where the effect of caffeine on DNA damaged by known genotoxins was assessed. Based on *in vitro* and *in vivo* findings, caffeine alone does not appear to be mutagenic even at high concentrations/dose.

However, caffeine has been shown to enhance mutagenic activity of known genotoxins (D'Ambrosio *et al.*, 1994; Legator and Zimmering, 1979; Roberts, 1984) and to increase the micronuclei formation (5-fold) in folate-deficient mice (MacGregor *et al.*, 1990).

## 9. Conclusion and Recommendations

The extensive nonclinical information submitted offers adequate support for the therapeutic indication (apnea of prematurity) and the safety of the proposed clinical dose of caffeine citrate at 10 mg/kg, IV followed by 2.5 mg/kg for maintenance.

Results of acute and repeat-dose toxicity studies suggest that neither morbidity nor mortality is a major risk at the doses proposed. Neither carcinogenicity nor mutagenicity has been observed in animals following long-term repeat-dose administration of caffeine at 5 to 10 times the proposed loading dose based on mg/kg comparison or approximately equal to 2 times the maximum recommended loading IV dose for children on a mg/m<sup>2</sup> basis.

Studies of caffeine actions in other organ systems suggest that the risk of cardiotoxicity or renal toxicity may be low in the proposed therapeutic dosage range. Caffeine may have similar irritating effects on the gut as theophylline and may cause mucosal necrosis and perforation (NEC), although caffeine seems to be slightly less toxic than theophylline and the conversion to theophylline from caffeine by N-7 demethylation is low (3-8%) due to undeveloped hepatic microsomal enzymes at birth.

Caffeine reduced male fertility (50 mg/kg/day, S.C. for 5 days) in rats, and repeat-dose exposure to high oral doses of caffeine (3.0g over 7 weeks) are toxic to rat testes with spermatogenic-cell degeneration. However, there was no association between caffeine and male fertility in humans in the literature, and this is not a relevant concern for the patient population for whom the caffeine is indicated.

The high dose of caffeine cause convulsion at 150 mg/kg in rats and 75 mg/kg in dogs, and low dose of 10-20 mg/kg can cause behavioral effects in rats. Behavioral changes in rats have been associated with short-term administration of caffeine in the therapeutic dosage range (1 and 9 mg/kg/day for six days). Changes were observed variably in suckling, locomotor activity, and operant learning in adults. Alterations in specific binding of adenosine in certain brain areas also have been associated with short-term administration of caffeine (15-20 mg/kg/day for 5 days).

Thus, there seems to be some risk associated with the use of caffeine in the treatment of apnea of prematurity based on results of nonclinical studies from the literature. However, the risks associated with alternatives or non treatment may be greater. Therefore, it is recommended that caffeine citrate injection be approved for the treatment of apnea of prematurity at the proposed dosing based on the clinical risk/benefit analysis.

## 10. Labeling

Nonclinical sections in the Proposed Labeling should be revised as follows:

### Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 2-year study in Sprague-Dawley rats, caffeine was not carcinogenic in male rats at doses up to 102 mg/kg or in female rats up to 170 mg/kg (approximately 2 and 4 times, respectively, the maximum recommended loading IV dose for [redacted] on a mg/m<sup>2</sup> basis). In an 18-month study in C57BL/6 mice, no evidence of tumorigenicity was seen at dietary doses up to 55 mg/kg

[redacted]

Caffeine increased the SCE/cell metaphase (exposure time dependent) in an *in vivo* mouse metaphase analysis. Caffeine also potentiated the genotoxicity of known mutagens and enhanced the micronuclei formation (5-fold) in folate-deficient mice. However, caffeine did not increase chromosomal aberrations in an *in vitro* CHO and Human lymphocyte assays and was not mutagenic in *in vitro* CHO/HGPRT gene mutation assay, except at cytotoxic concentrations. In addition, caffeine was not clastogenic in an *in vivo* mouse micronucleus assay.

Caffeine administered to male rats at 50 mg/kg/day subcutaneously (approximately equal to the maximum recommended loading IV dose for children on a mg/m<sup>2</sup> basis) for four days prior to mating with untreated females, caused decreased male reproductive performance in addition to causing embryotoxicity. In addition, long-term exposure to high oral doses of caffeine (3.0 g over 7 weeks) was toxic to rat testes with spermatogenic-cell degeneration.

### Pregnancy: Pregnancy Category C

The concern for the teratogenicity of caffeine is not relevant to infants. In studies performed in adult animals, caffeine administered to pregnant mice in

[redacted] at 50 mg/kg [redacted] caused a low incidence of cleft palate and exencephaly in the fetuses. There are no adequate and well-controlled studies in pregnant women.

APPEARS THIS WAY  
ON ORIGINAL

# BEST POSSIBLE COPY

NDA 20-793  
Page 90

APPEARS THIS WAY  
ON ORIGINAL

/S/

12/16/97

Misoon Y. Chun, Pharm.D., D.A.B.T.  
Pharmacologist/Toxicologist, HFD-570

- cc: NDA 20-793  
HFD-570/Div.File  
HFD-570/LPina  
HFD-570/LCobbs  
HFD-570/MChun, 12/4/97, 12/16/97  
HFD-570/MHimmel  
HFD-570/HSheevers, 11/14/97, 12/12/97

/S/

12/15/97

APPEARS THIS WAY  
ON ORIGINAL

Drug: **cafcit**

	age	mg/dose	# daily	doses	mg/da	kg	mg/kg	factor	mg/m <sup>2</sup>
Pediatric dose	0	30	1	30	3	10.00	25	250.00	
Adult dose	>12			0	50	0.00	37	0.00	

	route	mg/kg/day	factor	mg/m <sup>2</sup>	Dose Ratio		Rounded Dose Ratio	
					Adults	Children	Adults	Children
<b>Carcinogenicity:</b>								
mouse	m & f	55	3	165	—	0.66	—	1/2
rat	m	102	6	612	—	2.45	—	2
rat	f	170	6	1020	—	4.08	—	4
hamster			4	0	—	—	—	—
extra			—	—	—	—	—	—
<b>Reproduction and Fertility:</b>								
rat			6	0	—	N/A	—	N/A
rat			6	0	—	N/A	—	N/A
mouse			3	0	—	N/A	—	N/A
extra			—	—	—	N/A	—	N/A
<b>Teratogenicity:</b>								
rat			6	0	—	N/A	—	N/A
rat			6	0	—	N/A	—	N/A
rabbit			12	0	—	N/A	—	N/A
rabbit			12	0	—	N/A	—	N/A
mouse			3	0	—	N/A	—	N/A
<b>Overdosage:</b>								
mouse			3	0	—	—	—	—
mouse			3	0	—	—	—	—
rat			6	0	—	—	—	—
rat			6	0	—	—	—	—
<b>Other: (Describe studies here)</b>								
dog			20	0	—	—	—	—
monkey			12	0	—	—	—	—
rabbit			12	0	—	—	—	—
extra			—	—	—	—	—	—
extra			—	—	—	—	—	—

**Conversion, Correction, and Rounding Factors:**

Human Age (yr)	Weight (kg)	Factor (kg/m <sup>2</sup> )	Species	Factor (kg/m <sup>2</sup> )	Exposure greater than x-times human	Round to nearest
0	3	25	dog	20	1	1
1	10	25	guinea pig	8	10	5
2	12	25	hamster	4	100	10
4	16	25	monkey	12	1000	100
6	20	25	mouse	3	10000	1000
12	50	37	rabbit	12		
			rat	6		