Integrated Laboratory Systems

Caffeine and Its Modulating Effects
[58-08-2]

Draft Review of Toxicological Literature

Prepared for

Errol Zeiger, Ph.D.
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709
Contract No. N01-ES-65402

Submitted by

Raymond Tice, Ph.D. (Principal Investigator)
Brigette Brevard, M.A. (Co-Principal Investigator)
Integrated Laboratory Systems
P.O. Box 13501
Research Triangle Park, North Carolina 27709

January 1999

EXECUTIVE SUMMARY

Caffeine was nominated by Drs. B. Ames, L. Gold, and T. Slone of Lawrence Berkeley Laboratories (Berkeley, CA) for testing based on its natural occurrence at high concentrations in food and the lack of carcinogenicity data.

Caffeine occurs naturally in over 60 plant species including tea, cacao, coffee, and cola. It is produced commercially by extraction and synthetic procedures. The majority of caffeine is used in the beverage industry. It is also used as a flavor enhancer in foods and as a flavoring agent in baked goods, frozen dairy desserts, gelatins, puddings, fillings, and soft candy. Caffeine is also used therapeutically.

In the United States, it has been detected in air, soil, and water, including drinking water and industrial effluents. It may be released into the environment as an escaped emission during its production and use, or via wastewater effluent, landfill leachate, or incinerator ash. It has an atmospheric half-life of ~2.5 hours.

The general population is exposed to caffeine via the ingestion of caffeine-containing drinks, foods, medicine, or drinking water. Humans ingest approximately seven billion kilograms of caffeine yearly, with ~90% consumed in the form of coffee or tea. The average daily caffeine intake in the United States is ~200 mg per individual. Occupationally, ~19,140 workers may have been exposed to caffeine from 1981 to 1983. Caffeine is regulated by the Food and Drug Administration (FDA).

Data from a number of epidemiologic studies on the long-term health effects of caffeine concluded that no marked elevations in risk have been found in the majority of studies for most diseases, including cardiovascular disease, ulcer, breast disease, and for effects on various target organs. In many of the studies, confounding factors, such as tobacco smoking, sex, and age were controlled for.

Absorption of caffeine from the gastrointestinal tract is pH-dependent, rapid, and virtually complete. After absorption, it is distributed rapidly into body fluids. It binds to plasma proteins, mainly albumins. Metabolism is by hepatic microsomal enzymes and does not appear to occur significantly in other organs. In humans, caffeine is metabolized into more than 25 metabolites, primarily paraxanthine, theobromine, and theophylline.

In humans, acute exposure to caffeine can cause gastric symptoms, insomnia, diuresis, restlessness, headache, and tremors. At concentrations up to 2 μg/mL (10 nmol/mL) in blood, caffeine stimulates the central nervous system (CNS). The oral LD_{50} is 127 mg/kg (0.654 mmol/kg) in mice, 192 mg/kg (0.989 mmol/kg) in rats, 230 mg/kg (1.18 mmol/kg) in hamsters and guinea pigs, and 150 mg/kg (0.772 mmol/kg) in rabbits. Symptoms of acute exposure in animals include convulsions, excitement, gastroenteritis, and edema of the liver, heart, lungs, spleen, and adrenal glands. No data on short-term and subchronic effects were located and there is limited data on the chronic effects of

caffeine. In a study reviewed by the International Agency for Research on Cancer (IARC), rats exposed to caffeine in drinking water for 104 weeks showed decreased body weight at the highest dose; a slight increase in mortality was observed in males.

Several epidemiologic studies reviewed by IARC found a correlation between congenital malformations and caffeine consumption; however, other reviews have concluded that studies on the effect of coffee (caffeine) intake on fertility and premature births were largely inconclusive.

In animal carcinogenicity studies, no significant increased incidence of bladder or mammary gland tumors were noted. Caffeine was shown to stimulate mammary gland lobulo-alveolar development and secretion. The development of mammary gland tumors was either stimulated or suppressed depending upon the animal species and strain, and the stage of tumorigenesis (initiation/promotion) at which caffeine was administered. Rats given caffeine in solution orally for 12 months showed decreased body weight and an increase in the weight of the pituitary gland caused by hyperplasia or the presence of a pituitary adenoma. Caffeine increased the survival time of Ehrlich ascites-bearing mice given adriamycin for 5 days by 39%.

In co-treatment studies, caffeine had no effect on the incidence of bladder tumors induced in rats by N-nitroso-N-butyl(4-hydroxybutyl)amine nor did it have an effect on 4-hydroxyaminoquinoline 1-oxide-induced pancreatic tumors in rats. However, caffeine did decrease the incidence of urethane-induced lung tumors and diethylstilbestrol-induced mammary tumors in mice. Caffeine appears to have a weak, yet significant potentiating effect on N-methyl-N-nitrosourea (MNU)-initiated rat urothelial tumors. In a study of urethane-induced skin tumorigenesis, caffeine significantly increased the incidence of papillomas when given 6 hours before initiation with urethane, but showed an inhibitory effect when given 6 hours after urethane initiation. In another study, adding caffeine to decaffeinated teas restored their inhibitory effects on UVB-induced skin carcinogenesis.

Prior to 1991, caffeine had been extensively studied for genotoxicity using a variety of acellular, bacterial, plant, *in vitro* mammalian cell, and *in vivo* test systems. Most *in vitro* tests, with the exception of *Salmonella typhimurium* gene mutation assays, were conducted in the absence of exogenous metabolic activation only. Caffeine was positive for differential toxicity in *Escherichia coli*, but was negative in the *Bacillus subtilis* rec assay. Various strains of *S. typhimurium* were tested with and without metabolic activation; all results were negative. Caffeine induced DNA single-strand breaks (SSB), double-strand breaks (DSB), and DNA-protein crosslinks in isolated L1210 cell nuclei and inhibited the formation of DSB induced by the topoisomerase II inhibitor ellipticine. Caffeine induced large scale deletions in the TK gene region in mouse L5178Y cells in the absence of S9. It was negative for fragile-X induction, but positive for induction of common fragile sites in human blood

lymphocytes. When present during DNA synthesis, it induced chromosomal aberrations and sister chromatid exchanges in cultured mammalian cells. Caffeine was a weak mutagen in the Drosophila white-ivory eye spot test. Caffeine did not induce chromosomal aberrations *in vivo* in mammals and the majority of results from micronucleus tests were negative. *In vivo*, caffeine had no significant effect on the frequency of aneuploidy or structural chromosomal aberrations in mouse oocytes or on oocyte maturation in female mice given caffeine i.p. Human population studies are in conflict on the association between caffeine intake and increased levels of chromosomal or DNA damage.

Without metabolic activation, caffeine administered prior to treatment markedly enhanced the ability of *N*-methyl-*N* -nitro-*N*-nitrosoguanidine (MNNG) to induce mutants in *S. typhimurium* strain TA1535, but reduced the mutagenicity of MNNG and *N*-methyl-*N*-nitrosourea (MNU) when co-administered or administered after treatment. In the yeast *S. cerevisiae* D5, the addition of caffeine with the antitumor agent N-[2-dimethylamino) ethyl]acridine-4-carboxamide (DCA) caused a significant decrease in the incidence of all types of mutations in yeast colonies. Caffeine synergistically enhanced the frequency of chromosomal aberrations induced by camptothecin in *Vicia faba* root tip cells. In mammalian cells, *in vitro and in vivo*, caffeine either enhanced or reduced DNA and chromosomal damage, micronuclei, and mutations induced by other chemical and physical agents.

Caffeine is cytotoxic in cultured mammalian cells at high concentrations. Studies have demonstrated the ability of millimolar concentrations of caffeine to inhibit cell proliferation in mitogen-stimulated human lymphocytes and to delay the entry of HeLa S3 cells into S-phase.

The ability of caffeine to modulate the cytotoxicity of genotoxic agents and specifically, antineoplastic agents, has been evaluated extensively. Caffeine has been shown to reduce the cytotoxicity of DNA damaging agents; however, in most studies, caffeine potentiated genotoxin-induced cell killing. There are also studies in which caffeine neither reduced nor potentiated chemical-induced cytotoxicity.

A number of investigations have demonstrated the ability of caffeine to alter cell cycle arrest in eukaryote cells induced by genotoxic agents. It is this mechanism that has been invoked most frequently as the means by which caffeine enhances clastogen-induced chromosomal damage and cytotoxicity. The modulation of cell cycle arrest by caffeine is cell-type and stage specific.

The antioxidant behavior of caffeine was suggested to be a possible mechanism for the reported anticarcinogenic effects of caffeine.

In an investigation of structure-activity relationship, 7-deazanthine derivatives compared to caffeine showed the same or more diuretic activity and more cardiac

activity. Results indicated that the nitrogen atom at the 7-position of the 7-deazanthine derivatives plays an important role in caffeine-like pharmacological activities.

TABLE OF CONTENTS

TABLES

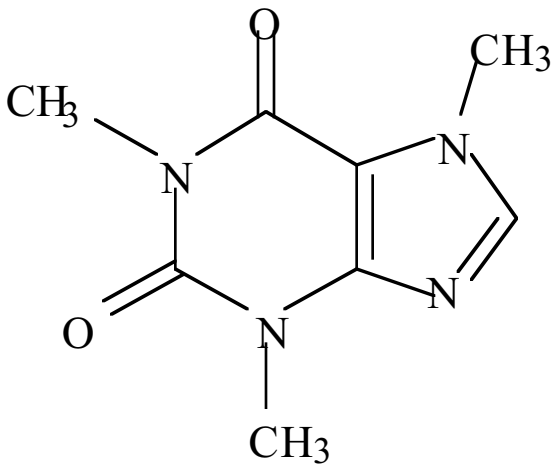
1.0 BASIS FOR NOMINATION

The nomination of caffeine by Drs. B. Ames, L. Gold, and T. Slone of Lawrence Berkeley Laboratories (Berkeley, CA) is based on its natural occurrence at high concentrations in food and the possible lack of adequate carcinogenicity test data.

2.0 INTRODUCTION

Caffeine

[58-08-2]



2.1 Chemical Identification

Caffeine ($C_8H_{10}N_4O_2$; mol. wt. = 194.19) is also called:

3,7-Dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione (9CI)
Anhydrous caffeine
Coffeine
Coffeinum
Guaranine
Methyltheobromine
Methyltheophylline
Thein
Theine
1,3,7-Trimethyl-2,6-dioxopurine
1,3,7-Trimethylxanthine

Trade names include: Caffeedrine, Dexitac, No Doz, Quick Pep, Tirend, and Vivarin (HSDB, 1997; Budavari, 1996; IARC, 1991).

2.2 Physical-Chemical Properties

Property	Information	Reference
Physical State	Glistening white needles	National Research Council (1981); Gennaro (1985); cited by IARC (1991)
Odor	Odorless	McElvoy (1989); cited by HSDB (1997)
Taste	Slightly bitter	McElvoy (1989); cited by HSDB (1997)
Sublimation Point (^{o}C)	178	Budavari (1996)
Melting Point (^{o}C)	238	Budavari (1996)
Density (18 ^{o}C/4^{o}C)	1.23	Budavari (1996)
Soluble in:	Acetone, benzene, chloroform, diethyl ether, ethanol, ethyl acetate pyrrole, tetrahydro-furan, water	IARC (1991); Budavari (1996)
Slightly soluble in:	Petroleum ether	Budavari (1996)

2.3 Commercial Availability

Caffeine for commercial use may be purchased from the following U.S. producers: Pfizer Inc. (HSDB, 1997); American Bio-Synthetic Corp.; Bell Flavors and Fragrances, Inc.; Certified Processing Corp.; Cultor Food Sciences, Inc. (SRI, 1997); Alfa Aesar; Allan Chemical Corp.; American Tartaric Products, Inc.; Ashland Chemical Co.; Barrington Chemical Corp.; BASF Fine Chemicals; B. I. Chemicals, Inc.; Chart Corp., Inc.; Mijac, Inc.; and SKW Chemicals, Inc. (Rodnan, 1997).

3.0 PRODUCTION PROCESSES AND ANALYSES

Caffeine is produced commercially by extraction and synthetic procedures. Extraction occurs by three methods: direct decaffeination of green coffee beans with water and solvents such as trichloroethylene and dichloromethane (McCutheon, 1969; cited by IARC, 1991), extraction from tea dusts and wastes and fragments of tea leaves (Menthe, 1985; cited by IARC, 1991) using pressurized carbon dioxide (Anon., 1986; cited by IARC, 1991), and extraction from cola nuts (Halsey and Johnston, 1987; cited by IARC, 1997).

Synthetic production of caffeine involves methylation of xanthines, mainly theobromine,

(Halsey and Johnston, 1987; cited by IARC, 1991) and theophylline (Stanovnik et al., 1982; Nesterov et al., 1985; both cited by IARC, 1991) or the reaction of theophylline with carbon monoxide and methanol (Bott, 1982; cited by IARC, 1991). Total synthesis is obtained using dimethyl carbamide and malonic acid (Anon., 1987b; cited by IARC, 1991).

Until the mid 1970s, ultraviolet spectroscopy was the technique employed for the analysis of caffeine in biological fluids (Axelrod and Reichenthal, 1953; Routh et al., 1969; both cited by IARC, 1991). Thin-layer chromatography (Welch et al., 1977; Riechert, 1978; Bradbrook et al., 1979; all cited by IARC, 1991), gas chromatography (Grab and Reinstein, 1968; Demas and Statland, 1977; Bradbrook et al., 1979; all cited by IARC, 1991), and gas chromatography-mass spectrometry (Merriman et al., 1978; cited by IARC, 1991) were subsequently used as a means for determining levels of caffeine in biological fluids. Currently, high performance liquid chromatography (HPLC) is the technique used most frequently for the analysis of caffeine and its metabolites and for the separation of caffeine from other xanthines and drugs in biological fluids, as well as in foods and beverages (IARC, 1991). Other procedures include chromatography on ion-exchange resins (Walton et al., 1979; cited by IARC, 1991), radioimmunoassays (Cook et al., 1976; cited by IARC, 1991), and enzyme immunoassay techniques (Aranda et al., 1987; cited by IARC, 1991).

4.0 PRODUCTION AND IMPORT VOLUMES

Caffeine is included on the U.S. Environmental Protection Agency s (EPA) Office of Pollution Prevention and Toxics (OPPT) High Production Volume Chemicals list with an estimated annual production volume of 3,397,842 to 5,615,973 lb (1,550 to 2,550 thousand kg) (USEPA, 1998). In 1962, production of caffeine in the United States totaled 1,959,000 lb (889,400 kg) and imports totaled 1,807,000 lb (820,400 kg) (Huber, 1964; cited by IARC, 1991). By 1988/89, imports had more than tripled to 6,345,310 lb (2,900 thousand kg) (U.S. Bureau of the Census, 1989; cited by IARC, 1991).

5.0 USES

The majority of caffeine (80-90%) is used in the beverage industry (Anon., 1987a, 1988; cited by IARC, 1991). It is also used as a flavor enhancer in foods and as a flavoring agent in baked goods, frozen dairy desserts, gelatins, puddings, fillings, and soft candy (Anon., 1987b; cited by IARC, 1991).

The remaining 10 to 20 percent of caffeine is used therapeutically. In combination with an analgesic such as aspirin or phenacetin, caffeine provides relief of headaches and menstrual tension (HSDB, 1997; IARC, 1991); in combination with an ergot alkaloid, it remedies migraines (HSDB, 1997; IARC, 1991); and, in combination with some antihistamines, it helps overcome their sedative effects (IARC, 1991). Caffeine has also been used intravenously (i.v.) in the treatment of prolonged apnea in preterm infants (HSDB, 1997; IARC, 1991), to control asthma, and to relieve bronchial spasms (Stavric, 1988; cited by IARC, 1991). Other therapeutic uses of caffeinated coffee include orthostatic and postprandial hypotension therapy (Pagano et al., 1988; Ahmad and Watson, 1990; Heseltine et al., 1991; all cited by Etherton and Kochar, 1993).

Studies have been conducted for over a century to evaluate a possible link between intake of caffeine and enhancement of exercise performance. Although ingestion of caffeine has been reported to enhance athletic performance in some studies (Jacobsen and Kulling, 1989; Tarnopolsky et al., 1988; Williams, 1992; all cited by Lamarine, 1994), a consensus regarding the benefits of the compound as an ergogenic aid has not been established (Lamarine, 1994). Research has also been conducted to assess caffeine s ability to help against obesity (Dulloo et al., 1989; cited by Lamarine, 1994).

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Caffeine occurs naturally in over 60 plant species, including tea, cacao, coffee, and cola (Hoskins, 1994). The caffeine content varies according to the plant species and growing conditions. For example, the level of caffeine in tea leaves is affected by the season, its genetic origin, and the use of nitrogen in fertilizers. The caffeine content in tea can be as high as 5%, but the average caffeine level in tea sold in the United States is about 3.0% (Graham, 1984a; Gilbert,

1984; both cited by IARC, 1991). In cacao plants, the bean is the main storage site for caffeine, with only trace amounts (concentrations not specified) present in the leaves and pods (Somorin, 1974; cited by IARC 1991). The dry green beans of arabica and robusta coffees contain caffeine at levels of 0.9-1.4% and 1.5-2.6%, respectively. Darkly roasted coffee beans may contain about 20% more caffeine by weight than green beans (Gilbert, 1981, cited by IARC, 1991). Caffeine occurs in the *Ilex paraguariensis* plant from which the South American beverage mat is prepared, and in other plants of the holly species. Caffeine levels in mat vary from 0.9 to 2.2%. The age of the leaf determines the caffeine content; young, growing leaves contain higher levels of caffeine than older leaves (Graham, 1984b; cited by IARC, 1991).

In the United States, caffeine has been detected in air, soil, and water (including drinking water and industrial effluents). Caffeine may be released into the environment as an escaped emission during its production and use, or via wastewater effluent, landfill leachate, or incinerator ash (HSDB, 1997). When released into the atmosphere, caffeine will exist absorbed to particulates; however, it may also undergo a gas-phase reaction with photochemically produced hydroxyl radicals, with an estimated half-life of 2.5 hours (HSDB, 1997). Caffeine has been detected in the air of New York and New Jersey due to emissions from coffee roasting plants (levels not reported) (Dong et al., 1977; cited by IARC, 1991). If released into the soil or water, caffeine will not volatilize into the atmosphere, but will biodegrade under aerobic conditions (HSDB, 1997).

7.0 HUMAN EXPOSURE

The general population is exposed to caffeine via the ingestion of caffeine-containing drinks, foods, medicine, or consumer products and also by the ingestion of contaminated drinking water (HSDB, 1997). It has been estimated that the human population ingests over seven billion kilograms of caffeine yearly (D Ambrosio, 1994), with approximately 90% of caffeine consumed in the form of coffee or tea (Gilbert, 1984; cited by IARC, 1991). Average daily caffeine intake in the United States has been estimated at 200 mg per individual on the basis of total U.S. consumption, although levels as high as 1022 mg/day (Stavric et al., 1988; cited by IARC, 1991)

have been reported. A survey by Morgan et al. (1982; cited by IARC, 1991) estimated the mean daily caffeine intake to be approximately 3 mg/kg body weight for all adults in the general population.

Occupational exposure to caffeine can occur via inhalation of dust or through dermal contact during its production or formulation (HSDB, 1997). A 1981-1983 National Institute for Occupational Safety and Health (NIOSH) survey estimated that approximately 19,140 workers may have been occupationally exposed to caffeine. An earlier NIOSH survey (1972-1974) estimated that 5,718 workers were exposed to caffeine (HSDB, 1997). No recent data on occupational exposure were located.

8.0 REGULATORY STATUS

U.S. government regulations pertaining to caffeine are summarized in **Table 1**.

Table 1. Regulations Relevant to Caffeine*

	Regulation	Summary of Regulation
FDA	21 CFR 182.1180 Subpart B	This section pertains to substances generally recognized as safe (GRAS) for their intended use (21 CFR 330.1). Subpart B specifically describes limits and conditions for the addition of multiple purpose GRAS food substances to foods. Caffeine is generally recognized as safe when used in cola-type beverages in accordance with good manufacturing practice with a tolerance of 0.02%.
	21 CFR 310.545 Subpart E	This section lists over-the-counter (OTC) drugs that contain certain active ingredients, but based on evidence currently available, there are inadequate data to establish general recognition of the safety and effectiveness of these ingredients for their specified uses. Caffeine appears as an ingredient in weight control drug products .
	21 CFR 340 Subpart A	Sets conditions for an oral OTC stimulant drug to be safe and effective and not misbranded . The intended use of a stimulant drug is to restore mental alertness or wakefulness during fatigue or drowsiness .
	21 CFR 340 Subpart B and C	Describes labeling of the stimulant drug product, caffeine. The label of a stimulant product containing caffeine should contain these elements: 1) Statement of identity - names the drug and identifies the product as an alertness aid or a stimulant 2) Indications — as a stimulant or used as provided in 21 CFR 330.1 3) Warnings: a) limit use of other caffeine containing substances b) use occasionally c) not for children under 12 years of age 4) Directions — adults and children 12 years of age and over: oral dosage is 100-200 mg not more often than every 3 to 4 hours

*Source: CPI, 1998.

9.0 TOXICOLOGICAL DATA

9.1 General Toxicology

9.1.1 Human Toxicological Data

The details of studies that have not previously been reviewed are presented in **Table 2**.

Ernster (1984) reviewed available data from a number of epidemiologic studies on the long-term health effects of caffeine and concluded that no marked elevations in risk have been found in the majority of studies for most diseases, including cardiovascular disease, ulcer, benign breast disease, and pregnancy outcome. For studies that showed elevated relative risks (RR), dose-response relationships with caffeine were not demonstrated.

Etherton and Kochar (1993) reviewed the literature on coffee (of which the primary component of physiological concern is caffeine) and its potential effects in humans, including cardiovascular, gastrointestinal, fertility, and pregnancy. The authors concluded that when consumed in moderation (fewer than 4 cups/day), coffee poses no threat to most people and the potential dangers of caffeine could be diminished by drinking decaffeinated, filter-brewed coffee. Many of the epidemiologic studies reviewed by Etherton and Kochar were deemed inconclusive. Additional epidemiological data on cardiovascular disease, breast cancer, pregnancy outcome, and effects on various target organs are discussed in detail below.

9.1.1.1 Bladder and Renal

There have been several reviews of studies investigating the association between coffee and caffeine consumption and the risk of bladder cancer. Many studies not addressed in IARC (1991) have been reviewed by other authors and are discussed below.

Studies Reviewed by Wallace (1971)

Wallace (1971), in examining the causes, assessment, and treatment of urothelial neoplasms (bladder tumors), noted that certain locations in Yugoslavia and Sweden have increased rates of nephropathy (papillary necrosis), which are associated with the abuse of phenacetin/caffeine powders. These same patients had increased rates of bladder and upper

respiratory tract tumors, although a causative agent was not identified. The details of these studies were not provided.

Studies Reviewed by Hopkins (1984)

Studies of cancer associated with coffee consumption were reviewed by Hopkins (1984). The association of cancer of the lower urinary tract with coffee consumption was first made by Cole (1971, cited by Hopkins, 1984), and soon after by Simon et al. (1975, cited by Hopkins, 1984). Cole et al. (1971) reported that coffee was responsible for bladder, renal cell, or ureter cancer in as many as half of all cases in women, and nearly one-fourth of all cases in men (although the incidence did not appear to be dose-related) and estimated that consumption of 1, 2-3, and 4 cups of coffee per day in women was associated with risks of 1.60, 3.76, and 2.19, respectively, compared to women who consumed less than one cup of coffee/day. Simon et al. (1975) estimated that consumption of more than 1 cup of coffee per day was associated with a risk of 2.1 (95% Confidence Interval (CI)=1.1-4.3). The studies all reported increased incidences of bladder cancer, but failed to present a clear, dose-response relationship. Studies by Howe et al. (1980, cited by Hopkins, 1984) and Mettlin and Graham (1979, cited by Hopkins, 1984) demonstrated the bladder carcinogenicity of coffee in males versus females more clearly. Howe et al. (1980) reported that the risk of developing bladder cancer was 1.4 (0.9-2.0) for all types of coffee, 1.5 (1.0-2.2) for regular coffee, and 1.5 (1.1-2.0) for instant coffee. A statistically significant risk of bladder cancer was associated with the consumption of 2 or more cups of coffee/day in men, and more than 3 cups of coffee/day in women, although in women, there was no difference in the increased risk of cancer between drinking 3-4 cups and 5 cups of coffee/day. Mettlin and Graham (1979) found that men who drank more than 3 cups of coffee per day had a cancer risk of 2.11; however, differences in amounts of coffee consumption and bladder cancer rates, although dose-dependent, were not statistically significant. Statistically significant, dose-dependent associations between increases in coffee consumption and corresponding increases in bladder cancer rates were reported in men, but not in women (Bross and Tidings, 1973; Wynder and Goldsmith, 1977; Morrison et al., 1982; all cited by Hopkins, 1984).

Marrett et al. (1983, cited by Hopkins, 1984) found that relative cancer risk in coffee drinkers (n=516) compared to non-drinkers was 1.5 (0.5-5.4) in women and 4.0 (1.2-2.08) in men. However, these results were not controlled for smoking. Drinking more than 7 cups of coffee per day was associated with increased risk of bladder cancer in all groups except the female 65 and older group. In a larger study of about 3,000 patients, risk from a lifetime intake of greater than 100 cups of coffee (adjusting for gender, age, race, and area of residence) was 1.8, which was lowered to 1.4 (1.1-1.8) when smoking was taken into consideration (Hartge et al., 1983, cited by Hopkins, 1984). Both values were statistically significant. Relative risk was not dependent on the duration of coffee drinking or whether the person was an ex-coffee drinker or recent coffee drinker. No increased risks were seen in women with greater than 7 cups/day consumption. Only in the highest exposure group in men (63-155 cups/week) was the relative risk statistically significant at 1.5 (1.1-1.9).

Studies Reviewed by Pozniak (1985)

Fifteen retrospective epidemiology studies associating coffee consumption with both bladder and renal cell cancer were reviewed by Pozniak (1985). Investigations of the bladder cancer rates in men versus women have been contradictory, such that it can not be concluded that a preponderance of bladder cancer occurs in one sex over the other. Likewise, somewhat mixed results have been reported in studies investigating the relationship among lower urinary tract or renal cell cancer and a variety of factors, including race, smoking, and coffee consumption (Dunham et al. 1968; Fraumeni et al., 1971; both cited by Pozniak, 1985). In one study of the effects of imported coffee in which a positive association was found, the study was not controlled for tobacco smoking (Sheman, 1973, cited by Pozniak, 1985). Pozniak concluded that while there was a strong correlation between national bladder cancer rates and per capita coffee intake (Weinburg et al. 1983, cited by Pozniak, 1985), most studies either found no correlation (Armstrong et al., 1976; Najem et al., 1982, Mommsen et al., 1983; all cited by Pozniak, 1985) or an association which was unrelated to either dose or duration of coffee use (McLaughlin et al., 1983; cited by Pozniak, 1985).

Studies Reviewed in IARC (1991)

Caffeine from coffee or tea was associated with a significant ($p<0.05$) incidence of cancer of the urinary bladder, when controlled for consumption of beer and soft drinks, age, and smoking in a Copenhagen, Denmark study (Jensen et al., 1986, cited by IARC, 1991).

Studies Not Previously Reviewed

Urinary bladder cancer mortality varies in different countries, and continues to rise for both men and women, although it is predominant in men (Paneau et al., 1992; details n.p., therefore, not included in **Table 2**). The highest rates are reported from Denmark, United Kingdom, Belgium, and Italy, with the lowest rates in Japan, Singapore, and Venezuela. In areas of the United States, Europe, and certain regions of Africa, the incidence is higher in males than in females. Although some researchers have found evidence to suggest that consumption of ground coffee may be associated with urinary bladder cancer (Paneau et al., 1992), there is conflicting evidence in the literature.

Several studies have found no definitive link between coffee consumption and the incidence of bladder cancer. In a study by Goodman et al. (1986), the consumption of caffeinated beverages such as coffee, soft drinks, and tea was found to be unrelated to the incidence of renal cell cancer in a hospital-based case-control study investigating 189 men and 78 women with renal cell carcinoma between 1977 and 1983. The individuals were patients of 18 hospital centers in six U.S. cities and were matched with an equal number of controls based on hospital, sex, race, age, and time of admission. A population-based case-control study of 173 bladder cancer cases in New York State (women, aged 20-49) diagnosed between 1975-1980 (confirmed in 93.7% of cases) matched to controls by sex, age, and residence within a particular area code was conducted by Piper et al. (1986). Although coffee drinking had no correlation with bladder cancer incidence, cases consumed more coffee per day than did controls (3.1 cups/day in cases compared to 2.5 cups/day in controls).

Slattery (1988a) studied the association between fluid intake and bladder cancer. Adjusting for cigarette smoking, age, sex, history of diabetes, and history of bladder infection, caffeinated coffee consumption was associated with increased risk of bladder cancer only when greater than 40 cups/week were consumed. Caffeinated tea consumption was associated with a significant increased risk of bladder cancer if consumption was at least 3 cups/week in non-smokers. Tea drinkers who smoked did not experience any increased risk beyond that associated with smoking. Slattery (1988b) studied the effect of cigarette smoking on risk factors for the development of bladder cancer. Risk factors for bladder cancer associated with coffee drinking decreased after controlling for cigarette smoking. Bladder cancer from tea drinking was only noted in those who never smoked. The results suggest that cigarette smoking may obscure the effects of other risk factors.

A case control study of the association between dietary factors and bladder cancer found no association between caffeine consumption and bladder cancer in 826 histologically verified cases (diagnosed between 1979 and 1982) (Risch et al., 1988).

The consumption of 7 or more cups of coffee/day was weakly associated with a RR of 1.8 in 187 patients with cancer of the renal pelvis or ureter, which decreased to 1.3 when controlled for smoking (Ross et al., 1989). Risk tended to increase with increased consumption, but this was not statistically significant.

The relationship between dietary factors and lower urinary tract cancers was examined in 195 men and 66 women in Hawaii (Nomura et al., 1991). There was no association found between the duration and amount of coffee consumption and the development of lower urinary tract cancer. Among women only, there was an inverse relationship between lower urinary tract cancer and the consumption of regular ground coffee ($p=0.02$), but not with other types of coffee. There were no significant differences in tea intake between cases and controls.

D Avanzo et al (1992) investigated the relationship between regular and decaffeinated coffee, other methylxanthine-containing beverages, and bladder cancer. The RR for coffee drinkers was lowest for those who drank 1 cup or less per day (1.2), and was highest for those who drank 3 cups per day (1.5). However, the results do not appear to be dose-dependent, since

those who drank 4 or more cups per day (1.4) were slightly less likely to develop bladder cancer than those who drank only 3 cups per day. With regard to duration, those who drank coffee regularly for less than 30 years had a slightly lower RR for development of bladder cancer than those consuming coffee regularly for greater than 30 years. The RR of bladder cancer for decaffeinated coffee, tea, and cola drinkers was 1.5 (0.9-2.4), 0.9 (0.6-1.2), and 0.6 (0.3-1.4), respectively. The results indicate a higher prevalence of coffee consumption in bladder cancer cases; however, the prevalence was again not clearly dose-dependent.

A higher incidence of bladder cancer in patients in the South of France compared to the general population prompted a study of the risk factors in bladder cancer in 219 patients in this region (Gremy et al., 1993). Although all forms of coffee studied (regular, decaffeinated, and instant, milled coffee) were associated with significant risk (>1.71), the RRs were highest (nearly 3 times control values) for regular, milled coffee, and lowest for instant coffee. The results indicate that the form of coffee may be more relevant to the risk of bladder cancer than the caffeine content.

There have been several cases studied regarding the relationship between abuse of analgesics containing caffeine and the development of renal cancers (Adam et al., 1970; Lomax-Smith and Seymore, 1980; Fokkens, 1979; Handa and Tewari, 1981; and Piper et al., 1986). While it is suspected that the breakdown products of phenacetin, an aniline derivative, are potentially carcinogenic, the possible contributory effect of caffeine and other factors is unknown (Adam et al., 1970)

9.1.1.2 Breast

Studies Reviewed in IARC (1991)

An association between fibrocystic breast disease and methylxanthine consumption was first reported by Minton et al. (1979a,b and 1981, cited by IARC, 1991). They also reported subsequent improvement in the disease with elimination of methylxanthines from the diet. Criticisms of the studies focused on the lack of randomization, the lack of disease baselines, failure to monitor caffeine consumption during the course of the study, and measurement of

outcome by those having knowledge of the caffeine consumption status of the study participants. Brooks et al. (1981; cited by IARC, 1991) also found improvement in signs of fibrocystic disease following restriction of dietary methylxanthines. No significant differences in methylxanthine consumption were found between women who experienced changes in mammary nodules and those who did not (Heyden and Muhlbaier, 1984; Heyden and Fodor, 1986, both cited by IARC, 1991). Caffeine, theophylline, and other methylxanthines increased the severity of fibrocystic disease (Hindi-Alexander et al., 1985, cited by IARC, 1991). In a randomized study, a reduction in palpable breast nodes was found in women on methylxanthine-restricted diets, which was unrelated to breast fluid caffeine levels (Ernster et al., 1982, cited by IARC, 1991). In other randomized studies, there was no difference in breast nodules in women on methylxanthine-restricted diets versus control groups (Parazzini et al., 1986; Allen and Froberg, 1987, both cited by IARC, 1991).

The IARC working group concluded that the positive association between benign breast disease and coffee intake in some epidemiologic studies (Lawson et al., 1981; Boyle et al., 1984; LaVecchia et al., 1985; all cited by IARC, 1991) could be due to difference in the likelihood of disease detection between consumers and non-consumers of methylxanthines. One cohort study (Odenheimer et al., 1984, cited by IARC, 1991) of twins found significant associations between self-reported caffeine consumption and biopsy-confirmed or clinically-confirmed benign breast disease. Marshall et al. (1982, cited by IARC, 1991) found no association between the occurrence of fibrocystic disease and coffee consumption, a slight, insignificant reduction in risk with tea consumption, and no relationship with combined coffee and tea consumption. Shairer et al. (1986, cited by IARC, 1991) found no significant differences in the incidence of benign breast disease for any of 5 different levels of caffeine consumption, nor with any type of fibrocystic disease. The only case-control study in which biopsy controls were used (Rohan et al., 1989, cited by IARC, 1991) found no association.

Four case-control studies of breast cancer in which an attempt was made to study methylxanthine (e.g., caffeine) consumption and breast cancer showed no association (Lubin et al., 1985b; Shairer et al., 1987; Iscovich et al., 1989; Pozner et al., 1986; all cited by IARC, 1991).

In one study, a slight increase in risk for breast cancer was reported for premenopausal women (Rohan and Michael, 1988; cited by IARC,1991). Overall results of these studies indicated that there was inadequate clinical data to associate consumption of caffeine or other methylxanthines (theophylline and theobromine) found in coffee, tea, cocoa, and chocolate with breast cancer.

Other Reviews

Several other reviews have been compiled on the association between caffeine consumption and the risk of breast cancer. None of the reviews found a significant association in the available literature between caffeine consumption and breast cancer (Lubin and Ron, 1990; Mesko et al., 1990; Menke et al., 1995).

Studies Not Previously Reviewed

A case-control study of 2,651 newly-diagnosed female breast cancer patients in the United States found a risk of 1.0 (95% C.I.=0.9-1.6) for up to 7 cups coffee/day, and 1.2 (95% C.I.=0.7-1.6) for at least 5 cups coffee/day (Rosenberg et al., 1985). Coffee consumption was not associated with an increased risk of breast cancer among women with a history of benign fibrocystic breast disease, nor were was consumption of tea or decaffeinated coffee associated with increased risk of breast cancer. The results suggest that coffee consumption is not associated with risk of breast cancer.

A case-control study of 1,617 women in New York state diagnosed with breast cancer found no association between consumption of coffee, tea, or colas and cancer risk (McLaughlin et al., 1992).

In a cohort study of 34,388 postmenopausal women from Iowa, no association was found between the occurrence of breast cancer and caffeine intake; adjustments were made for age and multiple breast cancer risk factors (Folsom et al., 1993). The women were monitored from 1986 through 1990, and caffeine intake was assessed by a food frequency questionnaire.

A case-control study of 755 female breast cancer patients under the age of 36 by the United Kingdom National Case-Control Study Group found no association between caffeine

consumption (>100 mg/day; 0.5 mmol/day) and risk of breast cancer, when controlled for smoking and other risk factors of breast cancer (Smith et al., 1994). Risks of breast cancer were reduced (non-significantly) at each level of caffeine consumption over baseline (0-100 mg daily).

9.1.1.3 Cardiovascular System

Studies reviewed by Etherton and Kochar (1993) suggest that coffee consumption is not a risk factor for cardiovascular disease when less than six cups per day are consumed. Ernster s (1993) review of epidemiologic data on the cardiovascular effects found that there is also little evidence relating caffeine consumption to myocardial infarction or fatal coronary heart disease (CHD). Coffee consumption has been shown to increase blood pressure; however, once an individual develops a tolerance to a particular level of consumption, blood pressure returns to normal (Etherton and Kochar, 1993). Studies that have reported an association between caffeine consumption and cardiovascular effects were found to be flawed and/or did not account for confounding factors, such as cigarette smoking (Etherton and Kochar, 1993).

A study by Grossarth-Maticek and Eysenck (1991) supports the theory that CNS drugs such as caffeine may play a role in tumor suppression by acting on neurotransmitters and second messengers that, when stimulated, trigger the repair of damaged DNA responsible for carcinogenesis. This study also suggests that personality and stress serve as mediating factors in the action of stimulant and depressant drugs and cancer. However, their results contradict other studies by suggesting an increased risk of coronary heart disease from consumption of stimulant drugs like caffeine. In 1973, data on cigarette smoking, alcohol consumption, and cola consumption were collected from 3,684 men and 3,018 women. Interviewers also collected data on personality and stress by questionnaire. Of the 6,702 subjects, 203 men and 191 women reported consumption of 2 to 3 liters of cola daily for 10 years or more. The groups were observed for 13 years and findings indicated a significant difference in mortality rates from cancer between cola drinkers (5.3%) and non-cola drinkers (1%).

A cohort study that followed 8,006 healthy and non-healthy Japanese men for 12 years found that coffee consumption was not associated with health or disease. Both healthy and non-

healthy men reported consuming an average of 3 cups of coffee per day (Reed and Benfante, 1985).

9.1.1.4 Colon

Colorectal adenomatous polyps are a precursor lesion for colorectal cancer (Lee et al., 1993; Neugut et al., 1993). A review of the literature by Neugut et al. (1993) found no association between coffee and the development of colorectal adenomas. Giovannucci (1998) conducted a literature review for data related to coffee consumption and the risk of colorectal cancer. The results of 12 case-control studies indicated a reverse association between coffee consumption and the risk of colorectal cancer for groups in the high consumption category. Many of the studies were deemed inconclusive because of inconsistencies such as the lack of control for covariates such as tobacco smoking.

Slattery et al. (1990) conducted a population-based case-control study using white males and females aged 40 to 79 identified through the Utah Cancer Registry. Little or no increase in risk of colon cancer from caffeine consumption was observed in women. Males who consumed higher levels of caffeine 2-3 years prior to the study were at higher risk than males who consumed low levels of caffeine (Slattery et al., 1990).

In a case-control study of the possible association of colorectal adenomatous polyps and caffeine consumption, 271 cases of patients with pathologically confirmed polyps and 457 controls were collected from three New York City colonoscopy practices. No significant associations were observed for caffeine or coffee consumption in males or in females after adjustment for cigarette smoking (Lee et al., 1993).

A study by Favero et al. (1998) investigated the modifying effect of coffee intake on colon cancer in a population from six areas of Italy. Coffee (not specifically caffeine) intake was inversely associated with cancer risk and exerted a modification effect for individuals who drank fewer than two cups per day. Frequent eating increased the excretion of bile acids which are suspected colon carcinogens. Frequent coffee intake decreased the excretion of bile acids, thereby counterbalancing the effect of frequent eating.

9.1.1.5 Gastrointestinal Tract

In studies reviewed by Ernster (1984), no correlation was demonstrated between the ingestion of coffee and gastric and duodenal ulcers in men or women. Studies that examined the consumption of decaffeinated coffee showed increases in peptic ulcers, suggesting that coffee components other than caffeine may be involved in their development. In a review by Etherton and Kochar (1993) of data on the gastrointestinal effects of caffeine, no associations between ingestion of regular or decaffeinated coffee and the incidence of duodenal ulcers or ulcerative colitis were found.

A 1987-1989 Spanish case-control study investigated gastric cancer in 235 men and 119 women with histologically confirmed adenocarcinomas; no association between the consumption of coffee or tea and the incidence of cancer was observed (Agudo et al., 1992).

N-nitroso compounds derived from caffeine and their causal relationship to oesophageal and gastric cancers in Kashmir, India were investigated by Kumar et al. (1992). Salted tea is prepared in Kashmir by adding sodium bicarbonate and shows high methylating activity upon *in vitro* nitrosation. When treated under the same conditions, caffeine forms caffeidine and caffeidine acid which also show high methylating activity upon nitrosation. The structure and activity of the N-nitroso compounds formed may be linked to the high occurrence of oesophageal and gastric cancers among the population of Kashmir.

9.1.1.6 Lymphatic System

A case-control study of non-Hodgkin s lymphoma patients in northern Italy indicated that there is inadequate information with which to establish a link between regular or decaffeinated coffee consumption and development of the disease (Tavani et al., 1994). A total of 429 patients with confirmed non-Hodgkin s lymphoma and 1,157 controls with non-neoplastic, non-immunological, non-digestive tract diseases were interviewed regarding consumption of coffee, tea, and cola-containing beverages. Other factors, such as smoking, alcohol, diet, and family history were also considered.

9.1.1.7 Pancreas

A review of the literature concerning the causes, diagnosis, and treatment of pancreatic cancer indicated that the link between coffee/caffeine consumption and pancreatic cancer in humans is either nonexistent or weak (Gordis and Gold, 1984, cited by Poston and Williamson, 1990).

Drinking up to 5 or more cups of coffee per day was not associated with pancreatic cancer in a study of 99 Swedish patients with pancreatic cancer (Norell et al., 1986).

A study of the relationship between consumption of decaffeinated coffee and incidence of pancreatic cancer was conducted in 127 men and 111 women with pancreatic cancer in the United States (Wynder et al, 1986). Decaffeinated coffee use was not associated with an increased risk of pancreatic cancer in men. An elevated risk of pancreatic cancer was associated with consumption of 1-2 cups/day in women, although the results were borderline significant. Coffee was related to an increased risk (RR=1.72; 95% CI=0.95-3.11) of pancreatic cancer in 150 Italian women with who drank 2 or more cups of coffee per day. The RR decreased to 1.44 (0.74-2.80) for those who drank 3-4 cup/day, and to 1.06 (0.41-2.70) for those who drank 5 or more cups/day (LaVecchia et al., 1987). When data from these patients were grouped with data from other case-control studies (n=1,464), the RR was decreased for moderate coffee drinkers (1.22) and increased for heavy coffee drinkers (1.44). It was suspected that the results of these studies are potentially confounded, at least in part, by tobacco smoking.

Coffee was associated with increased risk of pancreatic cancer in 63 French women and 98 men who drank 2 or more cups of coffee per day (Clavel et al., 1989). The increased risk seen may be due to the overrepresentation of noncoffee drinkers in patients used in the study.

9.1.1.8 Reproductive Organs

A study of the relationship between coffee drinking and the occurrence of ovarian cancer was investigated in 290 U.S. women with ovarian cancer (Miller et al., 1987). An increased risk (RR=1.6; 95% CI=0.8-3.1) was associated only with drinking 4-5 cups of coffee per day.

A study of 188 women with ovarian cancer in the San Francisco Bay area found that regular consumers of coffee for more than 40 years were 3.4 times more likely to develop ovarian cancer than controls (Whittenmore et al., 1988). However, there was no clear dose-dependent trend in the data.

Sturgeon et al. (1991) investigated the relationship between coffee consumption and vulvar cancer in 201 U.S. women with vulvar cancer. There was elevated risk for consumption of 1 cup of coffee/day, 2 cups/day, and for more than 4 cups/day, but not for 3 cups/day. There was no association of vulvar cancer with tea or decaffeinated coffee. Risk of vulvar cancer increased irregularly with coffee consumption.

A population-based study conducted in 362 white Utah men diagnosed with prostate cancer between 1983 and 1986 to determine the association between consumption of coffee tea, caffeine, and other factors and prostate cancer risk found no association (Slattery and West, 1993).

9.1.1.9 Skin

Forty-six basal cell carcinoma cases were compared with 46 age, sex, and skin type-matched control patients (Sahl et al., 1995). Sun exposure, alcohol, diet, and smoking did not appear to be significant factors; however, caffeine consumption was higher in the cancer group.

Table 2. Human Epidemiological Data

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
9.1.1.1 Bladder and Renal					
Case-control	Consumption of caffeinated beverages such as coffee, soft drinks, and tea	189 U.S. men and 78 U.S. women with renal cell carcinoma between 1977 and 1983	Controlled for tobacco smoking.	The consumption of caffeinated beverages such as coffee, soft drinks, and tea was found to be unrelated to the incidence of renal cell cancer.	Goodman et al. (1986)
Population-based, case-control	Coffee drinking and heavy phenacetin usage	173 women with bladder cancer in New York State	Controlled for sex, age, and residence within an area code.	Heavy phenacetin usage was strongly associated with bladder cancer. Cases of bladder cancer were more likely to be coffee drinkers (3.1 cups/d in cases versus 2.5 cups/d in controls). Bladder cancer rates followed a dose-response curve for coffee consumption.	Piper et al. (1986)
Population-based, case-control	Caffeinated Coffee: 0 cups (control), 1-20 cups, 21-40 cups, 40+ cups/wk Caffeinated Tea: 0 cups (control), 1-3 cups, 4+ cups/wk	424 bladder cancer patients (male and female) from Utah	Controlled for tobacco smoking, age, sex, history of diabetes, and history of bladder infection.	Consumption of greater than 40 cups/wk was associated with increased risk of bladder cancer. Consumption of one or more cups of caffeinated tea nearly doubled the odds ratio compared to controls for those having never smoked. For those having smoked, the odds were tripled for those consuming one or more cups of caffeinated tea versus controls.	Slattery et al. (1988a)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
Population-based, case-control	Consumption of 30+ cups coffee/wk or 3+ cups of tea/wk	332 white male bladder cancer patients from Utah	Controlled for nature, duration, and amount of tobacco smoking or use, age, sex, and alcohol use.	Men who consumed 30+ cups of coffee per week had 2.5-fold increased risk of developing bladder cancer versus controls. When controlled for smoking, those who never smoked had roughly the same odds for the development of bladder tumors (~1.5). Odds ratios were decreased further (to less than 1.3) in the smoking group with those who started smoking at the youngest age at greatest risk. Odds ratios were nearly identical to controls for those who had stopped smoking.	Slattery et al. (1988b)
Case-control	Consumption of coffee (caffeinated and decaffeinated), tea, cocoa, soda, and artificial sweetener, amounts n.p.	826 verified cases of bladder cancer (males and females)	Controlled for source of caffeine, types of coffee, duration, frequency and amount of coffee usage, effects of tobacco smoking, and use of artificial sweeteners.	No association was found between caffeine consumption and bladder cancer.	Risch et al. (1988)
Case-control	Consumption of up to 7+ cups of coffee/d	187 patients with cancer of the renal pelvis or ureter	Controlled for tobacco smoking.	The consumption of 7+ cups of coffee/day was weakly associated with a RR of 1.8, which was reduced to 1.3 when controlled for smoking. Risk tended to increase with increased consumption, but was not statistically significant.	Ross et al. (1989)
Case-control	Consumption of coffee (regular ground, instant, decaffeinated instant, and all types combined)	195 male and 66 female Japanese or Caucasian	Controlled for ethnic group, sex, age, and tobacco smoking.	No association was found between the duration and amount of coffee consumption and the development of lower urinary tract	Nomura et al. (1991)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
	(1-49, 50-109, and 109+ cup-years), and tea (black tea and all types combined) (1-10, and 11+ cup-years).	patients with cancer of the lower urinary tract		cancer. Among women only, there was an inverse relationship between lower urinary tract cancer and the consumption of regular ground coffee (p=0.02), but not with other types of coffee.	
Case-control	Consumption of regular and decaffeinated coffee, other methylxanthine-containing beverages, amounts n.p.	303 male and 61 female Italian bladder cancer patients	Controlled for age, sex, tobacco smoking, educational level, alcohol consumption, and occupation.	The relative risk for coffee drinkers was lowest for those who drank 1 cup or less per day (1.2), and was highest for those who drank 3 cups per day (1.5). The results were not dose-dependent, since those who drank 4 or more cups per day (1.4) were slightly less likely than those who drank only 3 cups per day to develop bladder cancer. Those who drank coffee regularly for less than 30 years had a slightly lower risk for developing bladder cancer versus those consuming coffee regularly for greater than 30 years. Results indicate a higher prevalence of coffee consumption in bladder cancer cases; however, the prevalence was not clearly dose-dependent.	D Avanzo et al. (1992)
Case-control	Coffee, amounts n.p.	219 subjects with bladder cancer from South of France, sex n.p.	Controlled for tobacco smoking, age, and alcohol consumption.	Although all forms of coffee studied (regular, decaffeinated, and instant, milled coffee) were associated with significant risk (>1.71), the relative risks were highest (nearly 3 times control values) for regular coffee, and lowest for instant coffee. The results indicate that the form of coffee may be more relevant to the risk of bladder cancer than the caffeine content.	Gremy et al. (1993)
9.1.1.2 Breast					
Case-control	Consumption of up to 7 cups of coffee/d	2,651 newly-diagnosed female U.S. breast cancer	Controlled for age, county of residence, race, menstrual status, age at first live birth,	No association between coffee consumption and risk of breast cancer was found.	Rosenburg et al. (1985)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
		patients	diagnosis of benign breast disease, family history of breast cancer, and alcohol consumption.		
Case-control	Consumption of coffee, tea, colas, chocolate drinks, and chocolate candies, amounts n.p.	1,617 women in New York state diagnosed with breast cancer	Controlled for age, race, religion, age at menarche, age at first live pregnancy, parity, type of menopause, age at menopause, history of fibrocystic breast disease, family history of breast cancer (in the mother or sister(s)), body mass index, and tea and alcohol consumption.	No association between consumption of coffee, tea, or colas and breast cancer risk was found.	McLaughlin et al. (1991).
Cohort study	median caffeine intake of 212 mg/d	580 post-menopausal women with breast cancer from Iowa	Controlled for age waist/hip ratio, number of live births, age at first live birth, age at menarche, family history of breast cancer, family history x waist/hip ratio, and	There was no association between breast cancer occurrence and intake of caffeine, coffee, or other caffeine-containing foods, either when controlled for age or for multiple risk factors of breast cancer.	Folsom et al. (1993)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
			family history x number of live births. No adjustment for tobacco smoking.		
Case control	>100 mg caffeine/d at ages 16 and 25	755 English women with breast cancer	Controlled for age at menarche, nulliparity, age at first full-term pregnancy, breastfeeding (ever/ never), family history of breast cancer, total oral contraceptive use, biopsy for benign breast disease, total alcohol consumption at age 18, and tobacco smoking.	There was no evidence of a significant association between caffeine consumption and breast cancer risk. Risks of breast cancer were reduced (non-significantly) at each level of caffeine consumption over baseline (0-100 mg daily).	Smith et al. (1994)
9.1.1.3 Cardiovascular					
Cohort study	Group 1: 1-2 liters of cola/d Group 2: 2-3 liters of cola/d	3,684 men, 3,018 women	Controlled for tobacco smoking, alcohol consumption, personality, and stress.	There was a significant difference in mortality rates from cancer between cola drinkers (5.3%) and non-cola drinkers (1%).	Grossarth-Maticek and Eysenck (1991)
Cohort study	Healthy: 3.4 cups of coffee/d Unhealthy: 3.3 cups/d	8,006 Japanese men	Controlled for alcohol consumption, tobacco smoking, age, physiological factors, physical activity, and blood pressure.	Coffee consumption was not associated with health or disease.	Reed and Benfante (1985)
9.1.1.4 Colon					
Population-based case-control	caffeine, 0 to >260 mg/d	0-36 mg/d: 32 M and 64 F cases, 80 M and	Controlled for diet, age, tobacco smoking, alcohol consumption.	Little or no increase in colon cancer from caffeine in women, males consuming higher caffeine levels had a higher risk than those	Slattery et al. (1990)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
		114 F controls 37-260 mg/d 37 M and 38 F, cases, 55 M and 56 F controls ≥260 mg/d: 43 M and 17 F cases, 50 M and 36 F controls		consuming lower levels.	
Case-control	caffeine, amount n.p.	271 cases of patients with confirmed adenomatous polyps (AP): 153 M AP cases, 118 F AP cases, 202 M controls, 255 F controls	Controlled for age and tobacco smoking.	Possible (not significant) association for caffeine consumption in males or females.	Lee et al. (1993)
Case-control	coffee: <2 to ≥3 cups/d from 1992-1996 (caffeine content n.p.)	688 M and 537 F with colon cancer; Controls: 2,073 M and 2,081 F with no history of cancer	Controlled for education, physical activity, intake of vegetables.	Coffee intake was inversely associated with cancer; odds ratio (OR) = 1.89 for those who drank <2 cups/d. High coffee intake decreased bile acid excretion.	Favero et al. (1998)
9.1.1.5 Gastrointestinal Tract					
Case-control	coffee, amount n.p. tea, amount n.p.	235 M, 119 F with confirmed gastric adeno-carcinoma; controls with no	Controlled for age, sex, tobacco smoking, alcohol intake.	No association between coffee or tea intake and risk of gastric cancer.	Agudo et al. (1992)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
		adenocarcinoma			
9.1.1.6 Lymphatic System					
Case-control	coffee: 1+ cups/d tea: amount n.p. cola: amount n.p. controls: same	cases: 249 M, 180 F controls: 709 M, 448 F	Controlled for alcohol intake, tobacco smoking, gender.	No association between coffee consumption and non-Hodgkin s lymphoma was found.	Tavani et al. (1994)
9.1.1.7 Pancreas					
Case-control	5+ cups of coffee/d	99 Swedish pancreatic cancer patients	Controlled for tobacco smoking.	Coffee consumption was not related to the incidence of pancreatic cancer.	Norell et al. (1986)
Case-control	decaffeinated coffee (3+ cups/d)	127 U.S. males and 111 U.S. females with pancreatic cancer	Controlled for tobacco smoking, education, occupation, religion, marital status, alcohol drinking, saccharin use, height, weight 5 years before hospitalization, history of previous diseases, and residence.	Decaffeinated coffee use was not associated with an increased risk of pancreatic cancer in men. An elevated risk of pancreatic cancer was associated with consumption of 1-2 cups/d in women, although the results were borderline significant.	Wynder et al. (1986)
Case-control	coffee (5+ cups/d), decaffeinated coffee (2+ cups/d), and tea (1+ cups/d)	150 Italian patients with pancreatic cancer	Potentially confounded by tobacco smoking.	Coffee was related to an increased incidence of pancreatic cancer in patients who drank 2 or more cups of coffee per day (RR=1.72; 95% CI=0.95-3.11), which decreased to 1.44 for those who drank 3-4 cup/day, and to 1.06 in those who drank 5 or more cups/day.	LaVecchia et al. (1987)
Case-control	coffee (5+ cups/d), decaffeinated coffee (2+ cups/d), or tea (1+ cups/d)	1,464 patients with pancreatic cancer (combined results of previous studies)	Potentially confounded by tobacco smoking.	When these patients were grouped with data from other case-control studies, the relative risk (95 % CI) was decreased for moderate coffee drinkers and increased for heavy coffee drinkers.	LaVecchia et al. (1987)
Case-control	coffee (4+ cups/d)	98 French men and 63 women	Controlled for foreign origin, education level,	Coffee was associated with increased risk of pancreatic cancer in women and men.	Clavel et al. (1989)

Table 2. Human Epidemiological Data (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls	Results	Reference
		with pancreatic cancer	alcohol consumption, and tobacco use.	Increased risk seen may be due to the overrepresentation of noncoffee drinkers in patients used in the study.	
9.1.1.8 Reproductive Organs					
Case-control	coffee (5+ cups/d), decaffeinated coffee (5+ cups/d) or tea (5+ cups/d)	290 U.S. women with ovarian cancer	Controlled for age.	An increased risk was associated only with drinking 4-5 cups of coffee/d. There was no association with decaffeinated coffee or tea consumption and ovarian cancer.	Miller et al. (1987).
Case-control	coffee (amount n.p.)	188 women with ovarian cancer in the San Francisco Bay area	Controlled for age and tobacco smoking.	Regular consumers of coffee for more than 40 years are more likely to develop ovarian cancer than controls. There was no clear dose-dependent trend in the data. Further study was suggested.	Whittenmore et al. (1988)
Case-control	caffeine, coffee, decaffeinated coffee, and tea (amounts n.p.)	201 U.S. women with vulvar cancer	Controlled for age, tobacco smoking, and number of sexual partners.	There was elevated risk for consumption of 1 cup of coffee/d, 2 cups/d, and more than 4 cups/d, but not for 3 cups/d. There was no association of vulvar cancer with tea or decaffeinated coffee. Risk of vulvar cancer increased irregularly with coffee consumption.	Sturgeon et al. (1991)
Case-control	coffee, tea, and caffeine (amounts n.p.)	362 white Utah men with prostate cancer	Controlled for tobacco smoking.	Coffee, tea, and caffeine consumption were not associated with prostate cancer risk.	Slattery and West (1993)
9.1.1.9 Skin					
Case-control	caffeine cases: 274-352 mg control: 201-257 mg	46 cancer patients, sex n.p. 46 controls, sex n.p.	Controlled for age, sex, tobacco smoking, skin-type, sun exposure, alcohol intake, and diet.	Caffeine consumption higher in cancer group, smoking was also higher in cancer group but was not reported as significant.	Sahl et al. (1995)

Abbreviation: ara-C = arabinofurosylcytosine; F = female; CHD = coronary heart disease; n.p. = not provided; M = male; RR = relative risk; NA = not

applicable; # = number; AP = adenomatous polyp; wk = week(s); + = or more; d = day(s); OR = odds ratio; CI = confidence interval.

9.1.1.10 Clinical Trials of Chemotherapeutics in Human Cancers

Details of these studies are presented in **Table 3**.

Bone

The use of caffeine in the treatment of osteosarcoma was studied by Tsuchiya et al. (1992). Nine patients with osteosarcoma received chemotherapy combined with caffeine (maximum 3 g/patient) treatment and surgery. In this study, the preoperative chemotherapeutic modality with intra-arterial infusion of cisplatin and caffeine with or without doxorubicin and systemic administration of high-dose methotrexate produced a 100% response rate for primary osteosarcoma. The usual response rate to cisplatin alone, or high-dose methotrexate combined with bleomycin, cyclophosphamide, and actinomycin D, or multi-drug combination chemotherapy including cisplatin and high-dose methotrexate is 30-68%. Caffeine enhanced the cytocidal effect of cisplatin. In a similar study that further supports the therapeutic use of caffeine in osteosarcoma patients, 22 patients were given preoperative courses of intra-arterial cisplatin (120 mg/m^2 for 1-2 hours) and caffeine (1.5 g/m^2/day for 3 days) (Tsuchiya et al., 1998). Following preoperative treatment, no viable tumor cells were seen in 19 patients, while only scattered foci of viable cells were seen in two patients. One patient had some areas of viable tumor cells.

Brain

Only one study on the effects of caffeine on the treatment of brain tumors was found. Stewart et al. (1986) looked at the efficacy of a three drug combination of cisplatin, arabinofuranosyl cytosine (ara-c), and caffeine in the treatment of human intracerebral tumors. Cisplatin, an antineoplastic agent active against primary and metastatic brain tumors, acts synergistically with other chemotherapeutic drugs *in vitro*. Twenty-five adult patients (19 males, 6 females) with astrocytomas were treated i.v. with cisplatin (75 mg/m^2). Ara-c (750 mg/m^2) was administered i.v. 2-4 hours after cisplatin treatment. Then, four doses of caffeine (250-700 mg/m^2) were given intramuscularly (i.m.) or peroral (p.o.) over 8 hours. Twelve of the 25 patients (48%) treated were classified as responders defined as improvement in the CT scan

with neurological worsening and without an increase in steroid dosage. Three patients (12%) had tumor stability for more than 12 weeks, 7 (28%) experienced rapid tumor regrowth, and 3 (12%) died less than 4 weeks after treatment. The overall results of the study suggest that the three drug regimen appears to be active against glioblastomas; however, when compared to previous studies, caffeine did not appear to have increased the efficacy of the cisplatin plus ara-c treatment.

Pancreas

A phase I-II study using platinol, ara-C, and caffeine in combination (PACE chemotherapy) was investigated in 28 patients with advanced pancreatic cancer (Dougherty et al., 1989). Treatment consisted of cisplatin i.v. at 100 mg/m^2 (Day 1), ara-C i.v. at 2 g/m^2 every 12 hours (2 doses), and caffeine subcutaneously (s.c.) at 200, 400, 600, or 800 mg/m^2 (1-4 mmol/m^2) after each ara-C dose. Courses were repeated every 28 days. The results indicated that the combination of cisplatin, ara-C, and caffeine was an active and tolerable therapy in the treatment of pancreatic cancer. No comparisons were made to treatment without caffeine.

Kelsen et al. (1991) conducted a phase III comparison of cisplatin, ara-C, and caffeine (CAC chemotherapy) versus standard treatment of mitomycin, streptozotocin, and 5-fluorouracil (5-FURA) in 82 patients with advanced pancreatic cancer. Treatment 1 consisted of cisplatin i.v. at 100 mg/m^2 (Day 1), ara-C i.v. at 2 g/m^2 every 12 hours (2 doses), and caffeine s.c. at 400 mg/m^2 (2 mmol/m^2) after each ara-C dose. Courses were repeated every 28 days. Treatment 2 consisted of i.v. streptozotocin (1 g/m^2), on days 1, 8, 29, and 36; mitomycin C (10 mg/m^2) on day 1; and 5-FURA (600 mg/m^2) on days 1, 8, 29, and 36. Cycles were repeated every 8 weeks. CAC treatment was inferior to standard therapy (without caffeine) for the treatment of pancreatic cancer.

A phase II study using PACE chemotherapy in combination with continuously-infused 5-FURA was investigated in 19 patients with advanced pancreatic cancer who had not previously received cytotoxic therapy (Kellogg et al., 1993). Treatment consisted of cisplatin i.v. at 100 mg/m^2 (Day 1), ara-C i.v. at 2 g/m^2 every 12 hours (2 doses), and caffeine s.c. at 400 mg/m^2

(2 mmol/m^2) after each ara-C dose. 5-FURA (250 mg/m^2) was administered by continuous infusion on days 3-21. Courses were repeated every 28 days. The addition of 5-FURA did not significantly improve the response rate of patients to PACE chemotherapy.

In most cases, the effectiveness of caffeine could not be determined because the appropriate controls were not performed.

Skin

A study by Cohen et al. (1980) found that caffeine did not enhance MeCCNU (methyl-1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, a chemotherapeutic agent) activity in patients with advanced metastatic malignant melanoma. Patients received either 200 mg/m^2 MeCCNU orally every 6 weeks or a combination of MeCCNU and caffeine (600 mg/m^2; 3.09 mmol/m^2) s.c. The response rate for MeCCNU alone was 11%, while in combination with caffeine it was 12%.

Table 3. Clinical Trials of Chemotherapeutics in Human Cancers

Study Type	Substance and Amount	Number and Sex of Individuals	Controls (Concurrent or Historical)	Results	Reference
Bone					
Clinical trial	caffeine: 1.2-1.5 g/m^2/d cisplatin: 120 mg/m^2/h	5 males, 4 females	Historical	100% response rate for primary osteosarcoma, versus response rate for traditional treatment of 30-68%. Seven osteosarcomas showed no tumor stain on angiograms after the first intraarterial infusion of cisplatin and caffeine. Caffeine enhanced the cytocidal effect of cisplatin.	Tsuchiya et al. (1992)
Clinical trial	caffeine: 1.5 g/m^2/d for 3 d cisplatin: 30 mg/m^2/d for 2 d	14 males, 8 females	Historical	Following preoperative treatment, no viable tumor cells were seen in 19 patients, while only scattered foci of viable cells were seen in two patients. One patient had some areas of viable tumor cells. 18 patients remained disease free after 61 months. Overall 5-year cumulative survival rate was 90%. 17 patients maintained excellent limb function. Caffeine is thought to enhance the cytotoxicity of anticancer agents by inhibiting post-replication repair of sublethally-damage DNA, with the degree of enhancement dependent upon the degree of lethal or sublethal effects of the anticancer agents.	Tsuchiya et al. (1998)
Brain					
Clinical trial	caffeine: 250-700 mg/m^2 cisplatin: 75 mg/m^2 ara-C: 750 mg/m^2	19 males, 6 females (20 with grade IV astro-cytomas; 5 with grade III astrocytomas)	Historical; results compared with a prior study in a similar patient population treated with cisplatin and ara-c without caffeine.	12/25 responded to 3 drug regimen, 3/25 showed stabilized tumors >12 wk, 7/25 had rapid tumor regrowth, 3/25 died < 4 wk after initiating treatment. The onset of caffeine-induced seizures prevented the use of caffeine levels necessary for a beneficial effect. As a result, caffeine did not significantly improve response to treatment.	Stewart et al. (1986)

Table 3. Clinical Trials of Chemotherapeutics in Human Cancers (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls (Concurrent or Historical)	Results	Reference
Pancreas					
Clinical Trial (Phase I-II)	Treatment consisted of cisplatin i.v. at 100 mg/m^2 (Day 1), ara-C i.v. at 2 g/m^2 every 12 h (2 doses), and caffeine s.c. at 200, 400, 600, or 800 mg/m^2 (1-4 mmol/m^2), after each ara-C dose. Courses were repeated every 28 days.	28 patients with advanced pancreatic cancer	Historical	Eighteen of the 28 patients had measurable disease, and of these, 7 of 18 patients (39%) responded to therapy, with mean survival rate of 9.5 mo, as compared with 15-20% response rate with traditional therapy. Mean survival rate of the group was 6.2 mo. No previous studies have been reported in with the combination of cisplatin, ara-c, and caffeine (PACE chemotherapy) were investigated for the treatment of pancreatic cancer, although the study design was based upon nude-mouse- to human equivalents for the same drug combination studied in a nude mouse-human xenograft model. The results indicate that the PACE chemotherapy is an active and tolerable therapy in the treatment of pancreatic cancer.	Dougherty et al. (1989)
Clinical Trial (Phase III)	Treatment 1 consisted of: cisplatin i.v. at 100 mg/m^2 (Day 1), ara-C i.v. at 2 g/m^2 every 12 h. (2 doses), and caffeine s.c. at 400 mg/m^2 (2 mmol/m^2), after each ara-C dose. Courses were repeated every 28 d. Treatment 2 consisted of i.v. streptozotocin (1 g/m^2), on days 1, 8, 29, and 36; mitomycin C (10 mg/m^2) on day 1, 5-FURA (600 mg/m^2) on days 1, 8, 29, and 36. Cycles were repeated every 8 wk.	82 patients with advanced pancreatic cancer, sex n.p.	Historical	The 95% confidence intervals for the two regimens overlapped, and neither therapy was found to be effective against advanced pancreatic cancer.	Kelsen et al. (1991)
Clinical Trial (Phase II)	Treatment consisted of cisplatin i.v. at 100 mg/m^2 (Day 1), ara-C i.v. at 2 g/m^2 every 12 h (2	19 patients with advanced	Historical	The addition of 5-FURA did not measurably improve the response rate of PACE chemotherapy. The individual effect of caffeine	Kellogg et al. (1993)

Table 3. Clinical Trials of Chemotherapeutics in Human Cancers (Continued)

Study Type	Substance and Amount	Number and Sex of Individuals	Controls (Concurrent or Historical)	Results	Reference
	doses), and caffeine s.c. at 400 mg/m^2 (2 mmol/m^2), after each ara-C dose. 5-FURA (250 mg/m^2) was administered by continuous infusion on days 3-21. Courses were repeated every 28 d.	pancreatic cancer who had not previously received cytotoxic therapy, sex n.p.		was not determined in this study.	
Skin					
Clinical Trial	Treatment with 600 mg/m^2 (3.09 mmol/m^2) caffeine s.c. and 200 mg/m^2 MeCCNU orally every 6 wk.	157 patients with malignant melanoma, sex n.p.	Concurrent, patients treated with MeCCNU without caffeine	Negative for enhancement of MeCCNU activity. 11% response to MeCCNU alone; 12% response with caffeine.	Cohen et al. (1980)

Abbreviations: n.p. = not provided; NA = not applicable; 5-FURA = 5-fluorouracil; h = hour(s); d = day(s); wk = week(s); i.v. = intravenous injection; s.c. = subcutaneous injection; mo = month(s); MeCCNU = methyl-CCNU.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

Absorption of caffeine from the gastrointestinal tract is pH-dependent, rapid, and virtually complete. After absorption, it is distributed rapidly into body fluids (Chvasta and Cooke, 1971; Marks and Kelly, 1973; Robertson et al., 1978; Bonati et al., 1982; all cited by IARC, 1991). *In vivo* and *in vitro* studies show that caffeine is bound to plasma proteins, mainly albumins (Bonati and Garattini, 1984; Yesair et al., 1984; both cited by IARC, 1991). In adults, the half-life of caffeine in the body is 2.5 to 4.5 hours (Aranda et al., 1979a,b; Parsons and Neims, 1981; Gorodischer and Karplus, 1982; all cited by IARC, 1991). The use of oral contraceptives can double the half-life of caffeine (Patwardhan et al., 1980; Callahan et al., 1983; both cited by IARC, 1991).

Metabolism of caffeine is by hepatic microsomal enzymes and significant metabolism does not appear to occur in other organs (Grant et al., 1987; Berthou et al., 1989; Arnaud, 1987; all cited by IARC, 1991). In humans, caffeine is metabolized into more than 25 metabolites, the primary metabolites being paraxanthine, theobromine, and theophylline (Etherton and Kochar, 1993). These metabolites can act in several ways, including as adenosine receptor antagonists, inhibitors of cyclic nucleotide phosphodiesterase activity, mobilizers of calcium, and inhibitors of monoamine oxidase activity. Antagonism to the adenosine receptor found in the brain, kidney, cardiovascular system, respiratory system, gastrointestinal system, and adipose tissue is the most important effect. Adenosine can either inhibit or stimulate cAMP production within a cell by binding to high-affinity A1 receptors (inhibition) or low-affinity A2 receptors (stimulation). Cyclic AMP acts as a second messenger to activate a cAMP-dependent protein kinase, which phosphorylates selected proteins within the cell. Depending on the protein, the process of phosphorylation can be either stimulatory or inhibitory. Some cell functions are regulated through the second messenger system. Caffeine blocks adenosine receptors (A1 and A2) and prevents adenosine from inhibiting or stimulating cAMP production. Through this mechanism of action, caffeine can cause such effects as diuresis, central nervous system stimulation, increased cardiac contractility, bronchodilation, and analgesia (Etherton and Kochar, 1993).

Horn et al. (1995) investigated the hypothesis that caffeine metabolism varies by gender.

A caffeine breath test (CBT) was performed on 113 adult volunteers given an oral dose of 3 mg/kg (15 μmol/kg) caffeine. The CBT measures 3-*N*-dimethylation, the primary route of caffeine metabolism which is catalyzed by P4501A2. An elevated CBT was observed in men, although the increase was not statistically significant. Women who had never given birth (nulliparous) had lower CBT levels compared to women who had given birth (parous). Parous women had CBT levels similar to men. Also, women taking oral contraceptives had lower CBT values. The results of this study indicate that there is no significant difference in caffeine metabolism between men and women in general, but suggests that women develop increased P4501A2 activity after childbirth.

In a recent study investigating the association between smoking and bladder cancer risk, caffeine and metabolites in 97 healthy male volunteers were measured following a single ingestion of a standardized cup of coffee (Landi et al., 1996). The results suggest that exposure to blond (flue-cured) tobacco smoke, meat consumption the day before the measurement, and regular ingestion of more than 4 cups of coffee per day were associated with increases in the level of cytochrome P4501A2-dependent N-oxidation. This metabolic pathway may be involved in an elevated bladder cancer risk via the formation of reactive intermediates that can form hemoglobin and DNA adducts.

In a caffeine excretion study by Lu et al. (1998), 120 volunteers provided urine samples at regular intervals between the 4th and 5th hours after consuming three cups of coffee. Five of at least 13 caffeine metabolites normally excreted in humans, 5-acetylamino-6-formylamino-3-methyluracil (AFMU); 1-methylxanthine (1MX); 1-methyluric acid (1MU); 1,7-dimethylxanthine (1,7MX); and 1,7-dimethyluric acid (1,7DMU), were measured in human urine using reverse-phase HPLC. The concentrations of these metabolites in urine were 5.7-371 μM for AFMU; 8.0-220 μM for 1MX; 6.1-207.0 μM for 1MU; 5.6-147 μM for 1,7MX; and 11.0-136 μM for 1,7DMU.

9.1.3 Acute Exposure

Limited data are available on the effects of acute exposure to caffeine. Some adverse health effects in humans such as gastric symptoms, insomnia, and diuresis have been observed (Lachance, 1982; Rall, 1985; Stavric, 1988; all cited by IARC, 1991). At doses up to 2 µg/mL (10 nmol/mL) in blood, caffeine stimulates the central nervous system (CNS). Concentrations of 10-30 µg/mL (51-154 nmol/mL) can cause restlessness, excitement, tremors, tinnitus, and headache (Lachance, 1982; Ashton, 1987; Stavric, 1988; all cited by IARC, 1991). In adults, the acute lethal dose (orally or i.v.) of caffeine is approximately 5 to 10 g (26-51 mmol), a quantity contained in about 75 cups of coffee or 125 cups of tea (HSDB, 1997).

Acute toxicity values for caffeine in animals are presented in **Table 4**. The details of studies discussed in this section are presented in **Table 5**.

Table 4. Acute Toxicity Values for Caffeine

Route	Species (sex and strain)	LD_{50}	Reference
i.p.	mouse (sex and strain n.p.)	168 mg/kg (0.855 mmol/kg)	Senda and Hirota (1974)
	rat (sex and strain n.p.)	240 mg/kg (1.24 mmol/kg)	RTECS (1997)
	rabbit (sex and strain n.p.)	150 mg/kg (0.772 mmol/kg)	RTECS (1997)
i.v.	mouse (M, CD2F1/Cr1BR)	62 mg/kg (0.319 mmol/kg)	Bonati et al. (1985)
	rat (sex n.p., Albino)	105 mg/kg (0.541 mmol/kg)	Scott and Chen (1944)
oral	mouse (M and F, Swiss CD)	127 mg/kg (0.654 mmol/kg)	Palm et al. (1978)
	rat (sex n.p., Albino)	192 mg/kg (0.989 mmol/kg)	Boyd (1965)
	hamster (M and F, Golden)	230 mg/kg (1.18 mmol/kg)	Palm et al. (1978)
	guinea pig (M, CBL)	230 mg/kg (1.18 mmol/kg)	Boyd (1960)
	rabbit (M and F, New Zealand white)	150 mg/kg (0.772 mmol/kg)	Palm et al. (1978)
s.c.	mouse (sex and strain n.p.)	242 mg/kg (1.25 mmol/kg)	RTECS (1997)
	rat (sex and strain n.p.)	170 mg/kg (0.875 mmol/kg)	Peters (1967)

Abbreviations: F = female; i.p. = intraperitoneal injection; i.v. = intravenous injection; M = male; n.p. = not provided; s.c. = subcutaneous; LD_{50} = dose lethal to 50% of test animals.

The acute effects of caffeine in mice were studied by Bonati et al. (1985). Mice administered caffeine i.v. (30-120 mg/kg; 0.154-0.618 mmol/kg) and orally (150-620 mg/kg;

0.772-3.19 mmol/kg) suffered convulsions approximately 10 seconds after treatment. Mortality occurred within 90 seconds and was the result of respiratory arrest.

Palm et al. (1978) administered oral doses of caffeine to mice, rats, hamsters, and rabbits. In all species, deaths occurred within 2 days of dosing but death in some animals was delayed to 14 days after dosing. Signs of toxicity included diarrhea and weight loss. In rats, increased mortality was present among females compared to males.

Guinea pigs experienced excitement, convulsions, gastroenteritis, and edema of the liver, heart, lungs, spleen, and adrenal glands 1 hour after oral administration of 230 mg/kg (1.18 mmol/kg) caffeine (Boyd, 1960).

Table 5. Acute Exposure to Caffeine

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Mouse						
CD2F1/Cr1BR mature, age n.p.	100 M	caffeine, purity n.p.	i.v.: 30-120 mg/kg (0.154-0.618 mmol/kg) oral: 150-620 mg/kg (0.772-3.19 mmol/kg)	Single dose; 1 wk observation period	Convulsions, seizures, death from respiratory arrest.	Bonati et al. (1985)
Swiss CD, young adult, age n.p.	15 M, 15 F dose group	caffeine dissolved in water, 50 mg/mL	oral: 30 mg/kg/day (0.154 mmol/kg/day)	Single dose, 15 day observation period	Diarrhea, weight gain or loss. Increased mortality among females compared to males.	Palm et al. (1978)
Rat						
Sprague-Dawley, young adult, age n.p.	15 M; 15 F /dose group	caffeine dissolved in water, 50 mg/mL	oral: dose n.p.	Single dose, 15 day observation period	Death within 2 days of dosing, occasionally delayed up to 14 days. Symptoms included diarrhea and weight loss. Increased mortality among females compared to males.	Palm et al. (1978)
Hamster						
Golden, age n.p.	15 M, 15 F /dose group	caffeine dissolved in water, 50 mg/mL	oral: dose n.p.	Single dose, 15 day observation period	Death within 2 days of dosing, occasionally delayed up to 14 days. Symptoms included diarrhea and weight loss. Increased mortality among females compared to males.	Palm et al. (1978)
Guinea Pig						
CBL, young, age n.p.	37 M	caffeine, purity n.p.	oral: 230 mg/kg (1.18 mmol/kg)	Single dose, 2 day observation period	Excitement, convulsions, gastroenteritis, and edema of the liver, heart, lungs, spleen, and adrenal glands after 1 h.	Boyd (1960)

Table 5. Acute Exposure to Caffeine (Continued)

Species Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rabbit						
New Zealand White, age n.p.	10 M, 10 F /dose group	caffeine dissolved in water, 50 mg/mL	oral: dose n.p.	Single dose, 15 day observation period	Death within 2 days of dosing, occasionally delayed up to 14 days. Symptoms included diarrhea and weight loss.	Palm et al. (1978)

Abbreviations: F = female; h = hour(s); M = male; n.p. = not provided; wk = week(s); i.v. = intravenous injection.

9.1.4 Short-Term and Subchronic Exposure

No data on the short-term and subchronic effects of caffeine were located.

9.1.5 Chronic Exposure

In a study reviewed by IARC investigating the chronic of caffeine, rats exposed to 200-2000 mg/L (1.03-10.30 mM) in drinking water for 104 weeks showed decreased body weight at the highest dose; a slight increase in mortality was observed in males (Mohr et al., 1984, cited by IARC, 1991).

The chronic toxicity of coffee was evaluated by Palm et al. (1984). In a two-year toxicity study of Sprague-Dawley CD rats (150 males, 150 females) exposed to three concentrations of fresh-brewed coffee (25, 50, and 100%) *in utero*, groups receiving 50 and 100% had smaller mean body weights. Bone calcium was reduced in females consuming 25 or 100% for 1 year, but not after 2 years. Increases in the relative weights of the lungs, kidneys, liver, and epididymides were observed. Serum cholesterol levels were elevated in males (25 and 100%) after 2 years and in females (100%) after 1 and 2 years. During the 2-year period, rats drank approximately 82-196 mL/kg caffeine per day in water and consumed 344-573 g/kg/week (1.77-2.95 mol/kg/week) in feed.

9.2 Reproductive and Teratological Effects

Details of these studies are presented in **Table 6**.

An IARC (1991) review reported that several epidemiologic studies found a correlation between congenital malformations and caffeine consumption (Borl e et al., 1978; Linn et al., 1982; Rosenberg et al., 1982; Tohnai et al., 1984; Furuhashi et al., 1985; all cited by IARC, 1991). Total caffeine intake was also positively associated with low birthweight babies after controlling for tobacco smoking and other variables (Caan and Goldhaber, 1989; cited by IARC, 1991). A more recent review by Etherton and Kochar (1993) concluded that studies on the effect of coffee intake on fertility and premature births were largely inconclusive.

Caan et al. (1998) investigated differences in fertility associated with caffeinated beverage

consumption in 210 women and reported that no significant associations were found for any caffeine-containing beverages, except tea. Daily consumption of a half cup or more of tea doubled the odds of conception. However, the authors noted that tea consumption may be associated with other lifestyle characteristics (not specified) that enhance fertility.

Treating semen *in vitro* with caffeine increased sperm motility but did not alter sperm morphology (Hommonai et al., 1976; Traub et al., 1982; Aitken et al., 1983; Barkay et al., 1984; all cited by IARC, 1991). The effect of caffeine intake on the frequency of aneuploidy in sperm has been investigated (Robbins et al., 1997). Aneuploidy is a common cause of poor reproductive outcomes in humans and can result in severe medical problems in offspring. The results, controlled for alcohol use, age, and urinary levels of cotinine, indicated that caffeine intake, measured as coffee cup equivalents per day, was associated with increased levels of numerical chromosomal abnormalities (for chromosomes X, Y, and 18) in sperm.

Mahony et al. (1996) studied the *in vitro* effects of caffeine on zona pellucida penetration by epididymal spermatozoa in Cynomolgus monkeys (*Macaca fascicularis*). The motility of sperm from the corpus and cauda epididymal regions of seven monkeys was observed before and after treatment with caffeine. Caffeine increased the curvilinear velocity but not the linear velocity of cauda sperm; the velocity of corpus sperm was not altered. Of interest is the observation that treatment with caffeine was necessary for penetration of the zona pellucida by both sperm types. No hypothesis was provided to explain these results.

Table 6. Reproductive and Teratological Effects

Species, Strain, and Age	Number and Sex	Chemical Form and purity	Dose	Exposure/ Observation Period	Results	Reference
Human, adults, age n.p.	210 F	Caffeinated beverages	Mean total caffeine intake was 600 mg/wk (3.09 mmol/kg)	Case control	Weight, smoking, alcohol consumption, fat intake, diet, and physical activity were controlled for. No significant association between fertility and coffee or soda; tea drinking appeared to double the odds of conception.	Caan et al. (1998)
In vitro human, study using sperm from Caucasian males age 18-35	Group 1: 19 M Group 2: 12 M Group 3: 14 M	Coffee	Group 1: no caffeine Group 2: 1 cup/day Group 3: 2+ cups/day	Case-control	Controlled for alcohol use, age, and urinary levels of cotinine. Mean aneuploid levels were as follows: XX18: Group 1 (1.9%), Group 2 (1.3%), Group 3 (2.3%) YY18: Group 1 (1.3%), Group 2 (0.6%), Group 3 (1.6%) XY18: Group 1 (9.0%), Group 2 (10.5%), Group 3 (12.1%) 18-18: Group 1 (3.5%), Group 2 (3.2%), Group 3 (4.8%)	Robbins et al. (1997)
Cauda and corpus sperm from epididymus of adult Cynomolgus monkeys	7 M	Caffeine, purity n.p.	1 mM (0.2 g/mL)	Treatment *in vitro* for 0.5 h	No change in VL, but significant increase in VCL of cauda sperm; no significant increase in VL or VCL of corpus sperm; gained ability to penetrate zona pellucida by corpus and cauda sperm.	Mahony et al. (1996)

Abbreviations: n.p. = not provided; F = female(s); M = male(s); h = hour(s); VCL = curvilinear velocity; VL = linear velocity; + = or more.

9.3 Carcinogenicity in Animals

Details of studies in this section not previously reviewed are presented in **Table 7**.

Caffeine was tested for carcinogenicity in mice in a drinking water study (Welsch et al., 1988a, cited by IARC), in mice administered caffeine i.p. (Theiss and Shimkin, 1978, cited by IARC), and orally in rats. The mouse studies were concluded by IARC to be inadequate for evaluation. In two of the rat studies (Takayama and Kuwabara, 1982; Mohr et al., 1984; cited by IARC, 1991), no differences in the incidence of tumors at any site were found. Four additional rat studies (Yamagami et al., 1983; Johansson, 1981; W rzner et al., 1977; Brune et al., 1981; all cited by IARC, 1991) were concluded to be inadequate for evaluation. The overall IARC evaluation indicated that there were inadequate experimental data to associate caffeine with rodent carcinogenicity.

The incidence of tumors in male and female Swiss mice (150/sex) fed diets containing 10-50 g instant coffee powder/kg/day was inversely proportional to the amount of coffee ingested (Stalder et al., 1984, 1990). The incidence of lymphosarcomas, bronchio-alveolar adenomas and adenosarcomas was 34.8 for males and 36.2 for females in the high dose group, versus 70.6 for males and 56.8 for females in the control group. There was a slightly increased incidence of leiomyomas in the uterus in the low and high dose groups (1.37 and 2.72%, respectively) compared to 0% in the controls. Ingestion of up to 5% coffee in the diet for 2 years did not increase the incidence of malignant neoplasms.

9.3.1 Bladder

Bladder cancer associated with caffeine consumption in rats was first reported by Takayama (1981a, cited by Pozniak, 1985), although the results were refuted later by both the Food and Drug Administration (FDA) and the author himself (Takayama, 1981b, cited by Pozniak, 1985). In Takayama (1981b), following consumption of 100 and 200 mg/kg (0.51 mmol and 1.03 mmol/kg) caffeine, respectively, Wistar rats showed no significant differences in the occurrence of tumors in control or treatment groups.

Studies Reviewed by IARC (1991)

No significantly increased incidences of bladder tumors (or tumors of any kind) were noted in the only rat studies considered adequate by IARC (Takayama and Kuwabara, 1982; Mohr et al., 1984; both cited by IARC, 1991).

9.3.2 Mammary Gland

No significantly increased incidences of mammary gland tumors were noted in the only two rat studies considered adequate by IARC (Takayama and Kuwabara, 1982; Mohr et al., 1984; both cited by IARC, 1991).

A review of the role of caffeine in the development of the normal and neoplastic mammary gland in rats and mice was conducted by Welsch (1994). In the studies reviewed, caffeine was shown to stimulate mammary gland lobulo-alveolar development and secretion. The development of mammary gland tumors can either be stimulated or suppressed depending upon the animal species and strain, and the stage of tumorigenesis (initiation/promotion) at which caffeine is administered. In mice, caffeine does not appear to act directly on the mammary gland, but via increased secretion of corticosterone.

Studied Not Previously Reviewed

A study of the appearance of hyperplastic alveolar nodules (HAN; a preneoplastic state of the mammary glands of mice) after caffeine administration was conducted (Nagasawa et al., 1985). Caffeine (500 mg/L [2.57 mM] *ad libitum* in drinking water for 60 days) administered to two groups of mice (Groups I and II, 4.5 and 2.5 months of age, respectively) promoted the growth of the mammary end-bud (lobulo-alveolar) system (of which HAN are an outgrowth) in Group I, but did not significantly affect HAN appearance in Group II. In a follow-up to this study, four strains of mice (SHN, SLN, GR/A, and C3H/HeMei) were given 500 mg/L (2.57 mM) caffeine *ad libitum* in drinking water from 20 days of age and sacrificed at 40, 60, 90, 120 and 150 days of age (Nagasawa and Konishi, 1987). An increase in HAN was present in

caffeine-treated mice of all four strains at 60, 120, 60, and 90 days of age, respectively. No such change was seen in controls. In another study, caffeine (500 mg/L [2.57 mM] *ad libitum* in drinking water from day 20 of age to day 60) had no effect on mammary gland growth in C3H/HeMei mice (Nagasawa and Sakurai, 1986). A third study showed that caffeine (500 mg/L [2.57 mM] *ad libitum* in drinking water from day 20) stimulated spontaneous mammary tumorigenesis and increased the number of HAN in virgin and breeder C3H/HeMei mice versus controls; however, caffeine had no effect on the number or growth of mammary tumors (Nagasawa and Konishi, 1988).

9.3.3 Pituitary Gland

Yamagami et al. (1983) studied the effect of caffeine on the pituitary gland in rats. Eighty female Wistar rats were given 13.5 g (69.5 mmol) of a 0.2% (2000 µg/mL; 10.3 µmol/mL) caffeine solution orally *ad libitum* for 12 months which resulted in decreased body weight and an increase in the weight of the pituitary gland caused by the growth of a pituitary adenoma or hyperplasia. The adenomas appeared to be endocrinologically nonfunctioning. Weight loss was attributed to either caffeine-induced diarrhea, promotion of lipolysis by phosphodiesterase inhibition in adipose tissue, loss of appetite, or a cachectic state resulting from a pituitary tumor.

Table 7. Carcinogenicity in Animals

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
Swiss mice (*in utero*)	150 M and F	caffeine, purity n.p.	*In utero*: lactating dams were fed 1% (10 g/day) instant coffee in the diet. Post-partum:10, 25, or 50 g (1-5%) instant coffee/kg daily in the diet	2 years	Incidence of common tumors (lymphosarcomas, bronchio-alveolar adenomas and adenosarcomas): High dose group (50 g/kg/day): 34.8% (M); 36.2% (F) Controls: 70.6% (M); 56.8% (F) Incidence of leiomyomas in the uterus: Low dose group (10 g/kg/day): 1.37% High dose group (50 g/kg/day): 2.72% Control group: 0%	Stalder et al. (1984, 1990)
Mice, SLN virgin, 2.5-4.5 months		caffeine, purity n.p.	500 mg/L (2.57 mM) *ad libitum* in drinking water for 60 days to two groups of mice (Groups I and II, 4.5 and 2.5 months of age, respectively)	2 month exposure, observation period n.p.	The growth of the mammary end-bud (lobulo-alveolar) system was promoted in Group I, but HAN appearance in Group II was not significantly affected.	Nagasawa et al. (1985)
Mice, SHN, SLN, GR/A and C3H/ HeMei, 40 to 150 days		caffeine, purity n.p.	500 mg/L (2.57 mM) *ad libitum* in drinking water from 20 days of age	exposure and observation period up to 150 days	Mice given caffeine and sacrificed at 40, 60, 90, 120 and/or 150 days of age showed HAN at 60, 120, 60, and 90 days of age in SHN, SLN, GR/A and C3H/HeMei mice, respectively versus no such change in controls.	Nagasawa and Konishi (1987)
Mice, C3H/HeMei, 20 days		caffeine, purity n.p.	500 mg/L (2.57 mM) *ad libitum* in drinking water from day 20 of age to day 60	exposure up to 70 days, observation period n.p.	No effect on mammary gland growth.	Nagasawa and Sakurai (1986)
Mice, C3H/HeMei, 20 days		caffeine, purity n.p.	500 mg/L (2.57 mM) *ad libitum* in drinking water from day 20 of age	exposure n.p., observation up to 20 months of age	Caffeine stimulated spontaneous mammary tumorigenesis and increased the number of HAN in virgin and breeder C3H/HeMei mice versus controls; however, caffeine had no effect on the	Nagasawa and Konishi (1988)

Table 7. Carcinogenicity in Animals

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
					number or growth of mammary tumors	
1 month old Wistar rats	80 F	caffeine, 0.2% (2000 µg/mL)	13.5 g (69.5 mmol)	dosed for 12 months	Hyperplasia and adenoma of the pituitary gland in 27/40 dosed rats and 9/30 of controls. Adenomas endocrinologically non-functioning	Yamagami et al. (1983)

Abbreviations: n.p. = not provided; F = female; M = male.

9.4 Antitumor Effect of Caffeine

In a study by Sadzuka (1995c), administration of adriamycin for 5 days at a dose of 0.5 mg/kg/day plus 100 mg/kg/day (0.5 mmol/kg/day) caffeine, increased the survival time of Ehrlich ascites-bearing mice by 39% over that seen with adriamycin without caffeine. However, caffeine (100 mg/kg/day; 0.5 mmol/kg/day) had no effect on survival following a dose of 2.0 mg/kg/day of adriamycin.

9.5 Modulation of Carcinogenicity

Studies Reviewed By IARC (1991)

The effect of caffeine on tumors induced by known carcinogens has been studied using a variety of animal models (IARC, 1991). In three separate studies, caffeine had no effect on the incidence of bladder tumors induced in rats by N-nitroso-N-butyl(4-hydroxybutyl)amine (Nakanishi et al., 1978, 1980; and Kunze et al., 1987, cited by IARC, 1991), nor did it have any effect on pancreatic tumors in rats induced by 4-hydroxyaminoquinoline 1-oxide (Denda et al., 1983, cited by IARC, 1991). Administration of caffeine resulted in a decreased incidence of lung tumors in mice treated with urethane (Theiss and Shimkin, 1978, cited by IARC 1991), of mammary tumors in rats treated with diethylstilbestrol (Petrek et al., 1985, cited by IARC, 1991), and of skin tumors in mice induced by UV light (Zajdela and Laterjet, 1973, 1975, 1978a,b, cited by IARC, 1991) or cigarette-smoke condensate (Rothwell, 1974, cited by IARC, 1991). Two other studies of urethane coadministered with caffeine in mice were deemed inadequate (Armuth and Berenblum, 1981; Nomura, 1983, both cited by IARC). A slightly protective effect against tumorigenesis was seen in mice administered caffeine in the drinking water 120-156 hours prior to s.c. administration of 4-nitroquinoline 1-oxide (Nomura, 1976, 1980, cited by IARC, 1991). A study which found a significant increase in squamous cell carcinomas in mice treated with caffeine, 4-nitroquinoline 1-oxide, and UV light was deemed inadequate by the IARC Working group (Hoshino and Tanooka, 1979, cited by IARC). Mice fed caffeine and morpholine in the diet along with sodium nitrite in the water had decreased numbers of lung tumors compared to mice without caffeine (Mirvish et al., 1975, cited by IARC, 1991).

Male Wistar rats receiving caffeine in drinking water three days prior to a 10-day daily treatment of i.p.-administered N-nitrosodiethylamine showed decreased incidences of liver tumors compared to controls; however, the IARC Working group noted that this reduction might be attributed to the high death rate in the caffeine-treated group. Caffeine had no effect on mammary tumors induced in mice or rats by 7,12-dimethylbenz[a]anthracene (Welsch et al., 1983, 1988a,b; Minton et al., 1983; Welsch and DeHoog, 1988; all cited by IARC, 1991). Studies in rats of the effect of caffeine on the incidence of tumors induced by 2-acetylaminofluorene (Hosaka et al.,1984; cited by IARC, 1991) and benzo[a]pyrene (Brune et al., 1981; cited by IARC, 1991) were deemed inadequate by the IARC Working Group.

Studies Not Previously Reviewed

The details of these studies are presented in **Table 8**.

Two studies by Sadzuka et al. (1995a and b) investigated the mechanism by which caffeine enhances the antitumor activity of adriamycin *in vivo* and *in vitro*. In the first study, the effects of adriamycin with and without caffeine on DNA and protein biosynthesis, and on the activities of DNA polymerase α and β were examined. Ehrlich ascites carcinoma cells were transplanted onto the backs of male CD1 mice (6 or 7 mice/group). Adriamycin was administered i.p. (2.0 mg/kg/day) into the carcinoma-bearing mice at 10, 12, 14, and 16 days after tumor initiation. Caffeine (100 mg/kg/day; 0.5 mmol/kg/day) was administered i.p. at 11, 13, 15, and 17 days after tumor initiation. The decrease in DNA and protein biosynthesis in tumors produced by caffeine combined with adriamycin were 2.4 and 2.5 times greater, respectively, compared to adriamycin alone. DNA polymerase activities in the tumor which was induced by adriamycin were 1.8 and 1.6 times greater, respectively, compared to controls. Conversely, adriamycin-induced effects in normal tissues were not enhanced by caffeine, although adriamycin concentrations were enhanced by 2.1-fold versus adriamycin administered alone. Caffeine (100 nM; 19.4 μg/mL) also inhibited the efflux of adriamycin (10 μg/mL) from cultured Ehrlich ascites

carcinoma cells *in vitro*. The results suggest the effect of caffeine on intracellular adriamycin concentrations may be related to its enhancement of adriamycin antitumor activity.

In the second study, the effects of caffeine as a biochemical modulator of adriamycin s antitumor activity were investigated *in vitro* and *in vivo* (Sadzuka et al., 1995b). *In vitro*, adriamycin (10 μg/mL) was incubated alone and in combination with caffeine (100 nM; 19.4 μg/mL) with Ehrlich ascites carcinoma cells which had been transplanted in male CDF_1 mice, and collected after the seventh day of transplantation. Immediately after the start of incubation with caffeine, an immediate decrease in cellular adriamycin concentration was seen. After 180 minutes, caffeine inhibited the efflux of adriamycin by 35.4% compared to controls. Adriamycin administered alone (0.5 mg/kg/day or 2.0 mg/kg/day) *in vivo* showed a 20% reduction of tumor weight versus control, an effect which was enhanced by 2.1-fold ($p<0.01$) by the addition of caffeine. However, there were no significant differences in heart, liver, or tumor adriamycin concentrations between adriamycin-alone and adriamycin-caffeine administered groups. The results suggest that the metabolism of caffeine may weaken its effect as a biochemical modulator.

9.5.1 Bladder

Female Wistar rats pretreated or not pretreated with N-methyl-N-nitrosourea (MNU) (approximately 1.5 mg) by urethral catheter were given either 0.1% caffeine, the equivalent of 20 mg (0.1 mmol) caffeine/day in drinking water, or coffee (Hicks et al., 1978). None of the rats ingesting coffee, alone, developed bladder tumors; however, caffeine-treated rats (20 mg/day; 0.1 mmol/day) had twice the number of MNU-induced tumors compared to rats ingesting caffeine without MNU-induction. The results indicate that coffee is not a bladder carcinogen in the rat, nor does it promote the growth of MNU-induced tumors. However, caffeine does appear to have a weak, yet significant ($p<0.05$), potentiating effect on MNU-initiated rat urothelium.

Female Fischer rats were fed either caffeine (0.1% in feed) alone (40 weeks) or co-administered N-[4-(5-nitro-1-furyl)-2-thizolyl]-formamide (FANFT) (0.188% in feed for 20 weeks), followed by caffeine alone (0.1% in feed for 20 weeks) (Wang and Hayashida, 1984).

Caffeine had no effect on bladder carcinogenesis, alone or in combination with FANFT. Similarly, caffeine (0.102% in the diet), alone or in combination with FANFT (2% in the diet) or antipyrene (0.535% in the diet), had no effect on the incidence of carcinogen-induced bladder tumors in male Sprague-Dawley rats (Johansson and Anderstrom, 1988).

9.5.2 Gastrointestinal

Intestinal tumors were induced in male Wistar and male BD6 rats by s.c. administration of 1,2-dimethylhydrazine (DMH) (20 mg/kg/day; 0.1 mmol/kg/day) for 20 consecutive weeks (Balansky et al., 1992). Caffeine (600 mg/L; 3.09 mmol/L) administered in the drinking water starting 7 days prior to administration of DMH had no significant effect on the size or number of the induced tumors.

Nishikawa et al. (1995) induced gastric adenocarcinomas in male Wistar rats with *N*-methyl-*N* -nitro-*N*-nitrosoguanidine (MNNG) and sodium chloride. MNNG is a known gastric carcinogen and sodium chloride has shown tumor co-initiating effects and tumor-promoting effects in the rat glandular stomach (Takahashi et al., 1984; cited by Nishikawa et al., 1995). Lipid peroxidation in the glandular stomach mucosa induced by 4% NaCl ingestion was inhibited by caffeine. This supports the hypothesis that caffeine (0.25%, comparable to 40-71 cups of coffee/day in humans) inhibits the gastric tumor promotion activity of NaCl in rats when administered during the post-initiation phase.

9.5.3 Liver

Caffeine had no significant protective effect on diethylnitrosamine-induced liver tumors in rats (Balansky et al., 1994).

9.5.4 Lymphatic System

Caffeine (100 mg/kg; 0.5 mmol/kg) enhanced the potentiating effect of benzo[a]pyrene (BP) on SJL/J mice infected with the Friend leukemia virus (FLV). However, caffeine alone or given in conjunction with FLV did not induce a carcinogenic effect (Raikow et al., 1981).

9.5.5 Mammary Gland

The effect of chronic caffeine consumption on 7,12-dimethylbenz(a)anthracene (DMBA) and N-methyl-N-nitrosourea (MNU)-induced mammary gland tumorigenesis was investigated in female Sprague-Dawley rats (VanderPloeg et al., 1991). In the initiation study, caffeine (500 mg/mL in drinking water) was administered for 30 days prior to and 3-4 days after carcinogen treatment. In the promotion study, caffeine was administered 3-4 days post carcinogen administration until experiment termination. Caffeine significantly decreased the number of carcinomatous and benign mammary tumors when given during the initiation phase in DMBA-dosed rats. However, when given during the promotion phase in DMBA-treated rats, no significant difference was found in the number of carcinomatous tumors, although a significant decrease in the number of benign tumors was seen. Caffeine had no effect on carcinomatous or benign tumors, either in the promotion or initiation phases of MNU-treated rats.

In another study, caffeine (500 mg/mL; 2.57 mmol/L) in drinking water was administered daily at 3-31 days post-carcinogen treatment to 4 different groups of female Sprague-Dawley rats, each having a high incidence of benign mammary fibroadenomas: 55 day old virgin rats (Group I), 53 day old ovariectomized, estrogen-treated virgin rats (Group II), 135 day old virgin rats (Group III), and 135 day old parous rats (Group IV) (Wolfrom et al., 1991). Caffeine treatment significantly reduced the incidence of benign mammary fibroadenomas in Groups I, II, and III. The decreased incidence of benign mammary fibroadenomas in Group IV was not significant. Caffeine also reduced the incidence of mammary gland cysts in Group II.

9.5.6 Pancreas

Caffeine s effects on the development of N-nitrosobis(2-oxypropyl)-amine (BOP)-initiated pancreatic tumors was investigated in Syrian hamsters (Nishikawa et al., 1992). The multiplicity of pancreatic tumors was significantly higher (p=0.05) in animals receiving 2000

µg/mL caffeine in drinking water in the post-initiation phase than in controls.

9.5.7 Pulmonary

Several studies relevant to the anticarcinogenic effects of caffeine or methylxanthines on lung neoplasms were found. Yun (1991) developed a test system to evaluate the anticarcinogenicity of natural products on lung tumors induced in NIH(GP) mice by s.c. administration of benzo[a]pyrene (BP) (0.5 mg). In this system, caffeine (0.1-1.0 mg; 0.5-5 nmol) in the diet significantly decreased the number of BP-induced lung adenomas in NIH(GP) mice. In another study, 1 or 2 mg/day (5 or 10 nmol/day) of caffeine in the diet significantly reduced the tumorigenic effects of BP (0.5 mg, s.c.) in both male and female N:GP(S) mice (Yun et al., 1995).

9.5.8 Skin

In a study of urethane-induced skin tumorigenesis, caffeine (100 µg/g; 0.5 µmol/g) significantly increased papilloma incidence when given 6 hours before initiation with urethane (topical anthranil treatment serving as the promoter). When administered 6 hours after urethane initiation, caffeine showed an inhibitory effect on tumor development. The results of this study demonstrate that the timing of caffeine administration in relation to initiation might determine the direction of its modulating effect (Armuth and Bereblum, 1981).

Huang et al. (1997) investigated the effects of caffeine on UVB light-induced carcinogenesis in the skin of SKH-1 mice. Oral administration of caffeine (0.24 or 0.72 mg/mL; 1.2 or 3.7 µmol/mL in water) inhibited tumorigenesis. Administration of doses (3.0-9.0 mg/mL) of decaffeinated tea enhanced the tumorigenic effect of UVB light. Adding caffeine to decaffeinated teas restored their inhibitory effects on UVB-induced carcinogenesis.

Table 8. Modulation of Carcinogenicity

Species, Strain, and Age	Number and Sex	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
Ehrlich ascites carcinoma cells transplanted onto the backs of 6 wk old CDF_1 mice	6-7 M per group	caffeine, purity n.p.	caffeine: 1, 10, 100 nM (0.2, 1.9, 19.4 ng/mL) adriamycin (ADR): 10 µg/mL	ADR administered 10, 12, 14, and 16 days after tumor inoculation; caffeine administered 11, 13, 15 and 17 days after inoculation, observation period n.p.	Caffeine inhibited the efflux of adriamycin from cultured Ehrlich ascites carcinoma cells. These results, together with the results of the *in vivo* study, suggest the effect of caffeine on intracellular adriamycin concentrations may be related to its enhancement of adriamycin antitumor activity.	Sadzuka et al. (1995a)
Ehrlich ascites carcinoma cells transplanted onto the backs of 6 wk old CDF_1 mice	10 M per group	caffeine, purity n.p.	caffeine: 10 mg/kg/day ADR: 2 mg/kg/day	4 day exposure, observation period n.p.	No effect on adriamycin influx; decrease in adriamycin concentration in cells; after 180 min caffeine inhibited efflux of adriamycin by 35.4%.	Sadzuka et al. (1995b)
9.5.1 Bladder						
Rats, Wistar, 6-8 wk	Group 1: 48 F Group 2: 87 F Group 3: 38 F Group 4: 58 F Group 5: F, # Group 6: 32 F n.p.	caffeine and coffee, purities n.p.	MNU: ~1.5 mg Coffee: ~20 mL/rat/day Caffeine: ~20 mg (0.1 mmol/rat/day) Dose schedule: Group 1: controls fed standard diet Group 2: MNU Group 3: Coffee Group 4: MNU and Coffee Group 5: Caffeine Group 6: MNU and Caffeine	2 year study duration	No tumors in groups 1 and 3; 15/81 tumors in group 2; 15/55 tumors in group 4; 11/25 tumors in group 6. No results were given for Group 5. None of the rats ingesting coffee (the equivalent of 15-20 mg of caffeine/day), alone or with MNU pretreatment, developed bladder tumors. However, treating rats with caffeine (20 mg/day) doubled the rate of MNU tumor induction. The results indicate that coffee is not a bladder carcinogen for the rat, nor does it promote the growth of MNU-induced tumors, despite its high caffeine content. However, caffeine, appears to have a weak, but significant (p=0.05) potentiating effect on MNU-initiated rat urothelial tumors.	Hicks et al. (1978)

Table 8. Modulation of Carcinogenicity (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats, Fischer 344, age n.p.	Group 1: 15 F Group 2: 47 F Group 3: 12 F Group 4: 15 F	caffeine, purity n.p.	Group 1: control diet Group 2: 0.188% FANFT in feed for 40 wk Group 3: 0.1% caffeine in feed for 40 wk Group 4: 0.1% caffeine in feed coadministered with 0.188% FANFT in feed for 20 wk followed by 0.1% caffeine alone in feed for 20 wk	20-40 week exposure, observation period n.p.	Group 2 had 8 carcinomas (17%). Group 4 had 10 carcinomas (20%). Groups 1 and 3 had no carcinomas. Caffeine had no effect on bladder carcinogenesis, alone or in combination with FANFT.	Wang and Hayashida (1984)
Rats, SPF Sprague-Dawley, 6 wk	Group 1: 30 M Group 2: 28 M Group 3: 27 M Group 4: 26 M Group 5: 28 M	caffeine, purity n.p.	Group 1: control diet Group 2: 2% FANFT in feed for 5 wk, followed by	88 wk exposure, observation period n.p.	Group 3, the only group with any tumors, had 10/27 rats develop urinary tract tumors (37%), 5 renal pelvic tumors, and 5 urinary bladder tumors. Caffeine had no effect on bladder carcinogenesis,	Johansson and Anderstrom (1988)

Table 8. Modulation of Carcinogenicity (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
	Group 6: 28 M		control diet Group 3: 2% FANFT in feed for 5 wk, followed by 0.535% antipyrene in the diet Group 4: 0.535% antipyrene in the diet Group 5: 0.102% caffeine in the diet. Group 6: 0.535% antipyrene and 0.102% caffeine in the diet.		alone or in combination with FANFT or antipyrene.	
9.5.2 Gastrointestinal						
Rats, Wistar and BD_6, 8-12 wk	180 M	caffeine, purity n.p.	600 mg/L in the drinking water starting 7 days prior to administration of 1,2-dimethylhydrazine (DMH)	20 wk exposure, rats sacrificed at 35 wk	Caffeine had no significant effect on the size or number of the induced tumors. Intestinal tumors were induced by s.c. administration of DMH for 20 consecutive weeks. Control groups of untreated rats were not included in this study.	Balansky et al. (1992)
Rats, Wistar, 5 wk	120 M	caffeine, >98.5% pure NaCl, >99.5% pure	100 ppm MNNG and 5% NaCl in drinking water (initiation) Group 1: 0.25% caffeine in water and 5% NaCl in	initiation: 8 wk post-initiation: 32 wk	Group 1 showed lowest body weight; incidence of adenocarcinomas in pyloric area significantly lower in group 1 compared to group 2, atypical hyperplasias in the fundic area were lower in group 1 than group 2. Group 3 showed no evidence of modulation of glandular stomach tumor development.	Nishikawa et al. (1995)

Table 8. Modulation of Carcinogenicity (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
			food Group 2: 5% NaCl in food Group 3: 0.25 % caffeine			
9.5.3 Liver						
Rats, BD_6, adult, age n.p.	30-42 F per group	caffeine, purity n.p. diethyl-nitrosamine, purity n.p.	caffeine: 300 or 600 mg/L orally diethylnitrosamine 80 mg/kg i.p.	5-9 wk exposure, observation period n.p.	Caffeine had no significant effect on diethylnitrosamine-induced liver tumors.	Balansky et al. (1994)
9.5.4 Lymphatic System						
FLV-infected mice, SJL/J, 10 wk	~ 138 F	caffeine, purity n.p. benzo[a]-pyrene (BP), purity n.p.	caffeine: 100 mg/kg (0.5 mmol/kg) BP: 500 µg/mL i.p.	single i.p. injection of caffeine, 300 day study period	Caffeine enhanced the potentiating effect of BP in SJL/J mice infected with FLV. However, caffeine alone or given in conjunction with FLV did not cause any carcinogenicity.	Raikow et al. (1981)
9.5.5 Mammary Gland						
Rats, Sprague-Dawley, 55 d.o.	Initiation stage: Control: 73 F, Caffeine: 70 F Promotion stage: Control: 72 F, Caffeine: 62 F	caffeine, purity n.p. DMBA, purity n.p. MNU, purity n.p.	caffeine: 500 mg/mL (2.57 mmol/L) in drinking water DMBA: 0.5 mg/100 g bw MNU: 2.5 mg/ 100 g bw	Initiation: caffeine given 30 days prior to and 3-4 days after carcinogen injection Promotion: caffeine given 3-4 days post carcinogen administration until	Caffeine significantly decreased the number of carcinomatous and benign mammary tumors in initiation phase in DMBA-dosed rats. However, in the promotion phase, no significant difference was seen in the number of carcinomatous tumors, and a significant decrease in the number of benign tumors occurred. Caffeine had no effect on carcinomatous or benign tumors, either in the promotion or initiation phases of MNU-treated	VanderPloeg et al. (1991)

Table 8. Modulation of Carcinogenicity (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
				study termination, 48 week study period	rats.	
Rats, Sprague-Dawley with elevated incidence of benign mammary fibroadenomas: Group 1: 55 d.o. virgin rats Group 2: 53 d.o. ovariectomized, estrogen-treated, 135 d.o. virgin rats Group 3: 35 d.o. virgin rats, Group 4: 35 d.o. parous rats	F, number n.p.	caffeine, purity n.p. DMBA, purity n.p.	caffeine: 500 mg/mL (2.57 mmol/L) in drinking water DMBA: 20 mg/mL i.g.	Daily at 3-31 days post-treatment with DMBA, 56 week study period	Caffeine treatment significantly reduced the incidence of benign mammary fibroadenomas in Groups 1, 2, and 3. Reduced incidence of benign mammary fibroadenomas in Group 4 was not significant. Caffeine also reduced the incidence of mammary gland cysts in Group 2.	Wolfrom et al. (1991)
9.5.6 Pancreas						
Hamsters, Syrian, 6 wk	270 F	caffeine, 98.5% pure BOP, purity n.p.	caffeine: 2000 µg/mL (10.30 mM) in drinking water BOP: 10 mg/kg s.c.	40 wk exposure period, animals sacrificed at 40 wk	The multiplicity of pancreatic tumors was significantly higher in animals receiving caffeine in the post-initiation phase than in controls.	Nishikawa et al. (1992)
9.5.7 Pulmonary System						
Mice, newborn NIH(GP)	80/group; sex n.p.	caffeine, purity n.p. BP, purity	caffeine: 0.1-1 mg/day (0.5-5 nmol/day) in the	BP injection within 24 h after birth, 9 wk exposure to caffeine,	47% of mice given 0.5 mg/kg BP s.c. developed pulmonary adenomas after 9 wk. Caffeine significantly reduced the number of BP-induced lung adenomas.	Yun (1991)

Table 8. Modulation of Carcinogenicity (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Purity	Dose	Exposure/ Observation Period	Results/Comments	Reference
		n.p.	diet BP: 0.5 mg/kg s.c.	observation period n.p.		
Mice, newborn A/J and N:GP(S)	60-80 M and F per group	caffeine, purity n.p. BP, purity n.p.	caffeine: 1 or 2 mg/day (5 or 10 nmol/day) in the diet BP: 0.5 and 1.0 mg s.c.	BP injection within 24 h after birth, 6 wk exposure to caffeine, mice sacrificed after 9 wk	42% of mice administered 0.5 mg/kg BP s.c. developed pulmonary adenomas. Caffeine reduced the percentage of BP-induced lung tumors by half. Caffeine alone induced no lung tumors.	Yun et al. (1995)
9.5.8 Skin						
Mice, ICR , 6-8 wk	390 F	caffeine, purity n.p.;	100 µg/g (0.5 µmol/g); urethane as initiator; anthranil as promoter	45 wk from beginning to promotion	Caffeine increased papilloma incidence when given 6 h before initiation (tumor incidence was augmented by 60%); inhibited tumorigenesis when given 6 h after initiation (tumor incidence decreased 23%).	Armuth and Berenblum (1981)
Mice, SKH-1 hairless, 6-7 wk	90 F (3 groups of 30)	caffeine, >99% pure	caffeine: 0.24 or 0.72 mg/mL (1.2 or 3.7 µmol/mL) UVB light: 30 mJ/cm^2 twice/wk	35 wk exposure, animals sacrificed at 39 wk	Number of UVB-induced tumors per mouse was decreased by 53% (0.24 mg/mL) and 61% (0.72 mg/mL) 35 wk after induction with UVB light.	Huang et al. (1997)

Abbreviations: n.p. = not provided; bw = body weight; h = hour(s); s.c. = subcutaneous injection; i.v. = intravenous injection; i.g. = intragastrically; d.o. = day(s) old; BP = benzo[a]pyrene; DMBA = 7,12-dimethylbenz(a)anthracene; DMH = 1,2-dimethylhydrazine; MNNG = *N*-methyl-*N* -nitro-*N*-nitrosoguanidine; M = male; F = female; wk = week(s); FANFT = N-[4-(5-nitro-1-furyl)-2-thizolyl]-formamide; MNU = N-methyl-N-nitrosourea; BOP = *N*-nitrosobis(2-oxypropyl)amine; wk = week(s); FLV = Friend leukemia virus.

9.6 Genotoxicity of Caffeine

In the following sections, a brief review of the published information on the genotoxicity of caffeine is provided. Information on the genotoxicity of caffeine published prior to 1991 was reviewed by IARC (1991), literature published between 1987-1993 was reviewed by D Ambrosio (1994). In addition to information summarized from these reviews, a detailed review is provided for articles published after 1990 that are not included in the D Ambrosio paper.

9.6.1 Studies Reviewed by IARC (1991)

Prior to 1991, caffeine had been extensively studied for genotoxicity using a variety of acellular, bacterial, plant, *in vitro* mammalian cell, and *in vivo* test systems. Almost all *in vitro* tests, with the exception of *Salmonella typhimurium* gene mutation assays, were conducted in the absence of exogenous metabolic activation only. As reviewed by IARC (1991), caffeine was positive for differential toxicity in *Escherichia coli* (DeFlora et al., 1984a; cited by IARC, 1991) but gave inconclusive results in the *Bacillus subtilis* rec assay (Kada et al., 1972; cited by IARC, 1991). Various strains of *S. typhimurium*, both those that mutate via base substitution and those that mutate via frameshift, were tested with and without exogenous metabolic activation; all results were negative (for example, see King et al., 1979; De Flora et al., 1984a, Dunkel et al., 1985; Mortelmans et al., 1986; Heddle and Bruce, 1977; all cited by IARC, 1991). In the *S. typhimurium* studies, the highest dose of caffeine tested was 5,000 g/plate (25.75 µM/plate) (Mortelmans et al., 1986; cited by IARC, 1991). Mutation studies using *E. coli* were typically but not exclusively negative (King et al., 1979; Demerec et al., 1951; Dunkel et al., 1985; all cited by IARC, 1991); however, caffeine induced lac^- reversion in *E. coli* K12 ND160 cells in the absence of metabolic activation (Clarke and Wade, 1975; cited by IARC, 1991).

Increased mitotic gene conversion and decreased meiotic recombination were induced in *Schizosaccharomyces pombe* (Loprieno et al., 1974; cited by IARC, 1991), while aneuploidy was induced in *Saccharomyces cerevisiae* (Parry et al., 1979; cited by IARC, 1991).

The induction of chromosomal aberrations by caffeine were reported for several plant test systems (for example, Kihlman et al., 1971a; Kesavan et al., 1973; Osiecka, 1976; all cited by IARC, 1991).

Numerous studies evaluated the effects of caffeine in *Drosophila melanogaster*, with conflicting results. Most tests for the induction of sex-linked recessive lethal (SLRL) mutations were negative (i.e., Clark and Clark, 1968; cited by IARC, 1991). However, two positive studies were reported, one in which larvae were treated (Ostertag and Haake, 1966; cited by IARC, 1991) and the other in which females were treated rather than males (Shakarnis, 1970; cited by IARC, 1991). Results from aneuploidy studies using *D. melanogaster* were also discordant, with studies investigating XO males mostly positive, and those evaluating for XXY females negative.

In cultured Chinese hamster cells, caffeine did not induce DNA single-strand breaks (SSB), unscheduled DNA synthesis (UDS), sister chromatid exchanges (SCE) (Swenberg, 1981; Casto et al., 1976; Kato, 1973; Palitti and Becchetti, 1977; Bowden et al., 1979; Speit, 1986; all cited by IARC, 1991), or gene mutations (*hprt*, *tk*, ouabain resistance) (Arlett and Harcourt, 1972; Amacher and Zelljadt, 1984; Trosko and Chu, 1971; Chang et al., 1977; Bowden et al., 1977; Amacher et al., 1980; all cited by IARC, 1991). In contrast, caffeine induced SCE in cultured human fibroblasts (Sasaki, 1977) and mitogen-stimulated human blood lymphocytes (Pant et al., 1976; Guglielmi et al., 1982; Andriadzee et al., 1986; Tohda and Oikawa, 1988; all cited by IARC, 1991). In the human blood lymphocyte study conducted by Guglielmi et al. (1982; cited by IARC, 1991), the induction of SCE occurred only when caffeine was present during cell proliferation and the shape of the dose response curve was consistent with caffeine interfering with DNA synthesis rather than interacting directly with DNA. Induction of chromosomal aberrations was frequently reported for cultured hamster cells (Kihlman et al., 1971a; Palitti et al., 1974; Sturelid, 1976; all cited by IARC, 1991) and for mitogen-stimulated human lymphocytes treated *in vitro* (Lee, 1971; Weinstein et al., 1972; Ceccherini et al., 1988; Ostertag et al., 1965; all cited by IARC, 1991).

In vivo in mammals, caffeine did not induce chromosomal aberrations, and the majority of results from micronucleus tests were negative. However, Aeschbacher et al. (1986; cited by

IARC, 1991) reported increased frequencies of micronucleated bone marrow cells in Chinese hamsters and Swiss CD-1 mice after oral administration of caffeine at LD_{50} dose levels. The frequency of SCE were increased in bone marrow cells by oral administration to Chinese hamsters (Basler et al., 1979; Renner, 1982; Aeschbacher et al., 1986; all cited by IARC, 1991) and mice (Panigrahi and Rao, 1983; cited by IARC, 1991). Numerous tests for induction of dominant lethal mutations in germ cells of male mice treated with caffeine via gavage, drinking water, or i.p. injection were negative (Lyon et al., 1962; Epstein and Shafner, 1968; Adler, 1969; Epstein et al., 1970; Rohrborn, 1972; Thayer and Kensler, 1973; Aeschbacher et al., 1978; all cited by IARC, 1991).

Two *in vivo* human studies were included in the IARC review. Caffeine intake was not associated with increased levels of chromosomal aberrations in human blood lymphocytes (Weinstein et al., 1972; cited by IARC, 1991). However, an increased frequency of micronucleated erythrocytes was reported for splenectomized humans who drank coffee and tea resulting in a total caffeine intake equivalent to drinking 5 cups of coffee per day (Smith et al., 1990; cited by IARC, 1991).

9.6.2 Studies Reviewed by D Ambrosio (1994)

D Ambrosio (1994) conducted an extensive review of published genotoxicity data on caffeine, covering the period from approximately 1987 through 1992, and overlapping with the more recent entries in the IARC review. Included in this review were test systems utilizing prokaryotic, *in vitro* eukaryotic, and *in vivo* mammalian systems. The genotoxic endpoints analyzed included structural chromosomal aberrations, micronuclei, and SCE. In general, the results reported in this review support the conclusions by IARC (1991) that caffeine is generally nonmutagenic in bacteria or mammalian cells, but clastogenic in proliferating eukaryote cells *in vitro*. *In vivo* genetic effects were mostly evaluated in studies testing caffeine in conjunction with other genotoxins, and exposures in human studies were not well controlled or defined. For example, caffeine (150 mg/kg four times/day) induced MN-PCE in folate-deficient mice but not in healthy mice (MacGregor et al., 1990; cited by D Ambrosio, 1994). Everson et al. (1988; cited

by D Ambrosio, 1994) reported that pregnant women who smoked and consumed more than 14 caffeinated beverages per week had significantly higher amounts of a specific adduct in placental DNA compared to pregnant smokers who did not consume caffeinated beverages. Chen et al. (1989; cited by D Ambrosio, 1994) reported a positive correlation between coffee consumption and structural chromosomal damage in mitogen-stimulated blood lymphocytes of individuals drinking more than 4 cups of coffee daily.

9.6.3 Review of Recent Literature

The details of these studies are presented in **Table 9**.

9.6.3.1 Acellular Systems

Caffeine induced DNA SSB, double-strand breaks (DSB), and DNA-protein crosslinks in isolated L1210 cell nuclei, and it inhibited the formation of DSB induced by the topoisomerase II inhibitor ellipticine (Russo et al., 1991).

9.6.3.2 Lower Eukaryotes

In the *Drosophila* white-ivory eye spot test, caffeine administered by feeding for 48 hours to 2 day-old larvae was non-recombinogenic, although a weak response was detected in the somatic mutation and recombination test (SMART) when caffeine was fed to 3-day old larvae (Graf and Wurgler, 1996). These results are in agreement with earlier *Drosophila* tests in which it was concluded that caffeine was a weak genotoxin (Graf and Wurgler, 1986; cited by Graf and Wurgler, 1996).

9.6.3.3 *In Vitro* Mammalian Systems

Further evidence of the ability of caffeine to induce chromosomal damage *in vitro* was provided by Glover et al. (1992, abstract) who showed that caffeine induced large scale deletions in the TK gene region in mouse L5178Y cells in the absence of S9. Caffeine was negative for fragile-X induction but positive for induction of common fragile sites in human blood

lymphocytes (Glover et al., 1986). Caffeine, in the absence of metabolic activation, induced fragile site expression (Yunis et al., 1987; Fundia et al., 1996; Wenger, 1995) and chromosomal aberrations but not SCE (Wenger, 1995) in human blood lymphocytes . In contrast, Curry et al. (1992, abstract), in a brief abstract, reported that caffeine without metabolic activation, at concentrations up 50 mM (9.7 mg/mL), did not increase the frequency of micronucleated monkey kidney cells *in vitro*.

9.6.3.4 *In Vivo* Mammalian Systems

Caffeine, 150 mg/kg (0.772 mmol/kg) administered i.p. to female mice, had no significant effect on the frequency of aneuploidy (compared to controls) or structural chromosomal aberrations in mouse oocytes, or on oocyte maturation (Mailhes et al., 1996).

9.6.3.5 Human Studies

Although clastogenic effects are easily demonstrated *in vitro*, human population studies are in conflict on the association between caffeine intake (measured as coffee drinking or caffeinated beverage consumption, both of which can be highly variable and inaccurate measures) and increased levels of chromosomal or DNA damage. Robbins et al. (1997) reported a significant correlation between caffeine intake and aneuploidy in sperm of healthy men aged 19-35 years, while caffeine intake was not associated with an increased frequency of 6-thioguanine mutations in lymphocytes of healthy men and women, aged 20-60 years (Davies et al., 1992). No significant relationship between caffeine intake and mutant frequency at the *hprt* locus in T-lymphocytes was seen in women with benign and malignant breast masses compared to healthy women (Branda et al., 1992).

Table 9. Genotoxicity of Caffeine

Test System or Species, strain, and Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.6.3.1 Acellular Systems							
Isolated L1210 cell nuclei	single and double strand DNA breaks (SSB and DSB, respectively)	-	caffeine, purity n.p.	0.1-10,000 µM (0.02-1942 µg/mL) for 30 min at 22°C	positive		Russo et al. (1991)
9.6.3.2 Lower Eukaryotes							
Drosophila melanogaster, white-ivory eye spot test	somatic recombination (eye spots)	NA	caffeine, purity n.p.	5, 10, and 20 mM (1, 1.9, 3.9 mg/mL) in feed to 2 day old larvae for 72 h	negative		Graf and Wurgler (1996)
D. melanogaster, somatic mutation and recombination test (SMART)	somatic recombination and mutation (wing spots)	NA	caffeine, purity n.p.	10 and 30 mM (1.9 and 5.8 mg/mL) in feed to 3 day old larvae for 48 h	weakly positive		Graf and Wurgler (1996)
9.6.3.3 In Vitro Mammalian Systems							
Mouse L5178Y lymphoma cells	deletions in the $tk^{+/-}$ region	-	caffeine, purity n.p.	n.p.	large scale deletions in the tk^{+} region		Glover et al. (1992, abstract)
Human blood lymphocytes (fragile-X patients)	fragile site expression	-	caffeine, purity n.p.	2.2 and 2.5 mM (0.43 and 0.49 mg/mL) for 3 or 6 hours prior to harvest	Negative for fragile-X induction but positive for induction of common fragile sites.	Total number of chromosomal lesions (gaps + breaks) was significantly increased by caffeine.	Glover et al. (1986)
Human blood lymphocytes	fragile site expression	-	caffeine, purity n.p.	2.2 and 2.5 mM (0.43 and 0.49 mg/mL) for 3 or 6 hours prior to harvest	positive		Yunis et al. (1987)
Human blood lymphocytes	fragile site expression	-	caffeine, purity n.p.	2.2 mM (0.43 mg/mL) 6 hours prior to harvest	positive		Fundia et al. (1996)

Table 9. Genotoxicity of Caffeine (Continued)

Test System or Species, strain, and Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
Human blood lymphocytes	fragile site expression, SCE, chromosomal aberrations	-	caffeine, purity n.p.	2.2 mM (0.43 mg/mL) 6 hours prior to cell harvest	Negative for SCE; positive for CA and fragile sites	50% of CA were induced at fragile cites	Wenger (1995)
Monkey kidney cells	Micronucleated binucleated (MN-BN) cells	NA	caffeine, purity n.p.	10 to 50 mM (1.9 to 9.7 mg/mL)	negative		Curry et al. (1992, abstract)
9.6.3.4 In Vivo Mammalian Systems							
Superovulated female ICR mice, 8-12 wk old	Aneuploidy in metaphase II oocytes	NA	caffeine, purity n.p.	150 mg/kg (0.772 mmol/kg) i.p. at several intervals prior to metaphase I	Negative for induction of aneuploidy (hyperploidy); no structural aberrations observed.	Oocyte maturation was normal. The negative effect may reflect the inability of caffeine to get to the ovaries.	Mailhes et al. (1996)
9.6.3.5 Human Studies							
Healthy men (45, 19-35 y.o.)	Aneuploidy in sperm, detected using FISH	NA	caffeine, purity n.p.	caffeine dose calculated from dietary data	positive correlation	Caffeine intake was significantly associated with increased frequencies of aneuploidy for chromosomes X, Y, and 18.	Robbins et al. (1997)
Healthy men (21, 22-60 y.o.) and women (20, 20-55 y.o.)	6-thioguanine mutations in blood lymphocytes	NA	coffee	n.p.	No significant increase in 6-thioguanine mutations in coffee drinkers.		Davies et al. (1992)
Healthy women (6, average age 44.7 y.o.) and women	mutation at the *hprt* locus in blood lymphocytes	NA	coffee, details n.p.	caffeine dose calculated from estimated coffee	No significant relationship between coffee intake and mutant frequency for women		Branda et al. (1992)

Table 9. Genotoxicity of Caffeine (Continued)

Test System or Species, strain, and Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
with benign (52, average age 45.8 y.o.) and malignant breast masses (49, average age 50.2 y.o.)				intake	with benign or malignant breast masses.		

Abbreviations: n.p. = not provided; NA = not applicable; DMSO = dimethyl sulfoxide; FISH = fluorescence in situ hybridization; SCE = sister chromatid exchanges; CA = chromosome aberration(s); MN-BN = micronucleated binucleated; SMART = somatic mutation and recombination test; wk = week(s); min = minute(s); y.o. = years old.

9.7 Modulation of Genotoxicity

The details of studies discussed in this section are presented in **Table 10**.

9.7.1 Prokaryotes

In *S. typhimurium* strains TA98 and TA100, the antimutagenic activity of various tea extracts to some mutagens (e.g., 3-amino-1,4-dimethyl-5*H*-pyrido(4,3-*b*)indole (Trp-P-1) were well correlated with the content of caffeine (Yen and Chen, 1996). Caffeine (up to 10 mol/plate (1.9 mg/plate)) did not reduce the mutagenic activity of either 4-nitroquinoline 1-oxide (4NQO) when tested without metabolic activation in *S. typhimurium* strain TA100 or mainstream cigarette smoke with metabolic activation in strain TA98 (Camoirano et al., 1994). However, when co-administered (1 or 2 mM; 0.2 or 0.4 mg/mL) or administered after treatment (0.8 mg/plate; 4 mol/plate) with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) or *N*-methyl-*N*-nitrosourea (MNU) to *S. typhimurium* strain TA1535, caffeine markedly reduced the mutagenicity of both compounds; post-treatment was not as effective as co-treatment (Balansky, 1992).

Caffeine at 0.5 mM (0.1 mg/mL), without metabolic activation, markedly enhanced the ability of MNNG to induce mutants in *S. typhimurium* strain TA1535 (Balansky, 1992).

9.7.2 Eukaryotes

In the yeast *S. cerevisiae* D5, the addition of caffeine (7.7 mM; 1.5 mg/mL) with the antitumor agent DACA (N-[(2-dimethylamino)ethyl]acridine-4-carboxamide) caused a significant decrease in the incidence of all types of mutations in yeast colonies (aberrant colonies) (Ferguson et al., 1993). At a higher concentration, caffeine (20 mM; 3.9 mg/mL) inhibited the induction of chromosomal aberrations by camptothecin in *Vicia faba* root tip cells (Kihlman and Andersson, 1992).

Caffeine (5 mM; 1 mg/mL) synergistically enhanced the frequency of chromosomal aberrations induced by camptothecin in *Vicia faba* root tip cells (Kihlman and Andersson, 1992).

9.7.3 *In Vitro* Mammalian Systems

DNA Damage: Treatment of A_L human-hamster hybrid cells with caffeine (1.5 mg/mL; 7.7 mM) for 16 hours (sufficient to reduce cell plating efficiency by 20%) synergistically enhanced the extent of ionizing radiation-induced DNA base pair deletions (McGuinness et al., 1995). Caffeine (10 mM; 1.9 mg/mL) was equally potent in enhancing *t*-butylhydroperoxide-induced DNA SSB in both oxygen-proficient and oxygen-deficient human myeloid leukemia U937 cells (Guidarelli et al., 1997). In human blood lymphocytes, caffeine (120 g/mL; 0.618 mM) in combination with chlorpromazine synergistically enhanced of the induction of SCE by adriamycin (Lialiaris et al., 1992).
Chromosomal Damage: When present in complete medium, caffeine (2.5 mM; 0.49 mg/mL) potentiated the expression of fragile sites by 4',6-diamidino-2-phenyl-indole in cultured human peripheral blood lymphocytes (Pelliccia and Rocchi, 1992). Caffeine (1 mM; 0.2 mg/mL) failed to potentiate the clastogenicity of mitoxantrone, an inhibitor of DNA synthesis, in the same test system (das Gra as Medeiros and Takahashi, 1994).

The effect of caffeine on the induction of chromosomal aberrations in X-irradiated normal human lymphoblastoid cell lines (RM341) and ataxia telangiectasia (AT) cells with intermediate radiosensitivity (AT9RM, AT10RM, and AT11RM) and classical radiosensitivity (AT8RM) was investigated by Antoccia et al. (1995). Caffeine (5 mM; 1 mg/mL) increased the frequency of chromosomal aberrations in RM341 and AT11RM cells (approximately 3-fold), moderately in AT9RM and AT10RM cells (1.6- and 1.8-fold, respectively), and weakly in AT8RM cells (1.3-fold). The damage produced by caffeine was similar to that of inhibitors of DNA polymerases and ribonucleotide reductase, indicating that caffeine may inhibit double-strand breakage repair. In a subsequent related study, caffeine (1 mM; 0.2 mg/mL) synergistically enhanced the

clastogenicity of ionizing radiation in normal and AT heterozygous human lymphoblastoid cells but not in AT homozygous lymphoblastoid cells (Bebb et al., 1998). Cells from AT patients prematurely pass through cell cycle checkpoints preventing the repair of DNA damage prior to entering a critical phase of cell division. The inability of caffeine to enhance the clastogenicity of ionizing radiation in AT cells supports a conclusion that caffeine interferes with these checkpoints in normal cells.

Caffeine (5 mM; 1 mg/mL), applied during G_2 after irradiation, synergistically enhanced the clastogenicity of X-rays when mitogen-stimulated lymphocytes from patients with the Nijmegen breakage syndrome (NBS) were used; carriers for the syndrome showed no such effect (Pincheira et al., 1998). In a related study, caffeine (5 mM; 1 mg/mL) enhanced radiation-induced chromosomal aberrations to a greater extent in RM341 cells than in NBS cells (line 1548) and AT cells, indicating an altered DNA repair pathway (Antoccia et al., 1997).

In vitro, in mouse zygotes, enhancement of chromosomal aberrations was also observed with caffeine (1, 2 mM; 0.2, 0.4 mg/mL), more when cells were irradiated in G_2 than in S phase (Matsuda and Tobari, 1995). In cultured Chinese hamster ovary (CHO) cells, caffeine (0.09-0.752 mg/mL; 0.5-3.87 mM) showed a dose-dependent potentiating effect on chromosomal aberrations induced by triphenyltin (Sasaki et al., 1994).
Micronuclei: In cultured monkey kidney cells, co-administration of caffeine (10-50 mM; 1.9-9.7 mg/mL) inhibited the induction of micronuclei (MN) by either ciprofloxacin (CIPRO) or PD 124816-2 (quinoline antiinfective drugs) (Curry et al., 1992, abstract). The investigators postulated that caffeine produced this effect through interference with the topoisomerase inhibiting effects of the quinolines. In metabolically competent human hepatoma cells, post-treatment with caffeine (0.185-500 g/mL; 0.953-2570 M) reduced by up to 75% the induction of MN by 2-amino-3-methylimidazo-[3,4-*f*]quinoline (IQ) (Sanyal et al., 1997). This reduction occurred also with other heterocyclic amines [e.g., 2-amino-3,8-dimethyl-imidazo-[4,5-*f*]quinoxaline (MeIQx), Trp-P-1].

Mutations: In cultured V79 Chinese hamster cells, caffeine (1 mM; 0.2 mg/mL) increased the yield of methotrexate-resistant colonies (Roy et al., 1993). Post-treatment of ^{137}Cs γ-radiation-irradiated A_L human-hamster hybrid cells doubled the slope of the dose-response curve for the induction of $S1^-$ mutants, compared to irradiated cells alone (McGuinness et al., 1995). The majority of the induced mutations (73-85%) involved large deletions, which were rare in cells exposed to radiation only (1/63=1.5%). Thus, treatment with caffeine after irradiation altered both the quantity and quality of γ-radiation-induced mutations.

9.7.4 *In Vivo* Mammalian Systems

Administration of caffeine, either acutely by i.p. injection (5 and 15 mg/kg bw; 0.03 and 0.077 mmol/kg bw) or chronically via drinking water for 5 weeks (4.208 and 0.772 mM; 0.8172 and 0.150 mg/mL) to mice prior to whole-body gamma-irradiation (1.5 Gy ^{60}Co γ-rays) decreased the frequency of radiation-induced chromosomal aberrations in bone marrow cells (Farooqi and Kesavan, 1992); the chronic caffeine pre-treatment was more effective than the acute pre-treatment. Intraperitoneal injection of caffeine (5, 15 mg/kg bw; 0.03, 0.077 mmol/kg bw) immediately post-irradiation also decreased the frequency of chromosomal aberrations induced by γ-rays, with the higher concentration providing more radioprotection.

In a host-mediated assay using *S. typhimurium* strain TA98 in female BALB/c mice, an average daily intake of 28 mg/kg bw (0.14 mmol/kg bw) caffeine for a month decreased the mutagenic activity of an oral dose of MeIQx (Alldrick et al., 1995). Based on *in vitro* data, the investigators postulated that the decrease reflected the ability of caffeine to inhibit the hepatic activation of MeIQx to a mutagen.

Caffeine (120 mg/kg; 0.618 mmol/kg), administered i.p. to mice in combination with chlorpromazine, enhanced the induction of SCE by adriamycin in inoculated Ehrlich ascites cells (Lialiaris et al., 1992).

Table 10. Modulation of Genotoxicity

Test System or Species, Strain, Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.10.1 Prokaryotes							
S. typhimurium strains TA98 and TA100	*his* gene mutations induced by 4NQO in TA100 and cigarette smoke in TA98	-TA100 +TA98	caffeine, purity n.p., plus 4NQO or cigarette smoke	caffeine: 0.33, 1.0, 3.3, 10 mol/plate (0.064, 0.19, 0.64, 1.9 mg/plate) 4NQO: 2 nmol/plate cigarette smoke: 360 mL	No inhibition or enhancement.	Positive response was defined as a 50% inhibition of agent-induced mutagenicity.	Camoirano et al. (1994)
S. typhimurium strain TA1535	*his* gene mutations	-	caffeine, purity n.p., plus MNNG or MNU	caffeine: 1, 2 mM (0.2, 0.4 mg/mL) MNNG: 0.0148 or 0.0166 mM MNU: 3.0 mM	Up to 75% reduction in mutagenicity for both compounds.	Test system was pre-treatment of bacteria with caffeine.	Balansky (1992)
				caffeine: 0.8 mg/plate (4 mol/plate) MNNG: 0.0166 mM MNU: 2.5 mM	Up to 18.6% decrease in mutagenicity of MNNG. Up to 38.1% decrease in mutagenicity of MNU.	Test system was post-treatment of bacteria with caffeine.	
				caffeine: 0.5 mM (0.1 mg/mL) MNNG: 0.0148 mM	An almost 4-fold enhancement of mutagenicity of MNNG.	Bacteria pre-treated with sodium selenite or caffeine as modulators.	

Table 10. Modulation of Genotoxicity (Continued)

Test System or Species, Strain, Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.10.2 Eukaryotes							
S. cerevisiae D5 and related BK strain series	Gene mutations	-	caffeine, purity n.p., plus DACA	caffeine: 7.7 mM (1.5 mg/mL) DACA: up to 1500 M	Significant decrease in DACA-induced mutations.		Ferguson et al. (1993)
Vicia faba root tip cells	Chromosomal aberrations	-	caffeine, purity n.p., plus camptothecin	caffeine: 5, 10, 20 mM (1, 1.9, 3.9 mg/mL) camptothecin: 0.05 M	Significant reduction in camptothecin-induced damage at 20 mM.	Enhancement occurred at 5 mM.	Kihlman and Andersson (1992)
9.10.3 In Vitro Mammalian Systems							
DNA Damage							
A_L human-hamster hybrid cells	DNA base pair deletions	-	caffeine, purity n.p., plus ^{137}Cs γ-radiation	caffeine: 1.5 mg/mL (7.7 mM) ^{137}Cs γ-radiation: 0.5-3.0 Gγ	Synergistic enhancement of radiation-induced DNA base pair deletions.	Caffeine added post-irradiation; the concentration of caffeine used was sufficient to reduce cell plating efficiency to 20%.	McGuinness et al. (1995)
Human myeloid leukemia U937 cells	DNA single-strand breaks (SSB)	-	caffeine, purity n.p., plus *t*-butylhydro-peroxide	caffeine: 10 mM (1.9 mg/mL) *t*-butylhydro-peroxide: 200 M	Synergistic enhancement in *t*-butylhydroperoxide-induced DNA damage.	Caffeine was equally potent in enhancing DNA damage in both oxygen-proficient and oxygen-deficient cells.	Guidarelli et al. (1997)
Human blood lymphocytes	SCE	-	caffeine, purity n.p., plus adriamycin, and chlorpromazine, purities n.p.	caffeine: 120 g/mL (0.618 mM) adriamycin: 15 ng/mL chlorpromazine: 6 g/mL	Caffeine and chlorpromazine synergistically enhanced SCE induction by adriamycin.	Caffeine alone did not induce SCE.	Lialiaris et al. (1992)
Chromosomal Aberrations							
Human peripheral	Chromosomal	-	caffeine, purity	caffeine: 2.5 mM	Potentiation of the	Caffeine was more active	Pelliccia and

Table 10. Modulation of Genotoxicity (Continued)

Test System or Species, Strain, Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
blood lymphocytes	aberrations at fragile sites		n.p., plus 4',6-diamidino-2-phenylindole (DAPI)	(0.49 mg/mL) DAPI: 50 g/mL	induction of fragile sites by DAPI when added to the same medium.	when administered in complete medium.	Rocchi (1992)
Human peripheral blood lymphocytes	Chromosomal aberrations	-	caffeine, purity n.p., plus mitoxantrone (MXN)	caffeine: 1 mM (0.2 mg/mL) MXN: $12x10^{-6}$ µg/mL	No potentiation of the clastogenic effects of MXN.	Caffeine was added with MXN to cultures in 30-min pulses.	das Gra as Medeiros and Takahashi (1994)
Human lymphoblastoid cells from ataxia telangiectasia (AT) patients	Chromosomal aberrations	-	caffeine, purity n.p., plus ^{137}Cs γ-radiation	caffeine: 1 mM (0.2 mg/mL) ^{137}Cs γ-radiation: 50 cGy	Potentiated the induction of chromosomal breaks in G_2 arrested normal and AT heterozygote cells but not in AT homozygous cells.	Lack of response in homozygote cells suggests that caffeine is acting as a cell cycle checkpoint.	Bebb et al. (1998)
Human lymphocytes from Nijmegen breakage syndrome (NBS) patient	Chromosomal aberrations	-	caffeine, purity n.p., plus x-rays	caffeine: 5 mM (1 mg/mL) x-rays: 40 rad/min	Enhanced clastogenicity in mitogen-stimulated lymphocytes from patients with Nijmegen breakage syndrome (NBS).	Caffeine was added 30 min after irradiation during G_2.	Pincheira et al. (1998)
Lymphoblastoid cell lines: normal individual (RM341); AT individuals with intermediate radiosensitivity (AT9RM, AT10RM, AT11RM) and classical radiosensitivity (AT8RM)	Chromosomal aberrations	-	caffeine, purity n.p., plus x-rays	caffeine: 5 mM (1 mg/mL) x-rays: 30 and 45 cGy RM341, AT9RM, AT10RM, and AT11RM; 15 and 30 cGy for AT8RM at a dose rate of 70 cGy/min	3-Fold increase in frequency of chromosomal aberrations in RM341 and AT11RM cells, 1.6- and 1.8-fold in AT9RM and AT10RM cells, respectively, and 1.3-fold in AT8RM cells.	The damage produced in irradiated cells was similar to that caused by inhibitors of DNA polymerases and ribonucleotide reductase, indicating that caffeine may inhibit double strand breakage repair.	Antoccia et al. (1995)
Human lymphoblastoid cell lines: RM341; NBS cells (1548); AT8RM	Chromosomal aberrations	-	caffeine, purity n.p., plus x-rays	caffeine: 5 mM (1 mg/mL) x-rays: 15 cGy for 1548 and 30 cGy for RM341 at a	Enhanced radiation-induced chromosomal aberrations in G_2 phase to a lesser extent in NBS and AT cells than RM341.	The results indicate an altered DNA repair pathway common to both syndromes.	Antoccia et al. (1997)

Table 10. Modulation of Genotoxicity (Continued)

Test System or Species, Strain, Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
				dose rate of 70 cGy/min			
Mouse zygotes	Chromosomal aberrations	-	caffeine, purity n.p., plus x-rays	caffeine: 1 and 2 mM (0.2 and 0.4 mg/mL) x-rays: 0.4 and 0.6 Gy in S phase; 2 and 3 Gy in G_2 phase	Enhancement of aberrations greater when irradiation in G_2 than in S phase.	Caffeine completely cancelled G_2 arrest.	Matsuda and Tobari (1995)
Chinese hamster ovary (CHO) cells	Chromosomal aberrations	-	caffeine, purity n.p., plus triphenyltin	caffeine: 0.09-0.752 mg/mL (0.5-3.87 mM) triphenyltin: 3.8-30 mg/mL	Dose-dependent potentiation of triphenyl-tin-induced aberrations.		Sasaki et al. (1994)
Micronuclei in vitro							
CV-1 monkey kidney cells	Micronuclei (MN)	-	caffeine, purity n.p., plus ciprofloxacin or DP 124816-2 (quinoline anti-infective drugs)	caffeine: 10, 25, 50 mM (1.9, 4.9, 9.7 mg/mL)	Significant decrease in quinoline-induced MN in binucleate cells.	Caffeine alone did not induce MN; effects proposed to be mediated through interference with topoisomerases.	Curry et al. (1992, abstract)
human hepatoma (Hep-G2) cells	MN	-	caffeine, purity n.p., plus IQ	caffeine: 0.185, 0.55, 1.66, 5.0, 50, 500 g/mL (0.953, 2.8, 8.55, 26, 260, 2570 M) IQ: 9 mM at ≤5	Significant decrease in IQ-induced MN.	Moderate but significant increase in MN by caffeine alone at 500 g/mL. Caffeine reduced MN induction by up to 75%.	Sanyal et al. (1997)

Table 10. Modulation of Genotoxicity (Continued)

Test System or Species, Strain, Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
				g/mL for 4h; 120 g/mL at ≥5 g/mL for 24 h			
Mutations							
Chinese hamster MTX-resistant V79 male lung fibroblast cells	Induction of SCE	-	caffeine, purity n.p., plus methotrexate (MTX) and UV light	caffeine: 1 mM (0.2 mg/mL) MTX: 200 nM UV light: 4-20 J/m^2	Enhancement of SCE induction rate of MTX-resistant clones by UV light.		Roy et al. (1993)
A_L human-hamster hybrid cells	Mutations at the S1 locus	-	caffeine, purity n.p., plus ^{137}Cs γ-radiation	caffeine: 1.5 mg/mL (7.7 mM) ^{137}Cs γ-radiation: 0.93 Gy/min	2-Fold increase in number of $S1^-$ mutants. The majority of the mutations (73-85%) were large deletions versus those induced by ^{137}Cs γ-radiation alone (1/63 = 1.5%).	Caffeine altered both the quantity and quality of radiation-induced mutations.	McGuinness et al. (1995)
9.10.4 In Vivo Mammalian Systems							
Male mice, Swiss albino, 6-8 wk old	Chromosomal aberrations in bone marrow cells	-	caffeine, purity n.p., plus ^{60}Co - γ-radiation	acute: 5, 15 mg/kg (0.03, 0.077 mmol/kg) i.p. 30 min before irradiation (1.5 Gy)	Significant reduction.		Farooqi and Kesavan (1992)
				chronic: 4.208,	Significant reduction.		

Table 10. Modulation of Genotoxicity (Continued)

Test System or Species, Strain, Age	Biological Endpoint	– S9	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
				0.772 mM (0.8172, 0.150 mg/mL) in drinking water for 5 wk before irradiation (1.5 Gy)			
S. typhimurium strain TA98r recovered from liver of female mice (BALB/c, 3-wk old)	*his* gene mutations	-	caffeine, >99% pure, plus MeIQx	caffeine: ~28 mg/kg/d (~0.14 mmol/kg/d) MeIQx: 1.5 mg/kg bw	47% reduction in MeIQx-induced mutations.	When added to hepatic S9, caffeine directly inhibited activation of MeIQx to a mutagen by almost 35%.	Alldrick et al. (1995)
Ehrlich ascites tumor cells in BALB/c mice	SCE	-	caffeine, purity n.p., plus chlorpromazine or adriamycin	caffeine: 120 mg/kg bw (0.618 mmol/kg) chlorpromazine: 5 g/g bw, i.p. adriamycin: 22 ng/g bw, i.p.	Caffeine with chlorpromazine resulted in synergistic enhancement of induction of SCE by adriamycin.	Caffeine alone did not induce SCE.	Lialiaris et al. (1992)

Abbreviations: n.p. = not provided; d = day(s); min = minute(s); bw = body weight; wk = week(s); DACA = *N*-[(2-dimethylamino)ethyl]acridine-4-carboxamide; IQ = 2-amino-3-methylimidazo-[3,4-*f*]quinoline; MN = micronuclei; 4NQO = 4-nitroquinoline 1-oxide; AT = *Ataxia telangiectasia*; DAPI = 4',6-diamidino-2-phenyl-indole; i.p. = intraperitoneal injection; MNNG = *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; MXN = mitoxantrone; MTX = methotrexate; NBS = Nijmegen breakage syndrome; SCE = sister chromatid exchange.

9.8 Cytotoxicity of Caffeine

The cytotoxicity of caffeine, at high concentrations, in cultured mammalian cells is well established (Kihlman, 1977; cited by Rowley, 1992). Studies have demonstrated the ability of millimolar concentrations of caffeine to inhibit cell proliferation in mitogen-stimulated human lymphocytes (Guglielmi et al., 1982; cited by IARC, 1991) and to delay the entry of HeLa S3 cells into S-phase (Saunders et al., 1997).

9.9 Modulation of Cytotoxicity

9.9.1 Studies Reviewed by D Ambrosio (1994):

The ability of caffeine to modulate the cytotoxicity of genotoxic agents and specifically, antineoplastic agents, has been extensively evaluated. D Ambrosio (1994) reviewed 39 caffeine studies that utilized prokaryotic, lower eukaryotic, and mammalian *in vitro* and *in vivo* model systems to investigate the modulation of cytotoxicity. In a few studies, caffeine reduced the cytotoxicity of DNA damaging agents; however, in most studies, caffeine potentiated genotoxin-induced cell killing. As examples of antagonism, in P388 mouse leukemia cells (Ganapathi et al., 1986) and V79 cells (Iliakis and Lazar, 1987), caffeine at 2 mM (0.4 mg/mL) and 0.01-20 mM (0.002-3.9 mg/mL) respectively, reversed the cytotoxicity of adriamycin. At 10 mM (1.9 mg/mL), caffeine decreased the cytotoxicity of ethidium bromide in Chinese hamster ovary (CHO) cells by interfering with its uptake into cells and inhibiting its binding to DNA (Kimura and Aoyama, 1989). As examples of potentiation, caffeine at 1 mM (0.2 mg/mL) increased the cytotoxicity of thiotepa and nitrogen mustard (HN_2) in the human T24 bladder tumor cell line (Fingert et al., 1986). Potentiation was reversed by treatment with cyclohexamide. In another study, caffeine at 3 mM (0.6 mg/mL), a nontoxic concentration, greatly potentiated the cell killing effects of methotrexate in mouse L cells (Golos and Malec, 1989). There were also studies in which caffeine neither reduced nor potentiated chemical-induced cytotoxicity.

9.9.2 Review of Recent Literature

The details of these studies are presented in **Table 11**.

In Vitro Mammalian Systems: Caffeine (1-4 mM; 0.2-0.8 mg/mL) increased the cytotoxicity of ultraviolet (UV) radiation in mouse hybrid cells (Zampetti-Bosseler et al., 1980). The effect of caffeine on UV-induced cell death in p53-null (-/-) and wild-type (+/+) mouse embryonic fibroblasts was investigated (DeFrank et al., 1996). Wildtype and p53-null cells showed equal sensitivity to UV light. Caffeine (2 mM; 0.4 mg/mL) enhanced the sensitivity of both wild-type and p53-null cells to UV light, with greater sensitivity occurring in p53-null cells. The investigators speculated that the greater enhancement of sensitivity in p53 null cells by caffeine may have been mediated by override of S-phase delay.

Hagan et al. (1997) examined the effect of caffeine on S-phase synchronized CHO cells in which DNA damage had been induced by incorporating bromodeoxyuridine (BrdUrd) into replicating DNA followed by exposure to UV-B light. Cell survival was dramatically decreased by post exposure to caffeine (1 or 2 mM; 0.2 or 0.4 mg/mL). Caffeine was maximally effective when it was present continuously for two successive mitotic cycles. In BrdUrd+UVB treated cells cultured in the presence of caffeine, there was a cessation of replicative synthesis after the second mitosis and the caffeine-treated cells exhibited changes characteristic of apoptosis.

Caffeine (1 mM; 0.2 mg/mL) enhanced the cytotoxicity of x-rays in human ovarian carcinoma B-1 cells (Wolloch et al., 1991), and at 2 mM (0.4 mg/mL) increased the radiosensitivity of three tumor cell lines (Musk, 1991). However, enhanced cell killing was not associated with inhibition of damage-induced G_2 block in 2 of 3 tumor cell lines investigated. Caffeine (1 mM; 0.2 mg/mL) also potentiated the cytotoxicity of ionizing radiation in the Thy1.2+ steroid-resistant murine T-lymphoma line EL-4 and CD20+ human B-lymphoma lines (Raji, HDL, Jiyoye) (Macklis et al., 1994).

At 2 mM (0.4 mg/mL), caffeine significantly enhanced the cytotoxicity of the anti-tumor drugs adriamycin, ellipticine, and actinomycin D in mouse leukemia L1210 cells (Ross et al., 1979); at 1 or 2.5 mM (0.2 or 0.49 mg/mL), it enhanced cisplatin- and cisplatin plus 3-aminobenzamide-induced cytotoxicity in human cervical and ovarian carcinoma cell lines (Boike et al., 1990; Petru et al., 1990). Caffeine (1 mM; 0.2 mg/mL) potentiated the cytotoxicity of carboplatin in human leukemic cell lines (Efferth et al., 1995) and of cisplatin in human

osteosarcoma Takase (OST) cells (Yasutake et al., 1995), and at 1.2 mM (0.23 mg/mL), the cytotoxicity of cisplatin and L-threitol-1,4-bismethanesulfonate (DHB) in human and rat ovarian tumor cell lines (Bernges and Zeller, 1996). Caffeine (0.5 mM; 0.1 mg/mL) enhanced by 8- to 16-fold the cytotoxic effects of adozelesin, a highly potent alkylating agent used for its broad-spectrum antitumor activity and unique mechanism of action, in several CHO cell lines (Smith et al., 1995).

Alaoui-Jamali et al. (1994) used caffeine (0.5 mM; 0.1 mg/mL) to increase the sensitivity of cultured rat mammary carcinoma cells to L-phenylalanine mustard (L-PAM) and *cis*-diamminedichloroplatinum (II) (CDDP). Belizario et al. (1993) exposed mouse and human cells to 0.1-100 ng/mL tumor necrosis factor-α (TNF) for 24 hours. The rates of cell death increased significantly when the cells were then exposed to 2.5-20 mM (0.49-3.9 mg/mL) caffeine. The cytotoxicity of methyl nitrosourea (MNU) to HeLa cells made resistant to MNU through repeated exposures was increased by incubation with 0.75 mM (0.15 mg/mL) caffeine; however, HeLa cells sensitive to MNU showed no increase in sensitivity in the presence of caffeine (Roberts and Basham, 1990).

Caffeine potentiated the ability of paclitaxel to induce apoptosis in MCF-7 breast cancer cells (Saunders et al., 1997). Paclitaxel, at doses up to 20 ng/mL, induced a dose-dependent increase in the frequency of apoptotic cells (up to 43% of cells). The induction was enhanced up to two-fold (to 88% of cells) by caffeine at concentrations up to 20 mM (3.9 mg/mL). In a similar manner, caffeine (1 mM; 0.2 mg/mL), when administered to Thy1.2^{+} steroid-resistant murine T-lymphoma EL-4 cells and CD20^{+} human B-lymphoma cells (Raji, HDL, Jiyoye) irradiated with low dose γ radiation, significantly enhanced cell killing associated with apoptosis (Macklis et al., 1994). In a related study using EL-4 cells, the addition of caffeine after ^{137}Cs γ irradiation more than doubled the proportion of cells in apoptosis and decreased the frequency of cells in the G_2/M block from 47.3% to 22% (Palayoor et al., 1995). In contrast, caffeine (1-10 mM; 0.2-1.9 mg/mL) was unable to modulate the apoptosis-inducing activity (measured by DNA fragmentation) of the topoisomerase inhibitors camptothecin (CPT) and teniposide (VM-26) in

cultured human promyelocytic HL-60 cells (Bertrand et al., 1993). Furthermore, when used at 1 mM (0.2 mg/mL), it attenuated the induction of apoptosis by daunorubicin in human leukemic KG-1a cells (Efferth et al., 1995).

Caffeine (1 mM; 0.2 mg/mL) had no effect on cyclophosphamide (CP)-induced cytotoxicity in either CP-resistant or non-resistant human melanoma cell lines (Boon and Parsons, 1984). In contrast to these potentiating effects, caffeine (450 µg/mL; 2.32 mM) reduced the cytotoxicity of cytosine arabinoside, hydroxyurea, and colcemid in Syrian baby hamster kidney (BHK) cells and in polyoma virus-transformed BHK cells, with greater reduction occurring in BHK cells (Pardee and James, 1975). The investigators hypothesized that caffeine caused BHK cells in G_1 to shift into G_0, an effect similar to that caused by high cell density or serum deprivation. In contrast, in transformed cells, caffeine was largely ineffective in causing this shift which allowed damaged cells to continue through the cell cycle and subsequently die.

Table 11. Modulation of Cytotoxicity

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
In Vitro Mammalian Systems						
Mouse hybrid cell line (mouse lymphoma L5178YS x mouse fibroblast A9)	Plating efficiency	caffeine, purity n.p., plus UV light	caffeine: 1-4 mM (0.2-0.8 mg/mL) UV light: 11 ergs/mm^2/sec	Caffeine enhanced the effect of UV damage resulting in decreased plating efficiency.		Zampetti-Bosseler et al. (1980)
Mouse embryonic fibroblasts: p53 wildtype +/+ and -/- deficient cells	Cell death	caffeine, purity n.p., plus UV light	caffeine: 0.1-5.0 mM (0.02-0.97 mg/mL) UV light: 10-30 J/m^2	p53 (+/+) and p53 (-/-) cells show equal sensitivity to UV light; however, addition of caffeine enhanced sensitivity by 1.8 fold in p53 (-/-) cells, but by 1.3-fold in p53 (+/+) cells. Caffeine had little effect on the cell cycle distribution of p53 (+/+) cells, but a greater ratio of p53 (-/-) cells were in S-phase following caffeine treatment.	The greater sensitization of p53 null cells to caffeine compared to p53 wild-type cells may have been mediated by override of the S-phase delay.	DeFrank et al. (1996)
Chinese hamster V79 cells	Cell survival	caffeine, purity n.p., plus bromodeoxy-uridine (BrdUrd) and UV-B light.	caffeine: 2 mM (0.4 mg/mL) BrdUrd: 1 mM UV-B: 1 kJ/m2	Cell survival was dramatically decreased by post exposure to caffeine.	Synchronized V79 cells were pulse-labeled (30 min) with BrdUrd in early or late S-phase, then exposed to 1 kJ/m2 of UV-B light at various times. Caffeine was administered post-exposure.	Hagen et al. (1997)
Human ovarian carcinoma B-1 cell line	Cell survival	caffeine, purity n.p., plus gamma radiation	caffeine: 1 mM (0.2 mg/mL) x-rays: 2-15 Gy	Caffeine enhanced radiation-induced cytotoxicity resulting in reduced cell survival.		Wolloch et al. (1991)

Table 11. Modulation of Cytotoxicity (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
RT112 bladder carcinoma cells, TE671 (a rhabdo-myosarcoma), and DAOY (a medullo-blastoma)	S-phase and G_2-phase block	caffeine, purity n.p., plus ^{60}Co γ x-rays	caffeine: 2 mM (0.4 mg/mL) x-rays: 2-12 Gy	Caffeine partially reduced the S-phase and G_2-phase blocks in all cell lines, but potentiated cytotoxicity in RT112 cells.		Musk (1991)
Thy 1.2+ steroid resistant murine T-lymphoma line EL-4 and CD20+ human B-lymphoma (Raji cells)	Cell death	caffeine, purity n.p.; plus $90\gamma_x$ - rays	caffeine: 1 mM (0.2 mg/mL) 90γ radiation: up to 1000cGγ	Caffeine significantly enhanced cell killing by radiation in EL-4 cells, but caffeine alone had little effect on cell killings.	Nontoxic doses of caffeine were used.	Macklis et al. (1994)
Mouse leukemia L1210 cell line	Cell survival	caffeine, purity n.p., plus adriamycin, ellipticine, and actinomycin D	caffeine: 2 mM (0.4 mg/mL) adriamycin: 0.2-1.4 µg/mL ellipticine: 3-20 µM actinomycin D: 0.2-0.75 µg/mL	Caffeine significantly enhanced the cytotoxicity of adriamycin, ellipticine, and actinomycin D.		Ross et al. (1979)
Human ovarian (CAOV-3) and cervical (Me-180)	Cell survival	caffeine, purity n.p., plus cisplatin	caffeine: 1 or 2.5 mM (0.2 or 0.49 mg/mL)	Caffeine enhanced the cytotoxicity of cisplatin by 2.5-2.9 times that of cisplatin alone in Me-180 cells, and 1.6-2.7 times that of cisplatin alone in		Boike et al. (1990)

Table 11. Modulation of Cytotoxicity (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
cancer cell lines		or 3-amino-benzamide	cisplatin: 2.5 µg/mL 3-aminoben-zamide: 5 mM	CAOV-3 cells. The cytotoxicity of caffeine with cisplatin and 3-aminobenzamide (an ADP ribosyl transferase inhibitor) was enhanced 2.7 times that of cisplatin alone versus 1.8-fold enhancement with 3-aminobenzamide.		
Human ovarian (CAOV-3) and cervical (Me-180) cancer cell lines	Modulation of cisplatin-induced cytotoxicity	caffeine, purity n.p. plus cisplatin	caffeine : 1 or 2.5 mM (0.2 or 0.49 mg/mL) cisplatin: 2.5 µg/mL	Dose-related enhancement of cytotoxicity. At 2.5 mM, caffeine enhanced cisplatin sensitivity 2.9-fold in CAOV-3 cells and 2.7-fold in Me-180 cells.	Caffeine alone was not cytotoxic.	Petru et al. (1990)
Human leukemic KG-1a cells	Potentiation of cytotoxicity	caffeine, purity n.p.	1 mM (0.2 mg/mL), immediately after drug treatment	Caffeine potentiated the cytotoxicity of carboplatin in human leukemic cell lines.		Efferth et al. (1995)
Human osteosarcoma (OST) cells (Takase strain)	Cell survival	caffeine, purity n.p., plus cisplatin	caffeine: 1 mM (0.2 mg/mL) cisplatin: 0.2, 1, 2, 5, and 10 µg/mL	Caffeine enhanced the cell killing by cisplatin but did not affect cell plating efficiency.	Cells were treated for 1 h, with cisplatin and then incubated for 2 wk with or without caffeine. DNA synthesis which was inhibited by cisplatin treatment was reversed by caffeine.	Yasutake et al. (1995)
4 human and rat ovarian cancer cell lines	Enhancement of the cyto-toxicity of cisplatin, carmustine, MNNG, and	caffeine, purity n.p.	unclear; in some treatments, 1.2 mM (0.23 mg/mL) was used	Small enhancement (< 2x) of MNNG, DHB, and cisplatin was seen in some cell lines; the cytotoxicity of carmustine was not enhanced by caffeine in any of the cell lines.		Bernges and Zeller (1996)

Table 11. Modulation of Cytotoxicity (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
	DHB					
Chinese hamster ovary (CHO) cells, B-16 cells	Cell survival	caffeine, purity n.p., plus adozelesin	caffeine: 0.5 mM (0.1 mg/mL) adozelesin: 0.1-0.4 ng/mL	Caffeine increased the cytotoxicity of adozelesin between 8 and 16-fold for the 2 highest concentrations.		Smith et al. (1995)
Rat mammary carcinoma cell lines, wild type and chemical resistant	Potentiation of L-PAM and CDDP cytotoxicity	caffeine, purity n.p.	0.5 mM (0.1 mg/mL); 18 h pretreatment	20% increase in cytotoxicity in wild-type cells treated with CDDP and resistant cells treated with L-PAM; GSH depletion further enhanced the effect.	No effect of caffeine in wild-type cells treated with L-PAM or resistant cells treated with CDDP.	Alaoui-Jamali et al. (1994)
L929 and WEHI-164 (mouse fibro-sarcomas), A-375 (human malignant melanoma), MCF-7 (human breast cancer cells), and HeLa (human cervix carcinoma)	Enhancement of tumor necrosis factor (TNF)-induced cytotoxicity	caffeine, purity n.p.	2.5-20 mM (0.49-3.9 mg/mL) for 24 h after 24 h treatment with TNF	Positive for enhanced cytotoxicity.	Caffeine alone induced dose-related increases in cytotoxicity at 5-20 mM.	Belizario et al. (1993)
HeLa cells and MNU-resistant HeLa cells	Enhancement of methyl nitrosourea (MNU)-induced cytotoxicity	caffeine, purity n.p.	0.75 mM (0.15 mg/mL) administered after MNU exposure	Positive in resistant cells; negative in sensitive HeLa cells.	Caffeine alone was not cytotoxic at this dose.	Roberts and Basham (1990)
MCF-7 human	Apoptosis	caffeine,	caffeine (0-20	Caffeine enhanced by 2-fold the induction	Caffeine added post-	Saunders et

Table 11. Modulation of Cytotoxicity (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
breast cancer cells	and DNA fragmentation (indicative of apoptosis)	purity n.p., plus Paclitaxel	mM; 0-3.9 mg/mL); paclitaxel (0-20 ng/mL)	paclitaxel-induced apoptotic cells. Also, enhanced the induction of cells with DNA fragmentation.	exposure enhanced the maximal induction of apoptotic cells from 43% to 88%.	al. (1997)
Thy1.2^{+} steroid-resistant murine T-lymphoma line EL-4 and CD20^{+} human B-lymphoma lines (Raji, DHL, Jiyoye)	DNA damage (indicative of apoptosis) and cytotoxicity	caffeine, purity n.p., plus ^{90}Y beta radiation	caffeine: 1 mM (0.2 mg/mL); radiation: up to 1000 cGy	Caffeine significantly enhanced gamma radiation-induced cell killing and DNA fragmentation.	Caffeine was added post-irradiation.	Macklis et al. (1994)
Murine T-lymphoma line EL-4	DNA damage (indicative of apoptosis)	caffeine, purity n.p., ^{137}Cs γ radiation	caffeine: 1 mM (0.2 mg/mL); ^{137}Cs γ radiation: 0.5-16 Gy	Caffeine more than doubled the frequency of apoptotic cells and decreased the G_2/M block from 47.3% to 22%.	Caffeine was added post-irradiation.	Palayoor et al. (1995)
Human promyelocytic HL-60 cells	DNA fragmentation	caffeine, reagent grade; plus CPT or VM-26	caffeine: 1-10 mM (0.2-1.9 mg/mL); CPT: .1-10 µM; VM-26: .1-10 µM	No enhancement or reduction in CPT or VM-26 induced DNA fragmentation.		Bertrand et al. (1993)
Human leukemic cell lines: KG-1a, K562, and HL-60; lymphocytes from 18 leukemia patients	Cytotoxicity and apoptosis	caffeine, purity n.p.; plus ionizing radiation, carboplatin, or daunorubicin	caffeine: 1 mM (0.2 mg/mL); ionizing radiation: 1-30 Gγ; carboplatin: 1-20 µg/mL; daunorubicin: 0.005-0.5 µg/mL	Positive for enhancement of radiation- and carboplatin-induced cytotoxicity and apoptosis; negative for potentiation of effects of daunorubicin.	Caffeine provided immediately after drug or radiation treatment. Caffeine alone did not induce apoptosis; GSH depletion was also enhanced by caffeine after carboplatin or irradiation treatment.	Efferth et al. (1995)

Table 11. Modulation of Cytotoxicity (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
Human melanoma MM253c1-4CG (subline) and MM253c1 (parent) cell lines	Cell survival	caffeine, purity n.p., plus cyclophos-phamide (CP)	caffeine: 1 mM (0.2 mg/mL) CP: up to 60 µM	Caffeine had no effect on CP-induced cytotoxicity in either cell line.		Boon and Parsons (1984)
Baby Syrian hamster kidney (BHK) and polyoma virus-transformed BHK cells	Cell death	caffeine, purity n.p., plus cytosine arabinoside, hydroxyurea, and colcemid	caffeine: 450 and 600 µg/mL (2.32 and 3.09 mM) cytosine arabinoside: 10 µg/mL hydroxyurea: 1 mM colcemid: n.p.	Caffeine inhibited cytosine arabinoside, hydroxyurea, and colcemid-induced cell death in BHK cells and polyoma virus. Inhibition of cytotoxicity was greater in normal BHK cells versus virus-transformed BHK cells.	The authors hypothesized that caffeine caused BHK cells in G_1 to shift into G_0 at the cell cycle check point, an effect similar to that caused by high cell density or serum deprivation. In contrast, in transformed cells, caffeine was largely ineffective in causing this shift allowing damaged cells to continue through the cell cycle and subsequently die.	Pardee and James (1975)

Abbreviations: CDDP = *cis*-diamminedichloroplatinum(II); DHB = L-threitol-1,4-bismethanesulfonate; GSH = glutathione; h = hour(s); i.p. = intraperitoneally; L-PAM = L-phenylalanine mustard; MNNG = 1-methyl-3-nitro-1-nitrosoguanidine; MNU = methyl nitrosourea; n.p. = not provided; TNF = tumor necrosis factor; CPT = camptothecin; VM-26 = teniposide; wk = week(s).

9.10 Modulation of Cell Cycle Arrest Induced by Genotoxic Agents

The details of studies discussed in this section are presented in **Table 12**.

A number of investigations have demonstrated the ability of caffeine to alter cell cycle arrest in eukaryote cells induced by genotoxic agents, and it is this mechanism that has been invoked most frequently as the means by which caffeine enhances clastogen-induced chromosomal damage and cytotoxicity. The modulation of cell cycle arrest by caffeine is cell-type and stage specific. For example, caffeine-induced release of the G_2 block and progression to mitosis of hamster cells damaged by clastogens results in premature chromosome condensation (PCC) (Schlegel and Pardee, 1986; Steinmann et al., 1991; both cited by Tam et al., 1995), chromosome shattering, or nuclear fragmentation (Demarcq et al., 1994). Human cells, in comparison, show a much reduced level of response (Tam et al., 1995). Using cell hybrids created by fusing hamster CHEF18 and human HT1080 cells, it was found that the level of cyclin B (synthesized in hamster, but not human cells) in S-phase arrested hybrids was directly related to their responsiveness to caffeine-induced PCC and thus to kinase activity.

In CHO/UV41 cells damaged with cisplatin at S phase, treatment with caffeine (2 and 5 mM; 0.4 and 1 mg/mL) overcame G_2 arrest (Demarcq et al., 1994). Human MCF-1 breast cancer cells were released from paclitaxel-induced G_2 block in a dose-dependent fashion by high concentrations of caffeine (5-20 mM; 1-3.9 mg/mL) (Saunders et al., 1997). Cells progressed through mitotic division, resulting in enhanced damage. Caffeine also decreased the ability of γ-irradiation to inhibit cell progression in irradiated HeLa S3 or human myeloblastic leukemia ML-1 cells into S-phase.

In human bladder carcinoma (RT112) cells, caffeine (0.5-10 mM; 0.1-1.9 mg/mL) enhanced cytotoxicity of methylxanthine derivatives in a dose-dependent manner (Musk and Steel, 1990). At a concentration of 1 mM (0.2 mg/mL) or greater, it inhibited G_2 block. Exposure of ML-1 cells to caffeine (4 mM; 0.8 mg/mL) inhibited the decrease in DNA synthesis in X-ray treated cells (Kastan et al., 1991). Caffeine inhibited the decrease in DNA synthesis after DNA damage and blocked the increase in p53 protein levels. This finding suggests that the effects of caffeine on cells after DNA damage may be due to a block of p53 protein induction and

subsequent alteration in cyclic nucleotide levels. Caffeine (1 mM; 0.2 mg/mL) prevented the G_2 cell cycle arrest in human ovarian carcinoma B-1 cells exposed to X-rays (Wolloch et al., 1991).

In contrast to these effects, Downes et al. (1992) reported that caffeine (2 mM; 0.4 mg/mL) prolonged 12-*O*-tetranodecanoyl-phorbol-13-acetate (TPA)-induced G_2 phase delay in HeLa cells.

Caffeine (1.5 mM; 0.29 mg/mL) applied for 72 hours inhibited the G_2 phase arrest of Fanconi Anemia (FA) lymphocytes (Hoehn et al., 1992). However, cells treated with caffeine were blocked in the subsequent G_1 phase following treatment, indicating that the DNA repair was transient and that caffeine appears to temporarily override the signal that blocks damaged nuclei in the G_2 phase. In a separate study, Seyschab et al. (1994) found that caffeine (0.5-1.5 mM; 0.1-0.29 mg/mL) completely resolved G_2 phase delay in X-irradiated FA mononuclear peripheral lymphocytes versus only partial resolution in similarly irradiated mononuclear peripheral lymphocytes from normal individuals. Caffeine (1.25-10 mM; 0.243-1.9 mg/mL) had similar dose-dependent effects in reducing the length of G2 phase in human lymphocytes from three separate donors (I=1-5 y.o., II=30-40 y.o., and III=60-70 y.o.); the delay was most pronounced in Group III (Pincheira et al., 1993).

Caffeine (1 mM; 0.2 mg/mL) prevented the accumulation of cells in G_2 in γ-irradiated human epidermal keratinocytes (Weller et al., 1996). At the same dose, it slightly reduced the accumulation of UV-B irradiated cells in G_2.

.

Table 12. Modulation of Cell Cycle Arrest Induced by Genotoxic Agents

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
Chinese hamster ovary (CHO) UV41 cells	Generation of G_1 daughter cells	caffeine, purity n.p. plus cisplatin	2 and 5 mM (0.4 and 1 mg/mL)	Caffeine, after 3 h, overcame G_2 arrest. After 4 h almost all cells were in G_1.	2 mM caffeine also overcame G_2 arrest but took twice as long.	Demarcq et al. (1994)
	DNA digestion induced by cisplatin		5 mM (1 mg/mL) added at 10 h	At 16 h following release with caffeine added at 10 h, DNA breaks were present in 1% of attached cells and 35% of detached cells. By 20 h, DNA breaks were present in <7% of attached cells and 90% of detached cells.	Results show that a temporary relationship exists between cell detachment and DNA fragmentation.	
Human fibrosarcoma cells (HT1080), Chinese hamster embryo fibroblasts (CHEP18), and whole cell hybrids	Premature chromosome condensation (PCC)	caffeine, purity n.p.	5 mM (1 mg/mL)	After caffeine treatment, PCC were as follows: HT1080 (<1%), CHEP18 (55%), and hybrids (2-45%). The level of cyclin B in S-phase arrested hybrids was directly related to the PCC, which is related to kinase activity.	The expression of cyclin B alone sensitizes human cells to caffeine-induced premature mitosis.	Tam et al. (1995)
MCF-7 human breast cancer cells	G_2 arrest	caffeine, purity n.p., plus paclitaxel	5-20 mM (1-3.9 mg/mL)	Cells were released from paclitaxel-induced G_2 block during S-phase in a dose-related fashion.		Saunders et al. (1997)

Table 12. Modulation of Cell Cycle Arrest Induced by Genotoxic Agents (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
Human bladder carcinoma cell line RT 112	Effect on radiation-induced G_2 phase delay	caffeine, purity n.p.	0.5-10 mM (0.1-1.9 mg/mL)	Caffeine enhanced cytotoxicity in a dose-dependent manner. Caffeine inhibited G_2 block at 1 mM only and had no further effect at higher concentrations.	The results indicate that override of G_2 block alone is insufficient to cause the dose-dependent cytotoxicity and that DNA repair must play a role in the mechanism of cytotoxicity.	Musk and Steel (1990)
ML-1 myeloblastic leukemia cells	Effect on cell cycle changes	caffeine, purity n.p., plus gamma radiation	4 mM (0.8 mg/mL) x-rays: 52, 104, or 416 rad	Caffeine caused an increase in the percentage of undamaged cells in G_1; caffeine treatment inhibited the usual decrease in DNA syntheses in X-ray treated cells. Caffeine inhibited the decrease in DNA synthesis after DNA damage and blocked the increase in p53 protein levels.		Kastan et al. (1991)
Human ovarian carcinoma B-1 cell line	Effect on radiation-induced G_2 arrest	caffeine, purity n.p., plus gamma radiation	1 mM (0.2 mg/mL) x-rays: 2-15 Gy	Caffeine prevented radiation-induced G_2 arrest, increasing cytotoxicity.		Wolloch et al. (1991)
HeLa cells	Effect on TPA-induced G_2 phase delay	caffeine, purity n.p., plus TPA	2 mM, 100 M, and 500 M (0.4, 0.0194, 0.0971 mg/mL)	Caffeine at 2 mM prolonged TPA-induced G_2 phase delay in HeLa cells.	Response was found to be dose-dependent, solutions of 100 and 500 M having no effect on the TPA-treated cells.	Downes et al. (1992)
Fanconi anemia	Effect on spontaneous	caffeine,	1.5 mM (0.29 mg/mL) for 72	Caffeine inhibited the spontaneous G_2	Cells treated with caffeine were blocked in the subsequent G_1	Hoehn et al.

Table 12. Modulation of Cell Cycle Arrest Induced by Genotoxic Agents (Continued)

Test System or Species, Strain, and Age	Biological Endpoint	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
(FA) lymphocytes	G_2 phase delay	purity n.p.	h	phase arrest.	phase following treatment, indicating that the DNA repair was transient and that caffeine temporarily overrides the signal that blocks damaged nuclei in the G_2 phase.	(1992)
Normal and FA mononuclear peripheral lymphocytes	Effect on G_2 phase delay	caffeine, purity n.p.	0.5-1.5 mM (0.1-0.29 mg/mL)	The G_2 phase delay in FA cells was completely resolved by 1.5 mM caffeine versus only partial resolution in x-irradiated normal cells.	The results indicate separate repair mechanisms for G_2 phase lesions in x-irradiated cells versus FA cells.	Seyschab et al. (1994)
Human lymphocytes from three donors: I=1-5 y.o.; II=30-40 y.o.; III=60-70 y.o.	Effect on G_2 phase delay and chromosomal aberrations	caffeine, purity n.p.	1.25-10 mM (0.243-1.9 mg/mL)	Caffeine had similar dose-dependent effects in reducing the length of G_2 phase in human lymphocytes from all three donors; the delay was most pronounced in Group III.		Pincheira et al. (1993)
Human epidermal keratinocytes	Effect on ^{137}Cs γ-radiation- and UV-B-induced cell cycle perturbations	caffeine, purity n.p.	1 mM (0.2 mg/mL)	Caffeine prevented accumulation of cells in G_2 in γ-irradiated cells but did not influence the frequency of micronuclei. Caffeine slightly reduced accumulation of cells in G_2 in UV-B-irradiated cells and moderately induced the frequency of micronuclei.	The results indicate that the different types of DNA damage produced by γ and UV-B irradiation may be repaired via separate pathways.	Weller et al. (1996)

Abbreviations: CHM = cycloheximide; CHO = Chinese hamster ovary; FA = Fanconi Anemia; n.p. = not provided; PCC = premature chromosome condensation; TPA = 12-*O*-tetranodecanoylphorbol-13-acetate; y.o. = years old; UV = ultraviolet; h = hour(s).

9.11 Modulation of Immunotoxicity

No data were located.

9.12 Other Data

Antioxidant Properties

The antioxidant behavior of caffeine was investigated using electron spin resonance (ESR) trapping (Shi et al., 1991). Both the Fenton reaction (Fe^{2+} plus peroxide) and the reaction of chromium with peroxide were used to generated free radicals which were then effectively scavenged by caffeine, as detected by ESR. The results suggested to the investigators a possible mechanism for the reported anticarcinogenic effects of caffeine.

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Two groups of compounds, 2,4-dioxo-1,2,3,4-tetrahydropyrrolo[2,3-*d*]pyrimidines and 2,4-dioxo-1,2,3,4,5,6-hexahydropyrrolo[2,3-*d*]pyrimidines, showed caffeine-like activities when investigated by Senda and Hirota (1974). These compounds, 7-deazaxanthine derivatives, showed the same or more diuretic activity than that of caffeine and more cardiac activity than caffeine. Results of the investigation of structure-activity relationships indicated that the nitrogen atom at the 7-position of the 7-deazaxanthine derivatives plays an important role in showing caffeine-like pharmacological activities.

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS (Structure and Nomenclature Search System)
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

Kirk-Othmer Encyclopedia of Chemical Technology

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

BIOSIS	EMBASE	Registry
CANCERLIT	HSDB	RTECS
CAPLUS	MEDLINE	TOXLINE
CHEMLIST		

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC®	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA

Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

Databases Available on the Internet

Phytochemeco Database (Agricultural Research Service)

In-House Databases

CPI Electronic Publishing Federal Databases on CD, 1998
Current Contents on Diskette ®
The Merck Index, 1996, on CD-ROM

11.2 Secondary References

Budavari, S. (ed.). 1996. The Merck Index. 12th edition. Whitehall, NJ: Merck & Co., Inc.

HSDB (Hazardous Substances Data Bank). 1997. Caffeine. HSDB Number 36. Bethesda, MD: National Library of Medicine (NLM).

RTECS (Registry of Toxic Effects of Chemical Substances). 1997. Caffeine. RTECS Number EV6475000. Cincinnati, OH: National Institute for Occupational Safety and Health (NIOSH).

SRI (Stanford Research Institute). 1997. Directory of Chemical Producers-United States of America. Menlo Park, CA: SRI International, p. 716.

12.0 REFERENCES

Adam, W. R., J. K. Dawborn, D. G. Price, J. Riddell, and H. Story. 1970. Anaplastic transitional-cell carcinoma of the renal pelvis in association with analgesic abuse. Med. J. Australia 1(22):1108-1109.

Adler, I.-D. 1969. Does caffeine induce dominant lethal mutations in mice? Humangenetik 7:137-148. (Cited by IARC, 1991)

Aeschbacher, H. U., H. Meier, and E. Jaccaud. 1986. The effect of caffeine in the *in vivo* SCE and micronucleus mutagenicity tests. Mutat. Res. 174:53-58. (Cited by IARC, 1991)

Aeschbacher, H. U., H. Milon, and H. P. W rzner. 1978. Caffeine concentrations in mice plasma and testicular tissue and the effect of caffeine on the dominant lethal test. Mutat. Res. 57:193-200. (Cited by IARC, 1991)

Agudo, A., C. A. Gonz lez, G. Marcos, M. Sanz, E. Saigi, J. Verge, M. Boleda, and J. Ortego. 1992. Consumption of alcohol, coffee, and tobacco, and gastric cancer in Spain. Cancer Causes Control 3:137-143.

Ahmad, R. A., and R. D. Watson. 1990. Treatment of postural hypotension: A review. Drugs 39:74-85. (Cited by Etherton and Kochar, 1993)

Aitken, R. J., F. Best, D. W. Richardson, R. Schats, and G. Simm. 1983. Influence of caffeine on movement characteristics, fertilizing capacity and ability to penetrate cervical mucus of human spermatozoa. J. Reprod. Fertil. 67:19-27. (Cited by IARC, 1991)

Alaoui-Jamali, M., B.-B. Loubaba, S. Robyn, H. Tapiero, and G. Batist. 1994. Effect of DNA-repair-enzyme modulators on cytotoxicity of L-phenylalanine mustard and *cis*-diamminedichloroplatinum (II) in mammary carcinoma cells resistant to alkylating drugs. Cancer Chemother. Pharmacol. 34:153-158.

Alldrick, A. J., W. E. Brennan-Craddock, and I. R. Rowland. 1995. Dietary caffeine reduces the genotoxicity of MeIQx in the host-mediated assay in mice. Nutr. Cancer 24(2):143-150.

Allen, S. S., and D. G. Froberg. 1987. The effect of decreased caffeine consumption on benign proliferative breast disease: A randomized clinical trial. Surgery 101:720-730. (Cited by IARC, 1991)

Amacher, D. E., and I. Zelljadt. 1984. Mutagenic activity of some clastogenic chemicals at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. Mutat. Res. 136:137-145. (Cited by IARC, 1991)

Amacher, D. E., S. C. Paillet, G. N. Turner, V. A. Ray, and D. S. Salsburg. 1980. Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. Mutat. Res. 72:447-474. (Cited by IARC, 1991)

Andriadzee, M. I., N. M. Pleskach, V. M. Mikhelson, and V. D. Zhestyanikov. 1986. Spontaneous and induced sister chromatid exchanges in blood lymphocytes of healthy donors and a xeroderma pigmentosum patient under the action of inhibitors of DNA synthesis and repair: Novobiocin, 3-methoxybenzamide, and caffeine. (Russ.). Tsitologia 28:69-85. (Cited by IARC, 1991)

Anon. 1986. Caffeine s no wide-awake market. Chem. Bus. 8:44. (Cited by IARC, 1991)

Anon. 1987a. Caffeine prices skyrocketing as total supplies tighten. Chem. Mark. Rep. 201. (Cited by IARC, 1991)

Anon. 1987b. Caffeine. Food Chem. News Guide 87:1, 25 May. (Cited by IARC, 1991)

Anon. 1988. Caffeine prices strengthened as coffee consumption slides. Chem. Mark. Rep., pp. 17-18. (Cited by IARC, 1991).

Antoccia, A., R. Ricordy, P. Maraschio, S. Prudente, and C. Tanzarella. 1997. Chromosomal sensitivity to clastogenic agents and cell cycle perturbations in Nijmegen breakage syndrome lymphoblastoid cell lines. Int. J. Rad. Biol. 71(1):41-49.

Antoccia, A., L.Chessa, R. Ricordy, and C. Tanzarella. 1995. Modulation of radiation-induced chromosomal damage by inhibitors of DNA repair and flow cytometric analysis in *Ataxia telangiectasia* cells with intermediate radiosensitivity. Mutagenesis 10(6):523-529.

Aranda, J. V., K. Beharry, J. Rex, R. J. Johannes, and L. Charest-Boule. 1987. Caffeine enzyme immunoassay in neonatal and pediatric drug monitoring. Ther. Drug Monit. 9:97-103. (Cited by IARC, 1991)

Aranda, J. V., J. M. Collinge, R. Zinman, and G. Watters. 1979a. Maturation of caffeine elimination in infancy. Arch. Dis. Child 54:946-949. (Cited by IARC, 1991)

Aranda, J. V., C. E. Cook, W. Gorman, J. M. Collinge, P. M. Loughnan, E. W. Outerbridge, A. Aldridge, and A. H. Neims. 1979b. Pharmacokinetic profile of caffeine in the premature newborn infant with apnea. J. Pediatr. 94:663-668. (Cited by IARC, 1991)

Arlett, C. F., and S. A. Harcourt. 1972. The induction of 8-azaguanine-resistant mutants in cultured Chinese hamster cells by ultraviolet light. The effect of changes in post-irradiation conditions. Mutat. Res. 14:431-437. (Cited by IARC, 1991)

Armstrong, B., A. Garrod, and R. Doll. 1976. A retrospective study of renal cancer with special reference to coffee and animal protein consumption. Br. J. Cancer 33:127. (Cited by Pozniak, 1985)

Armuth, V., and I. Berenblum. 1981. The effect of caffeine on two-stage skin carcinogenesis and on complete systemic carcinogenesis. Carcinogenesis 2(10):977-979.

Arnaud, M. J. 1987. The pharmacology of caffeine. Prog. Drug Res. 31:273-313. (Cited by IARC, 1991)

Ashton, C. H. 1987. Caffeine and health. Br. Med. J. 295:1293-1294. (Cited by IARC, 1991)

Axelrod, J., and J. Reichenthal. 1953. The fate of caffeine in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther. 107:519-523. (Cited by IARC, 1991)

Balansky, R. 1992. Effects of sodium selenite and caffeine on mutagenesis induced by *N*-methyl-*N*-nitrosourea, *N*-methyl-*N* -nitro-*N*-nitrosoguanidine and aflatoxin B_1 in *S. typhimurium*. Mutat. Res. 269(2):301-317.

Balansky, R., P. M. Blagoeva, Z. I. Mircheva, and S. DeFlora. 1994. Modulation of diethylnitrosamine carcinogenesis in rat liver and oesophagus. J. Cell. Biochem. 56(4):449-454.

Balansky, R., P. Blagoeva, Z. Mircheva, K. Pozharisski, and S. DeFlora. 1992. Effect of metabolic inhibitors, methylxanthines, antioxidants, alkali metals, and corn oil on 1,2-dimethylhydrazine carcinogenicity in rats. Anticancer Res. 12(3):933-940.

Barkay, J., B. Bartoov, S. Ben-Ezra, J. Langsam, E. Feldman, S. Gordon, and H. Zuckerman. 1984. The influence of *in vitro* caffeine treatment on human sperm morphology and fertilizing capacity. Fertil. Steril. 41:913-918. (Cited by IARC, 1991)

Basler, A., U. Bachman, G. Roszinsky-K cher, and G. R hrborn. 1979. Effects of caffeine on sister-chromatid exchanges (SCE) *in vivo*. Mutat. Res. 59:209-214. (Cited by IARC, 1991)

Bebb, D. G., P. P. Steele, P. J. Warrington, J. A. Moffat, and B. W. Glickman. 1998. Caffeine does not potentiate γ-radiation induced DNA damage in *Ataxia telangiectasia* lymphoblastoid cells. Mutat. Res. 401(1-2):27-32.

Belizario, J. E., J. L. Tilly, and S. W. Sherwood. 1993. Caffeine potentiates the lethality of tumour necrosis factor in cancer cells. Brit. J. Cancer 67(6):1229-1235.

Bernges, F., and W. J. Zeller. 1996. Combination effects of poly(ADP-ribose) polymerase inhibitors and DNA-damaging agents in ovarian tumor cell lines–with special reference to cisplatin. J. Cancer Res. Clin. Oncol. 122:665-670.

Berthou, F., D. Ratanasavanh, C. Riche, D. Picart, T. Voirin, and A. Guillouzo. 1989. Comparison of caffeine metabolism by slices, microsomes, and hepatocyte cultures from adult human liver. Xenobiotica 19:401-417. (Cited by IARC, 1991)

Bertrand, R., E. Solary, J. Jenkins, and Y. Pommier. 1993. Apoptosis and its modulation in human promyelocytic HL-60 cells treated with DNA topoisomerase I and II inhibitors. Exp. Cell. Res. 207:388-397.

Boike, G. M., E. Petru, B.-U. Sevin, H. E. Averette, T.-C. Chou, M. Penalver, D. Donato, M. Shiano, S. G. Hilsenbeck, and J. Perras. 1990. Chemical enhancement of cisplatin toxicity in a human ovarian and cervical cancer cell line. Gynecol. Oncol. 38:315-322.

Bonati, M., and S. Garattini. 1984. Interspecies comparison of caffeine disposition. In: Dews, P. B. (ed.). Caffeine. Perspectives from Recent Research. Berlin: Springer-Verlag, pp. 48-56. (Cited by IARC, 1991)

Bonati, M., R. Latini, F. Galletti, J. F. Young, G. Tognoni, and S. Garattini. 1982. Caffeine disposition after oral doses. Clin. Pharmacol. Ther. 32:98-106. (Cited by IARC, 1991)

Boon, M. H., and P. G. Parsons. 1984. Cyclophosphamide resistance developed in a human melanoma cell line. Cancer Treat. Rep. 68(10):1239-1246.

Borl e, I., M. F. Lechat, A. Bouckaert, and C. Misson. 1978. Coffee, risk factor during pregnancy. (Fr.). Louvain M d. 97:279-284. (Cited by IARC, 1991)

Bott, K. 1982. Caffeine from theophylline, carbon monoxide, and methanol. Chem. Abstract 98:53545m. (Cited by IARC, 1991)

Bowden, G. T., I. C. Hsu, and C. C. Harris. 1979. The effect of caffeine on cytotoxicity, mutagenesis, and sister-chromatid exchanges in Chinese hamster cells treated with dihydrodiol epoxide derivatives of benzo[a]pyrene. Mutat. Res. 63:361-370. (Cited by IARC, 1991)

Boyd, E. M. 1965. Caffeine addiction and drug toxicity. J. New Drugs 5:252.

Boyd, E. M. 1960. The acute oral toxicity in guinea pigs of acetylsalicylic acid, phenacetin, and caffeine, alone and combined. Toxicol. Appl. Pharmacol. 2:23-32.

Boyle, C. A., G. S. Berkowitz, V. A. LiVolsi, S. Ort, M. J. Merino, C. White, and J. L. Kelsey. 1984. Caffeine consumption and fibrocystic breast disease: A case-control epidemiologic study. J. Natl. Cancer Inst. 72:1015-1019. (Cited by IARC, 1991)

Bradbrook, I. D., C. A. James, P. J. Morrison, and H. J. Rogers. 1979. Comparison of thin-layer and gas chromatographic assays for caffeine in plasma. J. Chromatogr. 163:118-122. (Cited by IARC, 1991)

Branda, R. F., J. P. O Neill, D. Jacobson-Kram, and R. J. Albertini. 1992. Factors influencing mutation at the *hprt* locus in T-lymphocytes: Studies in normal and women with benign and malignant breast masses. Environ. Mol. Mutagen. 19:274-281.

Brooks, P. G., S. Gart, A. J. Heldfond, M. L. Margolin, and A. S. Allen. 1981. Measuring the effect of caffeine restriction on fibrocystic breast disease: The role of graphic stress telethermometry as an objective monitor of disease. J. Reprod. Med. 26:279-282. (Cited by IARC, 1991)

Bross, I. D., and J. Tidings. 1973. Another look at coffee drinking and cancer of the urinary bladder. Prev. Med. 2(3):445-451. (Cited by Hopkins, 1984)

Brune, H., R. P. Deutsch-Wenzel., M. Habs, S. Ivankovic, and S. Schm hl. 1981. Investigation of the tumorigenic response to benzo(a)pyrene in aqueous caffeine solution applied orally to Sprague-Dawley rats. J. Cancer Res. Clin. Oncol. 102:153-157. (Cited by IARC, 1991)

Caan, B., C. P. Quesenberry, and A. O. Coates. 1998. Differences in fertility associated with caffeinated beverage consumption. Am. J. Public Health 88(2):270-274.

Caan, B. J., and M. K. Goldhaber. 1989. Caffeinated beverages and low birthweight: A case-control study. Am. J. Public Health 79:1299-1300. (Cited by IARC, 1991)

Callahan, M. M., R. S. Robertson, A. R. Branfman, M. F. McComish, and D. W. Yesair. 1983. Comparison of caffeine metabolism in three nonsmoking populations after oral administration of radiolabeled caffeine. Drug Metab. Disposit. 11:211-217. (Cited by IARC, 1991)

Camoirano, A., R. M. Balansky, C. Bennicelli, A. Izzotti, F. D Agostini, and S. DeFlora. 1994. Experimental databases on inhibition of the bacterial mutagenicity of 4-nitroquinoline 1-oxide and cigarette smoke. Mutat. Res. 317(2):89-109.

Casto, B. C., W. J. Pieczynski, N. Janosko, and J. A. DiPaolo. 1976. Significance of treatment interval and DNA repair in the enhancement of viral transformation by chemical carcinogens and mutagens. Chem-Biol. Interact. 13:105-125. (Cited by IARC, 1991)

Ceccherini, I., N. Loprieno, and I. Sbrana. 1988. Caffeine post-treatment causes a shift in the chromosome aberration types induced by mitomycin C, suggesting a caffeine-sensitive mechanism of DNA repair in G_2. Mutagenesis 3:39-44. (Cited by IARC, 1991)

Chang, C.-C., C. Philipps, J. E. Trosko, and R. W. Hart. 1977. Mutagenic and epigenetic influence of caffeine on the frequencies of UV-induced ouabain-resistant Chinese hamster cells. Mutat. Res. 45:125-136. (Cited by IARC, 1991)

Chen, A. T. L., J. A. Reidy, J. L. Annest, T. K. Welty, and H.-G. Zhou. 1989. Increased chromosome fragility as a consequence of blood folate levels, smoking status, and coffee consumption. Environ. Mol. Mutagen. 13:319-324. (Cited by D Ambrosio, 1994)

Chvasta, T. E., and A. R. Cooke. 1971. Emptying and absorption of caffeine from the human stomach. Gastroenterology 61:838-843. (Cited by IARC, 1991)

Clark, A. M., and E. G. Clark. 1968. The genetic effects of caffeine in *Drosophila melanogaster*. Mutat. Res. 6:227-234. (Cited by IARC, 1991)

Clarke, C. H., and M. J. Wade. 1975. Evidence that caffeine, 8-methoxypsoralene and steroidal diamines are frameshift mutagens for *E. coli* K-12. Mutat. Res. 28:123-125. (Cited by IARC, 1991)

Clavel, F., E. Benhamou, A. Auquier, M. Tarayre, and R. Flamont. 1989. Coffee, alcohol, smoking and cancer of the pancreas. Int. J. Cancer 43:17-21.

Clive, D., P. Glover, M. Applegate, and J. Hozier. 1990. Molecular aspects of chemical mutagenesis in L5178Y/$tk^{+/-}$ mouse lymphoma cells. Mutagenesis 5(2):191-197.

Cohen, M. H., D. Schoenfeld, and J. Wolter. 1980. Randomized trial of chlorpromazine, caffeine, and methyl-CCNU in disseminated melanoma. Cancer Treat. Rep. 64(1):151-153.

Cole, P. 1971. Coffee drinking and cancer of the lower urinary tract. Lancet 1:1335. (Cited by Hopkins, 1984)

Cook, C. E., C. R. Tallent, E. W. Amerson, M. W. Myers, J. A. Kepler, G. F. Taylor, and H. D. Christensen. 1976. Caffeine in plasma and saliva by a radioimmunoassay procedure. J. Pharmacol. Expt. Ther. 199:679-686. (Cited by IARC, 1991)

Curry, P., M. Kropko, J. Garvin, R. Fiedler, and J. C. Theiss. 1992. Inhibition of quinolone induction of micronuclei by caffeine. Environ. Mol. Mutagen. 19(20):12. (Abstract)

D Ambrosio, S. M. 1994. Evaluation of the genotoxicity of caffeine. Regul. Toxicol. Pharmacol. 19:243-281.

das Gra as Medeiros, M., and C. S. Takahashi. 1994. Effects of treatment with mitoxantrone in combination with movobiocin, caffeine, and ara-C on human lymphocytes in culture. Mutat. Res. 307:285-292.

D Avanzo, B., C. LaVecchia, S. Franceshi, R. Talamini, and I. Buttino. 1992. Coffee consumption and bladder cancer risk. Eur. J. Cancer, Part A, Gen. Top. 28(8-9):1480-1484.

Davies, M. J., D. P. Lovell, and D. Anderson. 1992. Thioguanine-resistant mutant frequency in T-lymphocytes from a healthy human population. Mutat. Res. 265:165-171.

DeFlora, S., P. Zanacchi, A. Camoirano, C. Benicelli, and G. S. Badolati. 1984. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat. Res. 133:161-198. (Cited by IARC, 1991)

DeFrank, J. S., W. Tang, and S. N. Powell. 1996. P53-null cells are more sensitive to ultraviolet light only in the presence of caffeine. Cancer Res. 56:5365-5368.

Demarcq, C., R. T. Bunch, D. Creswell, and A. Eastman. 1994. The role of cell cycle progression in cisplatin-induced apoptosis in Chinese hamster ovary cells. Cell Growth Differ. 5(9):983-993.

Demas, T., and B. E. Statland. 1977. Prolonged half-life of serum caffeine in patients with hepatic insufficiency. Clin. Chem. 23:1156. [Abstract No. 203] (Cited by IARC, 1991)

Demerec, M., G. Bertani, and J. Flint. 1951. A survey of chemicals for mutagenic action on *E. coli*. Am. Naturalist 85:119-136. (Cited by IARC, 1991)

Denda, A., Y. Yokose, Y. Emi, Y. Murata, T. Ohara, N. Sunagawa, S. Mikami, S. Takahashi, and Y. Konishi. 1983. Effects of caffeine on pancreatic tumorigenesis by 4-hydroxyamino-quinoline 1-oxide in partially pancreatectomized rats. Carcinogenesis 4(1):17-22. (Cited by IARC, 1991)

Dong, M., D. Hoffmann, D. C. Locke, and E. Ferrand. 1977. The occurrence of caffeine in the air of New York City. Atmos. Environ. 11:651-653. (Cited by IARC, 1991)

Dougherty, J. B., D. Kelsen, N. Kemeny, G. Magill, J. Botet, and D. Neidzweiki. 1989. Advanced pancreatic cancer: A phase I-II trial of cisplatin, high-dose cytarabine, and caffeine. J. Natl. Cancer Inst. 81(22):1735-1738.

Downes, C. S., A. Ryan, and R. T. Johnson. 1992. Radiomimetic cell cycle delay induced by tetradecanoyl phorbol acetate is enhanced by caffeine and by the protein kinase inhibitor 2-aminopurine. Int. J. Radiat. Biol. 61(1):63-68.

Dulloo, A., C. Geissler, T. Horton, A. Collins, and D. Miller. 1989. Normal caffeine consumption: Influences on thermogenesis and daily energy expenditures in lean and post-obese human volunteers. Am. J. Clin. Nutr. 49:44-50. (Cited by Lamarine, 1994)

Dunham, L. J., A. S. Robson, H. I. Stewart, A. S. Frank, and J. C. Young. 1968. Rates, interveiw, and pathology of a study of cancer of the urinary bladder in New Orleans, Louisiana. J. Natl. Cancer Inst. 41:683. (Cited by Pozniak, 1985)

Dunkel, V. C., E. Zeiger, D. Brusick, E. McCoy, D. McGregor, K. Mortelmans, H. S. Rosenkranz, and V. F. Simmon. 1985. Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. Environ. Mutagen. 7(Suppl. 5):1-248. (Cited by IARC, 1991)

Efferth, T., U. Fabry, P. Glatte, and R. Osieka. 1995. Expression of apoptosis-related oncoproteins and modulation of apoptosis by caffeine in human leukemic cells. J. Cancer Res. Clin. Oncol. 121:648-656.

Epstein, S. S., W. Bass, E. Arnold, and Y. Bishop. 1970. The failure of caffeine to induce mutagenic effects or to synergize the effects of known mutagens in mice. Fd. Cosmet. Toxicol. 8:381-401. (Cited by IARC, 1991)

Epstein, S. S., and H. Shafner. 1968. Chemical mutagens in the human environment. Nature 219:385-387. (Cited by IARC, 1991)

Ernster, V. L. 1984. Epidemiologic studies of caffeine and human health. Prog. Clin. Biol. Res. 158:377-400.

Ernster, V. L., L. Mason, W. H. Goodson, E. A. Sickles, S. T. Sacks, S. Selvin, M. E. Dupuy, J. Hawkinson, and T. K. Hunt. 1982. Effects of caffeine-free diet on benign breast disease: A randomized trial. Surgery 91:263-267. (Cited by IARC, 1991)

Etherton, G. M., and M. S. Kochar. 1993. Coffee: Facts and controversies. Arch. Fam. Med. 2(3):317-322.

Everson, R. B., E. Randerath, R. M. Santella, T. A. Avitts, I. B. Weinstein, and K. Randerath. 1988. Quantitative associations between DNA damage in human placenta and maternal smoking and birth weight. J. Natl. Cancer Inst. 80:567-576. (Cited by D Ambrosio, 1994)

Farooqi, Z., and P. C. Kesavan. 1992. Radioprotection by caffeine pre- and post-treatment in the bone marrow chromosomes of mice given whole-body γ-irradiation. Mutat. Res. 269(2):225-230.

Favero, A., S. Franceschi, C. La Vecchia, E. Negri, E. Conte, and M. Montella. 1998. Meal frequency and coffee intake in colon cancer. Nutr. Cancer 30(3):182-105.

Ferguson, L. R., P. M. Turner, and B. C. Baguley. 1993. Induction of mitotic crossing-over by the topoisomerase II poison DACA (N-[2-dimethylamino)ethyl]acridine-4-carboxamide) in *Saccharomyces cerevisiae*. Mutat. Res. 289(2):157-163.

Fingert, H. J., J. D. Chang, and A. B. Pardee. 1986. Cytotoxic, cell cycle, and chromosomal effects of methylxanthines in human tumor cells treated with alkylating agents. Cancer Res. 46:2463-2467. (Cited by D Ambrosio, 1994)

Fokkens, W. 1979. Phenacetin abuse related to bladder cancer. Environ. Res. 20:192-198.

Folsom, A. R., D. R. McKenzie, K. M. Bisgard, L. H. Kushi, and T. A. Sellers. 1993. No association between caffeine intake and postmenopausal breast cancer incidence in the Iowa women s health study. Am J. Epidemiol. 138(6):380-383.

Fraumeni, J. F., J. Scotto, and L. J. Dunham. 1971. Coffee drinking and bladder cancer. Lancet 2:1204. (Cited by Pozniak, 1985)

Fundia, A., J. C. G mez, E. Mauri o, L. Boerr, J. C. Bai, I. Larripa, and I. Slavutsky. 1996. Chromosome instability in untreated adult celiac disease patients. Acta Paediatr. Suppl 412:82-84.

Furuhashi, N., S. Sato, M. Suzuki, M. Hiruta, M. Tanaka, and T. Takahashi. 1985. Effects of caffeine ingestion during pregnancy. Gynecol. Obstet. Invest. 19:187-191. (Cited by IARC, 1991)

Ganapathi, R., D. Grabowski, H. Schmidt, A. Yen, and G. Iliakis. 1986. Modulation of adriamycin and N-trifluoroacetyladriamycin-14-valerate induced effects on cell cycle traverse and cytotoxicity in P388 mouse leukemia cells by caffeine and the calmodulin inhibitor trifluoperazine. Cancer Res. 46:5553-5557. (Cited by D Ambrosio, 1994)

Gennaro, A. R. (ed.). 1985. Remington s Pharmaceutical Sciences. 17th edition. Easton, PA: Mack Publishing, pp. 1133-1135. (Cited by IARC, 1991)

Gilbert, R. M. 1984. Caffeine consumption. In: Spiller, G. A. (ed.). The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects. New York, NY: Alan R. Liss, pp. 185-213. (Cited by IARC, 1991)

Gilbert, R. M. 1981. Caffeine: Overview and anthology. In: Miller, S. A. (ed.). Nutrition and Behavior. Philadelphia, PA: The Franklin Institute Press, pp. 145-166. (Cited by IARC, 1991)

Giovannucci, E. 1998. Meta-analysis of coffee consumption and risk of colorectal cancer. Am. J. Epidemiol. 147(11):1043-1052.

Glover, T. W., J. Coyle-Morris, and R. Morgan. 1986. Fragile sites: Overview, occurrence in acute non-lymphocytic leukemia and effect of caffeine on expression. Cancer Genet. Cytogenet. 19:141-150.

Glover, P., R. Krehl, P. Poorman-Allen, and D. Clive. 1992. Distinctive aspects of mammalian cell mutagenesis. II. Most mutations induced by most mutagens involve several kilobases of DNA. Environ. Mol. Mutagen. 19(20). (Abstract)

Golos, B., and J. Malec. 1989. Enhancement of methotrexate-induced growth inhibition, cell killing, and DNA lesions in cultured L5178Y cells by the reduction of DNA repair efficiency. Biochem. Pharmacol. 38:1743-1748. (Cited by D Ambrosio, 1994)

Goodman, M. T., H. Morgenstern, and E. L. Wynder. 1986. A case-control study of factors affecting the development of renal cell cancer. Am. J. Epidemiol. 124(6):926-941.

Gordis, L., and E. B. Gold. 1984. Epidemiology of pancreatic cancer. World J. Surg. 8:808-821. (Cited by Poston and Williamson, 1991)

Gorodischer, R., and M. Karplus. 1982. Pharmacokinetic aspects of caffeine in premature infants with apnoea. Eur. J. Clin. Pharmacol. 22:47-52. (Cited by IARC, 1991)

Grab, F. L., and J. A. Reinstein. 1968. Determination of caffeine in plasma by gas chromatography. J. Pharm. Sci. 57:1703-1706. (Cited by IARC, 1991)

Graf, U., and F. E. W rgler. 1996. The somatic *white-ivory* eye spot test does not detect the same spectrum of genotoxic events as the wing somatic mutation and recombination test in *Drosophila melanogaster*. Environ. Mol. Mutagen. 27(3)219-226.

Graf, U., and F. E. W rgler. 1986. Investigation of coffee in Drosophila genotoxicity tests. Fd. Chem. Toxicol. 24:835-842. (Cited by Graf and W rgler, 1996)

Graham, H. N. 1984a. Tea: The plant and its manufacture, chemistry, and consumption of the beverage. In: Spiller, G. A. (ed.). The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects. New York, NY: Alan R. Liss, pp. 29-74. (Cited by IARC, 1991)

Graham, H. N. 1984b. Mat . In: Spiller, G. A. (ed.). The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects. New York, NY: Alan R. Liss, pp. 179-183. (Cited by IARC, 1991)

Grant, D. M., M. E. Campbell, B. K. Tang, and W. Kalow. 1987. Biotransformation of caffeine by microsomes from human liver. Kinetics and inhibition studies. Biochem. Pharmacol. 36:1251-1260. (Cited by IARC, 1991)

Gremy, F., I. Momas, and J. P. Daures. 1993. Risk factors in bladder cancer: A case-control study in the department of Herault. Bulletin de la Academie Nationale de Medicine 177(1):47-60.

Grossarth-Maticek, R., and H. J. Eysenck. 1991. Coca-cola, cancers, and coronaries: Personality and stress as mediating factors. Psychol. Rep. 68(3):1083-1087.

Guglielmi, G. E., T. F. Vogt, and R. R. Tice. 1982. Induction of sister chromatid exchanges and inhibition of cellular proliferation *in vitro*. I. Caffeine. Environ. Mutagen. 4:191-200. (Cited by IARC, 1991)

Guidarelli, A., L. Brambilla, E. Clementi, C; Sciorati, and O. Cantoni. 1997. Stimulation of oxygen consumption promotes mitochondrial calcium accumulation, a process associated with, and causally linked to, enhanced formation of t-butylhydroperoxide-induced DNA single-strand breaks. Exp. Cell Res. 237:176-185.

Hagan, M. P., K. L. Hopcia, F. C. Sylvester, and K. D. Held. 1997. Caffeine-induced apoptosis reveals a persistent lesion after treatment with bromodeoxyuridine and ultraviolet-B light. Rad. Res. 147:674-679.

Halsey, W. D., and B. Johnston (eds.). 1987. Caffeine. In: Collier s Encyclopedia, Vol. 5. New York, NY: MacMillan-Collier, p. 110. (Cited by IARC, 1991)

Handa, S. P., and H. D. Tewari. 1981. Urinary tract carcinoma in patients with analgesic neuropathy. Nephron 28(2):62-64.

Hartge, P., R. Hoover, D. W. West, and J. L. Lyon. 1983. Coffee drinking and the risk of bladder cancer. J. Natl. Cancer Inst. 70(6):1021-1026. (Cited by Hopkins, 1984)

Heddle, J. A., and W. R. Bruce. 1977. Comparison of tests for mutagenicity or carcinogenicity using assays for sperm abnormality, formation of micronuclei, and mutation in Salmonella. In: Hiatt, H. H., J. D. Watson, and J. A. Winsten (eds.). Origins of Human Cancer. Cold Spring Harbor, NY: CSH Press, pp. 1549-1557. (Cited by IARC, 1991)

Hestline, D., M. Kakkak, K. Woodhouse, I. A. MacDonald, and J. F. Potter. 1991. The effect of caffeine on postprandial hypotension in the elderly. J. Am. Geriatr. Soc. 39:160-164. (Cited by Etherton and Kochar, 1993)

Heyden, S., and J. G. Fodor. 1986. Coffee consumption and fibrocystic breasts: An unlikely association. Can. J. Surg. 29:208-211. (Cited by IARC, 1991)

Heyden, S., and L. H. Muhlbaier. 1984. Prospective study of fibrocystic breast disease and caffeine consumption. Surgery 96:479-484. (Cited by IARC, 1991)

Hicks, R. M., J. Chowaneic, and S. J. Wakefield. 1978. Experimental induction of bladder tumors by a two-stage system. In: Slaga, T. J., A. Sivak, and R. K. Boutwell (eds.). Carcinogenesis: A Comprehensive Survey. Vol. 2. Mechanisms of Tumor Promotion and Co-carcinogenesis. Symposium. Gaitlinburg, Tenn., USA, pp. 475-489.

Hindi-Alexander, M. C., M. A. Zielezny, N. Montes, B. Bullough, E. Middleton, D. H. Rosner, and W. M. London. 1985. Theophylline and fibrocystic breast disease. J. Allergy Clin. Immunol. 75:709-715. (Cited by IARC, 1991)

Hoehn, H., H. Seschab, G. Bretzel, D. Schindler, and M. Poot. 1992. The G_2 phase of the cell cycle as a functional parameter for the assessment of exogenous and endogenous DNA damage. Mutagenesis 7(2):159-160.

Homonnai, Z. T., G. Paz, Sofer, A. P. F. Kraicer, and A. Harell. 1976. Effect of caffeine on the motility, viability, oxygen consumption and glycolytic rate of ejaculated human normokinetic and hypokinetic spermatozoa. Int. J. Fertil. 21:163-170. (Cited by IARC, 1991)

Hopkins, J. 1984. Coffee drinking and bladder cancer. Fd. Chem. Toxicol. 22(6):481-495.

Horn, E. P., M. A. Tucker, G. Lambert, D. Silverman, D. Zametkin, R. Sinha, T. Hartge, M. T. Landi, and N. E. Caporaso. 1995. A study of gender-based cytochrome P4501A2 variability: A possible mechanism for the male excess of bladder cancer. Cancer Epidemiol., Biomark., Prevent. 4(5):529-533.

Hosaka, S., H. Jagayama, and I. Hirono. 1984. Suppressive effect of caffeine on the development of hepatic tumors induced by 2-acetylaminofluorene in ACI rats. Gann 75:1058-1061. (Cited by IARC, 1991)

Hoshino, H., and H. Tanooka. 1979. Caffeine enhances skin tumor induction in mice. Toxicol. Lett. 4:83-85. (Cited by IARC, 1991)

Hoskins, F. H. 1994. Food toxicants, naturally occurring. In: Howe-Grant, M. (ed.). Kirk-Othmer Encyclopedia of Chemical Technology. 4th edition. Volume 11. New York, NY: John Wiley and Sons, pp. 897-916.

Howe,G. R., J. D. Burch, A. B. Miller, G. M. Cook, J. Esteve, B. Morrison, P. Gordon, L. W. Chambers, G. Fodor, and G. M. Winsor. 1980. Tobacco use, occupation, coffee, various nutrients, and bladder cancer. J. Natl. Cancer Inst. 64(4):701-713. (Cited by Hopkins, 1984)

Huang, M.-T., J.-G. Xie, Z. Y. Wang, C.-T. Ho, Y.-R. Lou, C.-X. Wang, G. C. Hard, and A. H. Conney. 1997. Effects of tea, decaffeinated tea, and caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice: Demonstration of caffeine as a biologically important constituent of tea. Cancer Res. 57:2623-2629.

Huber, J., Jr. 1964. Caffeine. In: Kirk-Othmer Encyclopedia of Chemical Technology. Kirk, R. E., and D. F. Othmer (eds.). 2nd ed. Vol., 3. New York, NY: John Wiley and Sons, pp. 911-917. (Cited by IARC, 1991)

IARC (International Agency for Research on Cancer). 1991. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 51: Coffee, Tea, Mat , Methylxanthines and Methylglyoxal. Lyon, France: World Health Organization (WHO).

Iliakis, G., and W. Lazar. 1987. Reduction by caffeine of adriamycin-induced cell killing and DNA damage in Chinese hamster cells: Correlation with modulation in intracellular adriamycin content. Cancer Res. 47:2224-2229. (Cited by D Ambrosio, 1994)

Iscovich, J. M., R. B. Iscovich, G. Howe, S. Shiboski, J. M. Kaldor. 1989. A case-control study of diet and breast cancer in Argentina. Int. J. Cancer 44:770-776. (Cited by IARC, 1991)

Jacobson, B., and Kulling, F. 1989. Health and ergogenic effects of caffeine. Br. J. Sports Med. 23:34-40. (Cited by Lamarine, 1994)

Jensen, O. M., J. Wahrendorf, J. B. Knudsen, and B. L. S¿rensen. 1986. The Copenhagen case-control study of bladder cancer. II. Effect of coffee and other beverages. Int. J. Cancer 27:651-657. (Cited by IARC, 1991)

Johansson, S. L. 1981. Carcinogenicity of analgesics: Long-term treatment of Sprague-Dawley rats with phenacetin, phenazone, caffeine, and paracetamol (acetamidophen). Int. J. Cancer 27:521-529. (Cited by IARC, 1991)

Johansson, S. L., and C. Anderstrom. 1988. The influence of antipyrene on N-[4-(5-nitro-1-furyl)-2-thizolyl]-formamide-induced urinary tract carcinogenesis. Carcinogenesis 9(5):783-787.

Kada, T., K. Tutikawa, and Y. Sadaie. 1972. *In vitro* and host-mediated rec-assay procedures for screening chemical mutagens and phloxine, a mutagenic red dye detected. Mutat. Res. 16:165-174. (Cited by IARC, 1991)

Kastan, M. B., O. Onyekwere, D. Sidransky, B. Vogelstein, and R. W. Craig. 1991. Participation of p53 protein in the cellular response to DNA damage. Cancer Res. 51(23, Pt 1):6304-6311.

Kato, H. 1973. Induction of sister chromatid exchanges by UV light and its inhibition by caffeine. Exp. Cell Res. 82:383-390. (Cited by IARC, 1991)

Kellogg, C., C. Gordon, V. Vaitkevicius, E. Poplin, P. LoRusso, and L. Baker. 1993. PACE chemotherapy (platinol, ara-C, and caffeine) with continuously infused (CI) 5-fluorouracil

(5FURA) in the treatment of advanced pancreatic cancer. Proc. Annu. Meet. Am. Soc. Clin. Oncol. 12:A654. (Abstract)

Kelsen, D., C. Hudis, D. Neidzweiki, J. Dougherty, E. Casper, J. Botet, V. Vinciguerra, and R. Rosenbluth. 1991. A phase III comparison trial of streptozotocin, mitomycin, and 5-fluorouracil with cisplatin, cytosine arabinoside, and caffeine in patients with advanced pancreatic carcinoma. Cancer 68(5):965-969.

Kesavan, P. C., S. Trasi, and A. Ahmad. 1973. Modification of barley seed sensitivity by post-treatment with caffeine. I. Effect of post-irradiation heat shock and nature of hydration. Int. J. Rad. Biol. 24:581-587. (Cited by IARC, 1991)

Kihlman, B. A. 1977. Caffeine and Chromosomes. New York: Elsevier Scientific Pub. Co. (Cited by Rowley, 1992)

Kihlman, B. A., and H. C. Andersson. 1992. Enhancement and reduction by methylated oxypurines of the frequencies of chromatid aberrations induced by camptothecin in root-tip cells of *Vicia faba*. Mutat. Res. 269:259-267.

Kihlman, B. A., S. Sturelid, K. Norl n, and D. Tidriks. 1971a. Caffeine, caffeine derivatives and chromosomal aberrations. II. Different response of *Allium* root tips and Chinese hamster cells to treatments with caffeine, 8-ethoxycaffeine, and 6-methylcoumarin. Hereditas 69:35-50. (Cited by IARC, 1991)

Kimura, H., and T. Aoyama. 1989. Decrease in sensitivity to ethidium bromide by caffeine, dimethylsulfoxide or 3-aminobenzamide due to reduced permeability. J. Pharmacobiodyn. 12:589-595. (Cited by D Ambrosio, 1994)

King, M.-T., H. Beikirch, K. Eckhardt, E. Gocke, and D. Wild. 1979. Mutagenicity studies with X-ray contrast media, analgesics, antipyretics, antirheumatics and some other pharmaceutical drugs in bacterial, *Drosophila* and mammalian test systems. Mutat. Res. 66:33-43. (Cited by IARC, 1991)

Kumar, R., P. Mende, C.-D. Wacker, B. Spiegelhalder, R. Preussman, and M. Siddiqi. 1992. Caffeine-derived N-nitroso compounds—I: Nitrosatable precursors from caffeine and their potential relevance in the etiology of oesophageal and gastric cancers in Kashmir, India. Carcinogenesis 13(11):2179-2182.

Kunze, E., G. Rath, and T. Graewe. 1987. Effect of phenacetin and caffeine on N-butyl-N-(4-hydroxybutyl)nitrosamine-initiated urothelial carcinogenesis in rats. Urol. Int. 42:108-114. (Cited by IARC, 1991)

Lachance, M. P. 1982. The pharmacology and toxicology of caffeine. J. Fd. Saf. 4:71-112. (Cited by IARC, 1991)

Lamarine, R. J. 1994. Selected health and behavioral effects related to the use of caffeine. J. Commun. Health 19(6):449-466.

Landi, M. T., C. Zocchetti, I. Bernucci, F. F. Kadlubar, S. Tannenbaum, P. Skipper, H. Bartsch, C. Malaveille, P. Shields, N. E. Caporaso, and P. Vineis. 1996. Cytochrome P4501A2: Enzyme induction and genetic control in determining 4-aminobiphenyl-hemoglobin adduct levels. Cancer Epidemiol., Biomark., Prevent. 5(9):693-698.

La Vecchia, C., P. Liati, A Decarli, E. Negri, and S. Franceschi. 1987. Coffee consumption and risk of pancreatic cancer. Int. J. Cancer 40(3):309-313.

La Vecchia, C., S. Franceschi, F. Parazzini, M. Regallo, A. Decarli, G. Gallus, S. DiPietro, and G. Tognoni. 1985. Benign breast disease and consumption of beverages containing methylxanthines. J. Natl. Cancer Inst. 74:995-1000. (Cited by IARC, 1991)

Lawson, D. H., H. Jick, and K. J. Rothman. 1981. Coffee and tea consumption and breast disease. Surgery 90:801-803. (Cited by IARC, 1991)

Lee, S. 1971. Chromosome aberrations induced in cultured human cells by caffeine. Jpn. J. Genet. 46:337-344. (Cited by IARC, 1991)

Lee, W. C., A. I. Neugut, G. D. Garbowski, K. A. Forde, M. R. Treat, J. D. Waye, and C. Fenoglio-Preiser. 1993. Cigarettes, alcohol, coffee, and caffeine as risk factors for colorectal adenomatous polyps. Ann. Epidemiol. 3:239-244.

Lialiaris, T., A. Pantazaki, E. Sivridis, and D. Mourelatos. 1992. Chlorpromazine-induced damage on nucleic acids: A combined cytogenic and biochemical study. Mutat. Res. 265:155-163.

Linn, S., S. C. Schoenbaum, R. R. Monson, B. Rosner, P. G. Stubblefield, and K. J. Ryan. 1982. No association between coffee consumption and adverse outcomes of pregnancy. New Engl. J. Med. 306:141-145. (Cited by IARC, 1991)

Lomax-Smith, J. D., and A. E. Seymore. 1980. Neoplasia in analgesic nephropathy: A urothelial field change. Am. J. Surg. Pathol. 4(6):565-572.

Loprieno, N., R. Barale, and S. Baroncelli. 1974. Genetic effects of caffeine. Mutat. Res. 26:83-87. (Cited by IARC, 1991)

Lu, J. F., X. M. Cao, T. Yi, H. T. Zhuo, and S. S. Ling. 1998. Simultaneous determination of five caffeine metabolites in human urine with reverse-phase HPLC method. Anal. Lett. 31(4):613-620.

Lubin, R., and E. Ron. 1990. Consumption of methylxanthine-containing beverages and the risk of breast cancer. Cancer Lett. 53:81-90.

Lubin, R., E. Ron, Y. Wax, and B. Modan. 1985b. Coffee and methylxanthines and breast cancer: A case-control study. J. Natl. Cancer Inst. 74:569-573. (Cited by IARC, 1991)

Lyon, M. F., R. J. S. Phillips, and A. G. Searle. 1962. A test for mutagenicity of caffeine in mice. Z. Verebungsl. 93:7-13. (Cited by IARC, 1991)

MacGregor, J. T., R. Schlegel, C. M. Wehr, P. Alperin, and B. N. Ames. 1990. Cytogenetic damage induced by folate deficiency in mice is enhanced by caffeine. Proc. Natl. Acad. Sci. USA 87:9962-9965. (Cited by D Ambrosio, 1994)

Macklis, R. M., B. A. Beresford, and J. L. Humm. 1994. Radiologic studies of low-dose-rate 90Y-lymphoma therapy. Cancer 73:966-973.

Mahony, M. C., S. Lanzendorf, K. Gordon, and G. D. Hodgen. 1996. Effects of caffeine and dbcAMP on zona pellucida penetration by epididymal spermatozoa of Cynomolgus monkeys (*Macaca Fascicularis*). Mol. Reprod. Dev. 43:530-535.

Mailhes, J. B., D. Young, and S. N. London. 1996. Cytogenetic effects of caffeine during *in vivo* mouse oocyte maturation. Mutagenesis 11(4):395-399.

Marks, V., and J. F. Kelly. 1993. Absorption of caffeine from tea, coffee, and Coca Cola. Lancet i:827. (Cited by IARC, 1991)

Marrett, L. D., S. D. Walter, and J. W. Meigs. 1983. Coffee drinking and bladder cancer in Connecticut Am. J. Epidemiol. 117(2):113-127. (Cited by Hopkins, 1984)

Marshall, J., S. Graham, and M. Swanson. 1982. Caffeine consumption and benign breast disease: A case-control comparison. Am. J. Public Health 72:610-612. (Cited by IARC, 1991)

Matsuda, Y., and I. Tobari. 1995. Radiosensitivity and effects of repair inhibitors for X-ray-induced chromosomal damage in mouse zygotes in S and G_2 phases. Int. J. Rad. Biol. 68(6):615-623.

McCutheon, G. F. 1969. Caffeine. In: Snell, F. D. and L. S. Ettre (eds.). Encyclopedia of Industrial Chemical Analysis. Vol. 8. New York, NY: Interscience, pp. 55-71. (Cited by IARC, 1991)

McElvoy, G. K. 1989. American Health Formulary Service (AHFS) Drug Information. Bethesda, MD, American Society of Hospital Pharmacists, pp. 1179-1181. (Cited by HSDB, 1991)

McGuinness, S. M., M. L. Shibuya, A. M. Ueno, D. B. Vannais, and C. A. Waldren. 1995. Mutant quantity and quality in mammalian cells (A_L) exposed to cesium-137 gamma radiation: Effect of caffeine. Rad. Res. 142:247-255.

McLaughlin, C. C., M. C. Mahoney, P. C. Nasca, B. B. Metzger, M. S. Baptiste, and N. A. Field. 1992. Breast cancer and methylxanthine consumption. Cancer Causes Control 3:175-178.

McLaughlin, J. K., W. J. Blot, J. S. Mandel, L. M. Schuman, E. S. Mehl, and J. F. Irameni. 1983. Etiology of cancer of the renal pelvis. J. Natl. Cancer Inst. 71:287. (Cited by Pozniak, 1985)

Menke, C. H., J. V. Biazus, J. A. Cavalheiro, and E. G. Rabin. 1995. Diet and breast cancer. In: Figueira, F. O. et al. (eds.). International Congress Series, 1067. Mastology: Breast Disease. 8th International Congress on Senology. May 8-12, 1994. New York: Elsevier Science Publishers, pp. 357-359.

Menthe, J. 1985. Caffeine A commodity in demand. Tea Coffee Trade J. February, 18. (Cited by IARC, 1991)

Merriman, R. L., A. Swanson, M. W. Anders, and M. E. Sladek. 1978. Micro-determination of caffeine in blood by gas chromatography-mass spectrometry. J. Chromatrogr. 146:85-90. (Cited by IARC, 1991)

Mesko, T. W., J. N. Dunlap, and C. M. Sutherland. 1990. Risk factors for breast cancer. Compr. Ther.. 16(11):3-9.

Mettlin, C., and S. Graham. 1979. Dietary risk factors in human bladder cancer. Am. J. Epidemiol. 110(3):255-263. (Cited by Hopkins, 1984)

Miller, D. R., L. Rosenberg, D. W. Kaufman, S. P. Helmrich, D. Schottenfeld, J. Lewis, P. D. Stolley, N. Rosenshein, and S. Shapiro. 1987. Epithelial ovarian cancer and coffee drinking. Int. J. Epid. 16(1):13-17.

Minton, J. P., H. Abou-Issa, M. K. Foecking, and M. G. Sriram. 1983. Caffeine and unsaturated fat diet significantly promotes DMBA-induced breast cancer in rats. Cancer 51(7):1249-1253.

Minton, J. P., M. K. Foecking, D. J. T. Webster, and R. H. Matthews. 1979a. Response of fibrocystic disease to caffeine withdrawl and correlation of cyclic nucleotides with breast disease. Am. J. Obstet. Gynecol. 135:157-158. (Cited by IARC, 1991)

Minton, J. P., M. K. Foecking, D. J. T. Webster, and R. H. Matthews. 1979b. Caffeine, cyclic nucleotides, and breast disease. Surgery 86:105-109. (Cited by IARC, 1991)

Minton, J. P., H. Abou-Issa, N. Reiches, and J. M. Roseman. 1981. Clinical and biochemical studies on methylxanthine-related fibrocystic breast disease. Surgery 90:299-304. (Cited by IARC, 1991)

Mirvish, S. S., A. Cardesa, L. Wallcave, and P. Shubik. 1975. Induction of mouse lung adenomas by amines or ureas plus nitrite and by N-nitroso compounds: Effects of ascorbate, gallic acid, thiocyanate, and caffeine. J. Nat. Cancer Inst. 55:633-636. (Cited by IARC, 1991)

Mohr, U., J. Althoff, M. B. Ketkar, P. Conradt, and K. Morgareidge. 1984. The influence of caffeine on tumor incidence in Sprague-Dawley rats. Fd. Chem. Toxicol. 22:377-382. (Cited by IARC, 1991)

Mommsen, S., A. Jorgen, and A. Sell. 1983. A case control study of female bladder cancer. Eur. J. Cancer Clin. Oncol. 9:725. (Cited by Pozniak, 1985)

Morgan, K. J., V. J. Stults, and M. E. Zabik. 1982. Amount and dietary sources of caffeine and saccharin intake by individuals aged 5 to 18 years. Regul. Toxicol. Pharmacol. 2:296-307. (Cited by IARC, 1991)

Morrison, A. S., J. E. Buring, W. G. Verhoek, K. Aoki, I. Leck, Y. Ohno, and K. Obata. 1982. Coffee drinking and cancer of the lower urinary tract. J. Natl. Cancer Inst. 68(1):91-94. (Cited by Hopkins, 1984)

Mortelmans, K., S. Haworth, T. Lawlor, W. Speck, B. Tainer, and E. Zeiger. 1986. *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8(Suppl. 7):1-119. (Cited by IARC, 1991)

Musk, S. R. 1991. Reduction of radiation-induced cell cycle blocks by caffeine does not necessarily lead to increased cell killing. Rad. Res. 125:262-266.

Musk, S. R., and G. G. Steel. 1990. Override of the radiation-induced mitotic block in human tumor cells by methylxanthines and its relationship to the potentiation of cytotoxicity. Int. J. Rad. Biol. 57(6):1105-1112.

Nagasawa, H., and R. Konishi. 1988. Stimulation by caffeine of spontaneous mammary tumorigenesis in mice. Eur. J. Cancer Clin. Oncol. 24(4):803-805.

Nagasawa, H., and R. Konishi. 1987. Precancerous mammary hyperplastic alveolar nodules in four strains of virgin mice with different mammary tumor potentials: Influence of caffeine ingestion. Eur. J. Cancer Clin. Oncol. 23(7):1019-1023.

Nagasawa, H., and N. Sakurai. 1986. Effects of chronic ingestion of caffeine on mammary growth and reproduction in mice. Life Sci. 39:351-357.

Nagasawa, H., N. Sakurai, and M. Ishida. 1985. Estimation by caffeine of the onset time of precancerous mammary hyperplastic alveolar nodules in mice. IRCS Med. Sci. 13:29.

Najem, G. R., D. B. Louria, J. J. Seebode, I. S. Thind, and J. M. Prusakowski. 1982. Life time occupation, smoking, caffeine, saccharine, hair dyes, and bladder carcinogenesis. Int. J. Epidemiol. 11(3):212-217. (cited by Pozniak, 1985)

Nakanishi, K., M. Hirose, T. Ogiso, R. Hasegawa, M. Arai, and N. Ito. 1980. Effects of sodium, saccharin, and caffeine on the urinary bladder of rats treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine. Gann 71:490-500. (Cited by IARC, 1991)

Nakanishi, K., S. Fukushima, M. Shibata, T. Shirai, T. Ogiso, and N. Ito. 1978. Effects of phenacetin and caffeine on the urinary bladder of rats treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine. Gann 69:395-400. (Cited by IARC, 1991)

National Research Council. 1981. Food Chemicals Codes. 3rd edition. Washington, DC: National Academy Press, p. 44. (Cited by IARC, 1991)

Nesterov, M., L. A. Kucherya, R. G. Zaval nyuk, and T. D. Alibaeva. 1985. Improvement of caffeine synthesis. Khim.-Farm. Zh. 19:1389-1390. (Cited by IARC, 1991)

Neugut, A. I., J. S. Jacobson, and I. DeVivo. 1993. Epidemiology of colorectal adenomatous polyps. Cancer Epidemiol., Biomark., Prevent. 2:159-176.

Nishikawa, A., F. Furukawa, T. Imazawa, H. Yoshimura, K. Mitsumouri, and M. Takahashi. 1992. Effects of caffeine, nicotine, ethanol, and sodium selenite on pancreatic carcinogenesis in hamsters after initiation with N-nitrosobis(2-oxypropyl)-amine. Carcinogenesis 13(8):1379-1382.

Nishikawa, A., F. Furukawa, T. Imazawa, S. Ikezaki, T. Hasegawa, and M. Takahashi. 1995. Effects of caffeine on glandular stomach carcinogenesis induced in rats by *N*-methyl-*N* -nitro-*N*-nitrosoguanidine and sodium chloride. Fd. Chem. Toxicol. 33(1):21-26.

Nomura, T. 1980. Timing of chemically-induced neoplasia in mice revealed by the antineoplastic action of caffeine. Cancer Res. 40(4):1332-1340. (Cited by IARC, 1991)

Nomura, T. 1983. Comparative inhibiting effects of methylxanthines on urethan-induced tumors, malformations, and presumed somatic mutations in mice. Cancer Res. 43(3):1342-1346. (Cited by IARC, 1991)

Nomura, T. 1976. Diminution of tumorigenesis initiated by 4-nitroquinoline-1-oxide by post-treatment with caffeine in mice. Nature 260:57-549. (Cited by IARC, 1991)

Nomura, A. M. Y., L. N. Kolonel, J. H. Hankin, and C. N. Yoshizowa. 1991. Dietary factors in cancer of the lower urinary tract. Int. J. Cancer 48(2):199-205.

Norell, S. E., A. Ahlblom, R. Erwald, G. Jacobson, I. Lindberg-Navier, R. Olin, B. Tornberg, and K. L. Wiechel. 1986. Diet and pancreatic cancer: A case-control study. Am. J. Epidemiol. 124(6):894-902.

Odenheimer, D. J., M. V. Zunzunegui, M. C. King, C. P. Shipler, and G. D. Friedman. 1984. Risk factors for benign breast disease: A case-control study of discordant twins. Am J. Epidemiol. 120:565-571. (Cited by IARC, 1991)

Osiecka, R. 1976. Effect of the treatment-temperature on induction of chromosome aberrations by caffeine in *Vicia faba*. Genet. Pol. 17:159-163. (Cited by IARC, 1991)

Ostertag, W., and J. Haake. 1966. The mutagenicity in *Drosophila melanogaster* of caffeine and other compounds which produce chromosome breakage in human cells in culture. Z. Vererbungsl. 98:299-308. (Cited by IARC, 1991)

Ostertag, W., E. Duisberg, and M. St rmann. 1965. The mutagenic activity of caffeine in man. Mutat. Res. 2:293-296. (Cited by IARC, 1991)

Pagano, R., E. Negri, A. Decarli, and C. LaVecchia. 1988. Coffee drinking and prevalence of bronchial asthma. Chest 94:386-389. (Cited by Etherton and Kochar, 1993)

Palayoor, S. T., R. M. Macklis, E. A. Bump, and C. N. Coleman. 1995. Modulation of radiation-induced apoptosis and G_2/M block in murine T-lymphocyte cells. Rad. Res. 141:235-243.

Palitti, F., and A. Becchetti. 1977. Effect of caffeine on sister chromatid exchanges and chromosomal aberrations induced by mutagens in Chinese hamster cells. Mutat. Res. 45:157-159. (Cited by IARC, 1991)

Palitti, F., M. Rizzoni, M. Gatti, and G. Olivieri. 1974. Chromosomal aberrations induced by caffeine in endoreduplicated Chinese hamster cells. Mutat. Res. 26:145-150. (Cited by IARC, 1991)

Palm, P. E., E. P. Arnold, M. S. Nick, J. R. Valentine, and T. E. Doerfler. 1984. Two-year toxicity/carcinogenicity study of fresh-brewed coffee in rats initially exposed *in utero*. Toxicol. Appl. Pharmacol. 74:364-382.

Palm, P. E., E. P. Arnold, P. C. Rachwall, J. C. Leyczek, K. W. Teague, and C. J. Kensler. 1978. Evaluation of the teratogenic potential of fresh-brewed coffee and caffeine in the rat. Toxicol. Appl. Pharmacol. 44:1-16.

Paneau, C., P. Schaffer, and C. Bollock. 1992. Epidemiologie du cancer del la vessie. Ann. D. Urologie 26(5):281-293.

Panigrahi, G. B., and A. R. Rao. 1983. Influence of caffeine on arecoline-induced SCE in mouse bone-marrow cells *in vivo*. Mutat. Res. 122:347-353. (Cited by IARC, 1991)

Pant, G. S., N. Kamada, and R. Tanaka. 1976. Sister chromatid exchanges in peripheral lymphocytes of atomic bomb survivors and of normal individuals exposed to radiation and chemical agents. Hiroshima J. Med. Sci. 25:99-105. (Cited by IARC, 1991)

Parazzini, F., C. La Vecchia, R. Riundi, S. Pampallona, M. Regallo, and A. Scanni. 1986. Methylxanthine, alcohol-free diet and fibrocystic breast disease: A factorial clinical trial. Surgery 99:576-580. (Cited by IARC, 1991)

Pardee, A. B., and L. J. James. 1975. Selective killing of transformed baby hamster kidney (BHK) cells. Proc. Nat. Acad. Sci. USA 72 (12):4994-4998.

Parry, J. M., D. Sharp, R. S. Tippins, and E. M. Parry. 1979. Radiation-induced mitotic and meiotic aneuploidy in the yeast *Saccharomyces cerevisiae*. Mutat. Res. 61:37-55. (Cited by IARC, 1991)

Parsons, W. D., and A. H. Neims. 1981. Prolonged half-life of caffeine in healthy term newborn infants. J. Pediatr. 98:640-641. (Cited by IARC, 1991)

Patwardan, R. V., P. V. Desmond, R. F. Johnson, and S. Schenker. 1980. Impaired elimination of caffeine by oral contraceptive steroids. J. Lab. Clin. Med. 95:603-608. (Cited by IARC, 1991)

Pelliccia, F., and A. Rocchi. 1992. The effect of caffeine on DAPI-inducible fragile sites. Mutat. Res. 282:43-48.

Peters, J. M. 1967. Factors affecting caffeine toxicity. J. Clin. Pharmacol. 7:131-141.

Petrek, J. A., W. A. Sandberg, M. N. Cole, M. S. Silberman, and D. C. Collins. 1985. The inhibitory effect of caffeine on hormone-induced rat breast cancer. Cancer 56(8):1977-1981.

Petru, E., G. Boike, and B. U. Sevin. 1990. Potentiation of cisplatin cytotoxicity by methylxanthines *in vitro*. J. Cancer Res. Clin. Oncol. 116:431-433.

Pincheira, J., M. Bravo, and M. J. Santos. 1998. G_2 repair in Nijmegen breakage syndrome: G_2 duration and effect of caffeine and cycloheximide in control and X-ray irradiated lymphocytes. Clin. Genet. 53:262-267.

Pincheira, J., C. Gallo, M. Bravo, M. H. Naverrete, and J. F. L pez-S ez. 1993. G_2 repair and aging: Influence of donor age on chromosomal aberrations in human lymphocytes. Mutat. Res. 295(2):55-62.

Piper, J. M., G. M. Matanosky, and J. Tonascia. 1986. Bladder cancer in young women. Am. J. Epidemiol. 123(6):1033-1042.

Poston, G. E., and R. C. N. Williamson. 1990. Causes, diagnosis, and management of exocrine pancreatic cancer. Compr.Ther. 16(1):36-42.

Pozner, J. A., E. Paptestas, R. Fagerstron, I. Schwartz, J. Saevitz, M. Feinbert, and A. H. Aufses. 1986. Association of tumor differentiation with caffeine and coffee intake in women with breast cancer. Surgery 100:482-488. (Cited by IARC, 1991)

Pozniak, P. C. 1985. The carcinogenicity of caffeine and coffee: A review. J. Am. Diet. Assoc. 85(9):1127-1133. Ref: 115

Raikow, R. B. , J. P. Okunewick, M. J. Buffo, D. L. Jones, B. J. Brozovich, P. R. Seeman, and T. M. Koval. 1981. Potentiating effect of benzo[a]pyrene and caffeine on Friend viral leukemogenesis. Carcinogenesis 2(1):1-6.

Rall, T. W. 1985. The methylxanthines. In: Gilman, A. G., L. S. Goodman, T. W. Rall, and F. Murad (eds.). Goodman and Gilman s The Pharmacological Basis of Therapeutics. 7th edition. New York, NY: MacMillan, pp. 589-603. (Cited by IARC, 1991)

Reed, D., and R. Benfante. 1985. Predictors of health in men of Japanese ancestry in Hawaii. Hawaii Med. J. 44(8):307-308.

Reichert, M. 1978. Micromethod for the determination of caffeine and theophylline allowing direct application of biological fluids to thin-layer chromatography plates. J. Chromatogr. 146:175-180. (Cited by IARC, 1991)

Renner, H. W. 1982. Sister chromatid exchanges induced by methylxanthines contained in coffee, tea, and cocoa. Experientia 38:600. (Cited by IARC, 1991)

Risch, H. A., J. D. Burch, A. B. Miller, G. B. Hill, R. Steele, and G. R. Howe. 1988. Dietary factors and the incidence of cancer in the urinary bladder. Am. J. Epidemiol. 127(6):1179-1191.

Robbins, W. A., M. F. Vine, K. Y. Truong, and R. B. Everson. 1997. Use of fluoresence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. Environ. Mol. Mutagen. 30(2):175-183.

Roberts, J. J., and C. Basham. 1990. Properties of mer$^-$ HeLa cells sensitive or resistant to the cytotoxic effects of MNU; effects on DNA synthesis and of post treatment with caffeine. Mutat. Res. 223:253-263.

Robertson, D., J. C. Fr lich, R. K. Carr, J. T. Watson, J. W. Hollifield, D. G. Shand, and J. A. Oates. 1978. Effects of caffeine on plasma renin activity, catecholamines, and blood pressure. New Engl. J. Med. 298:181-186. (Cited by IARC, 1991)

Rodnan, N. 1997. Caffeine. In: Chemcyclopedia, 98. Washington, D. C.: American Chemical Society, p. 69.

Rohan, T. E., and A. J. McMichael. 1988. Methylxanthines and breast cancer. Int. J. Cancer 441:390-393. (Cited by IARC, 1991)

Rohan, T. E., M. G. Cook, and A. J. McMichael. 1989. Methylxanthines and benign proliferative epithelial disorders of the breast in women. Int. J. Epidemiol. 18:626-633. (Cited by IARC, 1991)

R hrborn, G. 1972. Mutagenicity studies on mouse after chronic administration of caffeine (Ger.). Z. Ern hrungswiss Suppl. 14:54-67. (Cited by IARC, 1991)

Rosenburg, L., D. R. Miller, S. P. Helmrich, D. W. Kaufman, D. Schottenfeld, P. D. Stolley, and S. Shapiro. 1985. Breast cancer and the consumption of coffee. Am. J. Epidemiol. 122(3):391-399.

Rosenberg, L., A. A. Mitchell, S. Shapiro, and D. Slone. 1982. Selected birth defects in relation to caffeine-containing beverages. J. Am. Med. Assoc. 247:1429-1432. (Cited by IARC, 1991)

Ross, W. E., L. A. Zwelling, and K. W. Kohn. 1979. Relationship between cytotoxicity and DNA strand breakage produced by adriamycin and other intercalating agents. Int. J. Rad. Oncol. Biol. Phys. 5:1221-1224.

Ross, R. K., A. Paganini-Hill, J. Landolf, V. Gerkins, and B. E. Henderson. 1989. Analgesics, cigarette smoking, and other risk factors for cancer of the renal pelvis and ureter. Cancer Res. 49(4):1045-1048.

Rothwell, K. 1974. Dose-related inhibition of chemical carcinogenesis in mouse skin by caffeine. Nature 252:69-70. (Cited by IARC, 1991)

Routh, J. I., N. A. Shane, E. G. Arrendondo, and W. D. Paul. 1969. Determination of caffeine in serum and urine. Clin. Chem. 15:661-668. (Cited by IARC, 1991)

Rowley, R. 1992. Reduction of radiation-induced G_2 arrest by caffeine. Rad. Res. 129:224-227.

Roy, M., S. Sengupta, N. P. Bhattacharyya, S. K. Dey, and S. B. Bhattacharjee. 1993. Response of MTX-resistant V79 cells to some DNA-damaging agents. Mutat. Res. 285:199-207.

Russo, P., L. Poggi, S. Parodi, A. M. Pedrini, K. W. Kohn, and Y. Pommier. 1991. Production of protein-associated DNA breaks by 8-methoxycaffeine, caffeine, and 8-chlorocaffeine in isolated nuclei from L1210 cells: Comparison with those produced by topoisomerase II inhibitors. Carcinogenesis 12(10):1781-1790.

Sadzuka, Y., E. Mochizuki, and Y. Takino. 1995a. Mechanism of caffeine modulation of the antitumor activity of adriamycin. Toxicol. Lett. 75:39-49.

Sadzuka, Y., A. Iwazaki, A. Miyagishima, Y. Nozawa, and S. Hirota. 1995b. Effects of methylxanthine derivatives on adriamycin concentration and antitumor activity. Jpn. J. Cancer Res. 86:594-599.

Sadzuka, Y., E. Mochizuki, A. Iwazaki, S. Hirota, and Y. Takino. 1995c. Caffeine enhances adriamycin antitumor activity in Ehrlich ascites carcinoma-bearing mice. Biol. Pharmaceut. Bull. 18(1):159-161.

Sahl, W. J., S. Glore, P. Garrison, K. Oakleaf, and S. D. Johnson. 1995. Basal cell carcinoma and lifestyle characteristics. Int. J. Dermatol. 34(6):398-402.

Sanyal, R., F. Darroudi, W. Parzefall, M. Nagao, and S. Knasmuller. 1997. Inhibition of the genotoxic effects of heterocyclic amines in human derived hepatoma cells by dietary bioantimutagens. Mutagenesis 12(4):297-303.

Sasaki, M. S. 1977. Sister chromatid exchange and chromatid interchange as possible chromosome breakage by DNA cross-linking agents. Cancer Res. 33:1829-1836. (Cited by IARC, 1991)

Sasaki, Y. F., M. Sakaguchi, H. Yamada, T. Miyahara, and H. Kozuka. 1994. Antagonizing effect of triphenyltin chloride on cytosine-1-beta-D-arabinofuranoside potentiation of chromosome aberration induced by mitomycin C. Mutat. Res. 323(3):99-104.

Saunders, D. E., W. D. Lawrence, C. Christensen, N. L. Wappler, H. Ruan, and G. Deppe. 1997. Paclitaxel-induced apoptosis in MCF-7 breast-cancer cells. Int. J. Cancer 70:214-220.

Schlegel, R., and A. B. Pardee. 1986. Premature mitosis induced in mammalian cells by the protein kinase inhibitors 2-aminopurine and 6-dimethylaminopurine. Cell Growth Differ. 1:171-178. (Cited by Tam et al., 1995)

Scott, C. C., and K. K. Chen. 1944. Comparison of the action of 1-ethyl theobromine and caffeine in animals and man. J. Pharmacol. Exp. Therap. 82:89-97.

Senda, S., and K. Hirota. 1974. Pyrimidine derivatives and related compounds. XXII. Synthesis and pharmacological properties of 7-deazaxanthine derivatives. Chem. Pharm. Bull. 22(7):1459-1467.

Seyschab, H., G. Bretzel, R. Freidl, D. Schindler, and Y. Sun. 1994. Modulation of the spontaneous G_2 phase blockage in Fanconi anemia cells by caffeine: Differences from cells arrested by x-irradiation. Mutat. Res. 308(2):149-157.

Shairer, C., L. A. Brinton, and R. N. Hoover. 1987. Methylxanthines and breast cancer. Int. J. Cancer 40:469-473. (Cited by IARC, 1991)

Shairer, C., L. A. Brinton, and R. N. Hoover. 1986. Methylxanthines and benign breast disease. Am. J. Epidemiol. 124:603-611. (Cited by IARC, 1991)

Sharkarnis, V. F. 1970. A comparative study on the action of caffeine on the X-chromosome nondisjunction and recessive sex-linked lethal mutation of different *Drosophila melanogaster* stocks (Russ.). Genetica 6:83-87. (Cited by IARC, 1991)

Sheman, P. 1973. Renal cancer and coffee consumption in 16 countries. Br. J. Cancer 28:473. (Cited by Pozniak, 1985)

Shi, X., N. S. Dalal, and A. C. Jain. 1991. Antioxidant behavior of caffeine: Efficient scavenging of hydroxyl radicals. Fd. Cosmet.Toxicol. 29(1):1-6.

Simon, D., S. Yen, and P. Cole. 1975. Coffee drinking and cancer of the lower urinary tract. J. Natl. Cancer Inst. 54(3):587-591. (Cited by Hopkins, 1984)

Slattery, M. L., and D. W. West. 1993. Smoking, alcohol, coffee, tea, caffeine, and theobromine: Risk of prostate cancer in Utah (United States). Cancer Causes Controls 4(6):559-563.

Slattery, M. L., D. W. West, L. M. Robison, T. K. French, M. H. Ford, K. L. Schuman, and A. W. Sorenson. 1990. Tobacco, alcohol, coffee, and caffeine as risk factors for colon cancer in a low-risk population. Epidemiology 1(2):141-145.

Slattery, M. L., D. W. West, and L. M. Robison. 1988a. Fluid intake and bladder cancer in Utah, USA. Int. J. Cancer 42(1):17-22.

Slattery, M. L., M. C. Schumacher, D. W. West, and L. M. Robison. 1988b. Smoking and bladder cancer: The modifying effect of cigarettes on other factors. Cancer (Phila) 61(2):402-408.

Smith, D. F., J. T. MacGregor, R. A. Hiatt, N. K. Hooper, C. M. Wehr, B. Peters, L. R. Goldman, L. A. Yuan, P. A. Smith, and C. E. Becker. 1990. Micronucleated erythrocytes as an index of cytogenetic damage in humans: Demographic and dietary factors associated with micronucleated erythrocytes in splenectomized subjects. Cancer Res. 50:5049-4054. (Cited by IARC, 1991)

Smith, K. A., B. A. Folz, E. G. Adams, and B. K. Bhuyan. 1995. Synergistic and additive combinations of several antitumor drugs and other agents with the potent alkylating agent adozelesin. Cancer Chemother. Pharmacol. 45:471-482.

Smith, S. J., J. M. Deacon, and C. E. Chilvers. 1994. Alcohol, smoking, passive smoking, and caffeine in relation to breast cancer risk in young women. Br. J. Cancer 70(1):112-119.

Somorin, O. 1974. Caffeine distribution in *C. acuminata, T. cacao, and C. arabica*. J. Fd. Sci. 39:1055-1056. (Cited by IARC, 1991)

Speit, G. 1986. The relationship between the induction of SCEs and mutations in Chinese hamster cells. I. Experiments with hydrogen peroxide and caffeine. Mutat. Res. 174:21-26. (Cited by IARC, 1991)

Stalder, R., H. Luginbuehl, A. Bexter, and H. P. W rzner. 1984. Preliminary findings of a carcinogen bioassay of coffee in mice. In: MacMahon, B. and T. Sugimura (eds.). Banbury Report, Vol. 17, Coffee and Health; Symposium, October, 1993. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 79-88. (Final results published in 1991).

Stalder, R., A. Bexter, H. P. W rzner, and H. Luginbuehl. 1990. A carcinogenicity study of instant coffee in Swiss mice. Fd. Chem. Toxicol. 28(12):829-838.

Stanovnik, B., T. Mirtic, B. Koren, M. Tisler, and B. Belcic. 1982. Methylation of some oxopurines and 1,2,4-triazoles with N,N-dimethylformamide dimethyl acetal. Vestn. Slov. Kem. Drus. 29:331-343. (Cited by IARC, 1991)

Stavric, B. 1988. Methylxanthines: Toxicity to humans. 2. Caffeine. Fd. Chem. Toxicol. 26:645-662. (Cited by IARC, 1991)

Steinmann, K. E., G. S. Belinsky, D. Lee, and R. Schlegel. 1991. Chemically induced premature mitosis: Differential response in rodent and human cells and the relationship to cyclin B synthesis and $p34^{cdc2}$/cyclin B complex formation. Proc. Natl. Acad. Sci. USA 88:6843-6847. (Cited by Tam et al., 1995)

Stewart, J. D., B. Benoit, H. Hugenholts, J. A. Maroun, N. Russell, M. T. Richard, E. Peterson, J. Dennery, J. F. Nabwangu, Z. Grahovac, G. B langer, W. Assang, H. S. Hopkins, and A. Girard. 1986. Cisplatin, arabinofuranosyl cytosine, and caffeine before radiation for glioblastomas. In: Biology of Brain Tumours. Walker, M. D., and D. G. T. Thomas (eds.). Boston, MA: Martinus Nijhoff Publishers, pp. 393-398.

Sturelid, S. 1976. Enhancement by caffeine of cell killing and chromosome damage in Chinese hamster cells treated with thiotepa. Hereditas 84:157-162. (Cited by IARC, 1991)

Sturgeon, S. R., R. G. Zeiger, L. A. Brinton, P. C. Nasca, K. Mallin, and G. Gridley. 1991. Diet and the risk of vulvar cancer. Ann. Epidemiol. 1(5):427-437.

Swenberg, J. A. 1981. Utilization of the alkaline elution assay as a short-term test for chemical carcinogens. In: Stich, H. F., and R. H. C. San (eds.). Short-term tests for chemical carcinogens. New York, NY: Springer, pp. 48-58. (Cited by IARC, 1991)

Takahashi, M., T. Kokubo, F. Furukawa, H. Okamiya, K. Shinoda, K. Imaida, K. Toyoda, and Y. Hayashi. 1984. Effects of sodium chloride, saccharin, phenobarbital and aspirin after initiation with *N*-methyl-*N* -nitro-*N*-nitrosoguanidine. Jpn. J. Cancer Res. 75:365-371. (Cited by Nishikawa et al., 1995)

Takayama, S. 1981a. Studies on carcinogenicity testing of food additives in Japan. Japanese Ministry of Health and Welfare, Food Chemical Tech. Rep. Series No. 1. (Cited by Pozniak, 1985)

Takayama, S. 1981b. Private communication. In: Osler, B.L., and R.A. Ford. 1981. Caffeine: An update. Drug Chem. Toxicol. 4:311. (Cited by Pozniak, 1985)

Takayama, S., and N. Kuwabara. 1982. Long-term study on the effect of caffeine on Wistar rats. Gann 73:365-371. (Cited by IARC, 1991)

Tam, S. W., G. S. Belinsky, and R. Schlegel. 1995. Premature expression of cyclin B sensitizes human HT1080 cells to caffeine-induced premature mitosis. J. Cell. Biochem. 59:339-349.

Tarnopolsky, M., S. Atkinson, J. MacDougall, D. Sale, and J. Sutton. 1988. Physiological responses to caffeine during endurance running in habitual caffeine users. Med. Sci. Sports Exercise 21:428-424. (Cited by Lamarine, 1994)

Tavani, A., E. Negri, S. Francesche, R. Talamini, and C. LaVecchia. 1994. Coffee consumption and risk of non-Hodgkin s lymphoma. Eur. J. Cancer Prevent. 3:351-356.

Thayer, P. S., and C. J. Kensler. 1973. Genetic tests in mice of caffeine alone and in combination with mutagens. Toxicol. Appl. Pharmacol. 25:157-168. (Cited by IARC, 1991)

Theiss, J. C., and M. B. Shimkin. 1978. Inhibiting effect of caffeine on spontaneous and urethan-induced lung tumors in strain A mice. Cancer Res. 38:1757-1761. (Cited by IARC, 1991)

Tohda, H., and A. Oikawa. 1988. Characterization of the enhancing effect of caffeine on sister-chromatid exchanges induced by ultraviolet radiation in excision-proficient xeroderma pigmentosum lymphoblastoid cells. Mutat. Res. 201:1-8. (Cited by IARC, 1991)

Tohnai, I., Oka, T., and Y. Ohno. 1984. A case-control study of cleft lip and/or palate: Maternal dietary practices in early pregnancy (Abstract). Teratology 30:23A. (Cited by IARC, 1991)

Traub, A. E., J. C. Earnshaw, P. D. Brannigan, and W. Thompson. 1982. A critical assessment of the response to caffeine of human sperm motility. Fertil. Steril 37:436-437. (Cited by IARC, 1991)

Trosko, J. E., and E. H. Y. Chu. 1971. Effects of caffeine on the induction of mutations in Chinese hamster cells by ultraviolet light. Mutat. Res. 12:337-340. (Cited by IARC, 1991)

Tsuchiya, H., K. Tomita, Y. Mori, N. Asada, T. Morinaga, S. Kitano, and N. Yamamoto. 1998. Caffeine-assisted chemotherapy and minimized tumor excision for nonmetastatic osteosarcoma. Anticancer Res. 18:657-666.

Tsuchiya, H., H. Yasutake, A. Yokogawa, H. Baba, Y. Ueda, and K. Tomita. 1992. Effect of chemotherapy combined with caffeine for osteosarcoma. J. Cancer Res. Clin. Oncol. 118:567-569.

U.S. Bureau of the Census. 1989. US Exports and US Imports for Consumption (Customs Values) (Reports EM 522 and IM 146), Washington, DC: Foreign Trade Commission, pp. 5161-5162, 5179-5180. (Cited by IARC, 1991)

U.S. Environmental Protection Agency. 1998. OPPT High Production Volume Chemicals. Washington, DC: U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics. http://www.epa.gov/opptintr/chemtest/hpv.htm. (November 20, 1997).

VanderPloeg, L. C., D. M. Wolfrom, and C. W. Welsch. 1991. Influence of caffeine on the development of benign and carcinomatous mammary gland tumors in female rats treated with 7,12-dimethylbenz(a)anthracene and N-methyl-N-nitrosourea. Cancer Res. 51(13):3399-3404.

Wallace, D. M. 1971. Urothelial neoplasia: Causes, assessment, and treatment. Ann. Roy. Coll. Surg. Engl. 51:91-102.

Walton, H. F., G. A. Eiceman, and J. L. Otto. 1979. Chromatography of xanthines on ion-exchange resins. J. Chromatogr. 180:145-156. (Cited by IARC, 1991)

Wang, C. Y., and S. Hayashida. 1984. Enhancement of phenothiazine and 2,5-di-O-acetyl-D-glucosaccharo-(1,4)(6,3)-dilactone of bladder carcinogenicity of N-[4-(5-nitro-1-furyl)-2-thizolyl]-formamide in rats. Cancer Lett. 24(1):37-43.

Warters, R. L., B. W. Lyons, K. Kennedy, and T. Mua Li. 1989. Topoisomerase activity in irradiated mammalian cells. Mutat. Res. 216:43-55. (Cited by Rowley, 1992)

Weinberg, D. M., R. K. Ross, T. M. Mack, A. Pagnini-Hill, and B. E. Henderson. 1983. Bladder cancer etiology: A different perspective. Cancer 51:675. (Cited by Pozniak, 1985)

Weinstein, D., I. Mauer, and H. M. Solomon. 1972. Effect of caffeine on chromosomes of human lymphocytes: *In vivo* and *in vitro* studies. Mutat. Res. 16:391:399. (Cited by IARC, 1991)

Welch, R. M., S.-Y. Hsu, and R. L. DeAngelis. 1977. Effect of Aroclor 1254, phenobarbital, and polycyclic aromatic hydrocarbons on the plasma clearance of caffeine in rats. Clin. Pharm. Ther. 22:791-798. (Cited by IARC, 1991)

Weller, E. M., J. Hain, T. Jung, R. Kinder, M. Kofferlein, W. Burkart, and M. Nusse. 1996. UV-B-induced cell cycle perturbations, micronucleus induction, and modulation by caffeine in human keratinocytes. Intl. J. Radiat. Biol. 69(3):371-384.

Welsch, C. W. 1994. Caffeine and the development of the normal and neoplastic mammary gland. Proc. Soc. Exp. Biol. Med. 207(1):1-12.

Welsch, C. W., and J. V. DeHoog. 1988. Influence of caffeine consumption on 7,12-dimethylbenz(a)anthracene-induced mammary gland tumorigenesis in female rats fed a chemically defined diet containing standard and high levels of unsaturated fat. Cancer Res. 48:2074-2077. (Cited by IARC, 1991)

Welsch, C. W., J. V. DeHoog, and D. H. O Connor. 1988a. Influence of caffeine and/or coffee consumption on the initiation and promotion phases of 7,12-dimethylbenz(a)anthracene-induced rat mammary gland tumorigenesis. Cancer Res. 48:2068-2073.

Welsch, C. W., J. V. DeHoog, and D. H. O Connor. 1988b. Influence of caffeine consumption on carcinomatous and normal mammary gland development in mice. Cancer Res. 48:2078-2082.

Welsch, C. W., K. M. Scieszka, E. R. Senn, and J. V. DeHoog. 1983. Caffeine (1,3,7-trimethylxanthine), a temperate promotor of DMBA-induced rat mammary gland carcinogenesis. Int. J. Cancer 32:479-484.

Wenger, S. L. 1995. Chemical induction of sister chromatid exchange at fragile sites. Cancer Genet. Cytogenet. 85(1):72-74.

Whittenmore, A. S., M. L. Wu, R. S. Paffenbarger, Jr., D. L. Sarles, J. B. Kambert., S. Grosser, D. L. Jung, S. Ballon, and M. Hendrickson. 1988. Personal and environmental characteristics related to epithelial ovarian cancer II: Exposures to talcum powder, tobacco, alcohol, and coffeee. Am. J. Epidemiol. 128(6):1228-1240.

Williams, M. 1992. Ergogenic and ergolytic substances. Med. Sci. Sports Exercise 24:S344-S348. (Cited by Lamarine, 1994)

Wolfrom, D. M., A. R. Rao, and C. W. Welsch. 1991. Caffeine inhibits the development of benign mammary gland tumors in carcinogen-treated female Sprague-Dawley rats. Breast Cancer Res. Treat. 19(3):269-275.

Wolloch, E. H., B. U. Sevin, J. P. Perras, C. F. Gottlieb, A. Pollack, R. Ramos, M. A. Shiano, and H. E. Averette. 1991 Cell kinetic perturbations after irradiation and caffeine in the BG-1 ovarian carcinoma cell line. Gynecol. Oncol. 43:29-36.

W rzner, H.-P., E. Lindstr m, L. Vuataz, and H. Luginb hl. 1997. A 2-year feeding study of instant coffees in rats. II. Incidence and types of neoplasms. Fd. Cosmet. Toxicol. 15:289-296. (Cited by IARC, 1991)

Wynder, E. L., G. S. Deick, and N. E. Hall. 1986. Case-control study of decaffeinated coffee consumption and pancreatic cancer. Cancer Res. 46(10):5360-5363.

Wynder, E. L., and R. Goldsmith. 1977. The epidemiology of bladder cancer: A second look. Cancer 40(3):1246-1268. (Cited by Hopkins, 1984)

Yamagami, T., H. Handa, J. Takeuchi, H. Munemitsu, M. Aoki, and Y. Kato. 1983. Rat pituitary adenoma and hyperplasia induced by caffeine administration. Surg. Neurol. 20:323-331.

Yasutake, H., H. Tsuchiya, K. Tomita, T. Matsunaga, O. Nikaido, and T. Mori. 1995. Inhibitory effect of caffeine on potentially lethal damage repair in cisplatin-treated human osteosarcoma cells. Anticancer Res. 15:831-838.

Yen, G.-C., and H.-Y. Chen. 1996. Relationship between antimutagenic activity and major components of various teas. Mutagenesis 11(1):37-41.

Yesair, D. W., A. R. Branfman, and M. M. Callahan. 1984. Human disposition and some biochemical aspects of methylxanthines. In: Spiller, G. A. (ed.). The Methylxanthine Beverages and Foods: Chemistry, Consumption, and Health Effects. New York, NY: Alan R. Liss, pp. 215-233. (Cited by IARC, 1991)

Yun, T. K. 1991. Usefulness of medium-term bioassay determining formations of pulmonary adenoma in NIH(GP) mice for finding anticarcinogenic agents from natural products. J. Toxicol. Sci. 16(Suppl. 1):53-62.

Yun, T. K., S. H. Kim, and Y. S. Lee. 1995. Trial of a new medium-term model using benzo[a]pyrene induced lung tumor in newborn mice. Anticancer Res. 15(3):839-845.

Yunis, J. J., A. L. Soreng, and A. E. Bowe. 1987. Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1:59-69.

Zajdela, F., and R. Latarjet. 1973. Inhibitory effect of caffeine on the induction of skin cancers by UV light in the mouse. (Fr.). C. R. Acad. Sci. (Paris) 227:1073-1076. (Cited by IARC, 1991)

Zajdela, F., and R. Latarjet. 1975. The inhibitory effect of caffeine on the induction of cutaneous tumors in mice by ultraviolet rays. In: Bucalossi, P., U. Veronesi, and N. Cascinelli (eds.). Proceedings of the XI International Cancer Congress, Florence, 20-26 October 1974. Vol. 3. Amsterdam, Excerpta Medica, pp. 120-123. (Cited by IARC, 1991)

Zajdela, F., and R. Latarjet. (1978a). Ultraviolet light induction of skin carcinoma in the mouse; influence of cAMP modifying agents. Bull. Cancer 65:305-314. (Cited by IARC, 1991)

Zajdela, F., and R. Latarjet. (1978b). Inhibition of skin carcinogenesis *in vivo* by caffeine and other agents. Natl. Cancer Inst. Monogr. 50:133-140. (Cited by IARC, 1991)

Zamansky, G. B., L. F. Kleinman, J. B. Little, P. H. Black, and J. C. Kaplan. 1976. The effect of caffeine on the UV light induction of SV40 virus from transformed hamster cells. Virology 73:468-475.

Zampetti-Bosseler, F., P. Delhaise, and S. Limbosch. 1980. Ultraviolet survival and sensitizing effect of caffeine in mouse hybrid cells. Rad. Res. 84:77-86.

13.0 REFERENCES CONSIDERED BUT NOT CITED

Agapova, L. S., G. V. Ilyinskaya, N. A. Turovets, A. V. Ivanov, P. M. Chumakov, and B. P. Kopnin. 1996. Chromosome changes caused by alterations in p53 expression. Mutat. Res. 354:129-138.

Aida, T., and W. J. Bodell. 1987. Effect of caffeine on cytoxicity and sister chromatid exchange induction in sensitive and resistant rat brain tumor cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res. 47(19):5052-5058.

Andersson, H. C., and B. A. Kihlman. 1987. Effects of G_2 treatments with inhibitors of DNA synthesis and repair on chromosome damage induced by X-rays and chemical clastogens in root tips of *Vicia faba*. Mutat. Res. 181:173-185.

Anderstrom, C., S. L. Johansson, S. Pettersson, and L. Wahlqvist. 1989. Carcinoma of the ureter: A clinicopathologic study of 49 cases. J. Urol. 142(2 pt 1):280-283.

Andriadze, M. I., N. M. Pleskach, V. M. Mikhelson, and V. D. Zhestyanikov. 1987. Sister-chromatid exchanges in a special form of *Xeroderma pigmentosum* (form II). Mutat. Res. 180(1):89-92.

Balansky, R., Z. Mircheva., and P. Blagoeva. 1994. Modulation of the mutagenic activity of cigarette smoke, cigarette smoke condensate and benzo[a]pyrene *in vitro* and *in vivo*. Mutagenesis 9(2):107-112.

Balansky, R., H. Adomat, and L. Bryson. 1994. Additive clastogenicity of sodium selenite and caffeine in CHO cells treated with *N*-methyl-*N* -nitro-*N*-nitrosoguanidine. Biol. Trace Elem. Res. 42(1):53-61.

Blagoeva, P., Z. Mircheva, and R. Balansky. 1991. Potentiation by caffeine and ethanol of tobacco smoke-genotoxicity *in vivo* in mice. Eur. J. Cancer 27(Suppl. 3):S46. (Abstract)

Bonati, M., L. Jiritano, A. Bortolotti, F. Gaspari, S. Filippeschi, A. Puidgemont, and S. Garattini. 1985. Caffeine distribution in acute toxic response among inbred mice. Toxicol. Lett. 29:25-31.

Boothman, D. A., R. Schlegel, and A. B. Pardee. 1988. Anticarcinogenic potential of DNA-repair modulators. Mutat. Res. 202:393-411.

Bowden, G. T., B. Giesselbach, and N. E. Fusenig. 1978. Postreplication repair of DNA in ultraviolet light-irradiated normal and malignantly transformed mouse epidermal cell cultures. Cancer Res. 38(9):2709-2718.

Bronzetti, G., C. Della Croce, and A. Galli. 1992. Antimutagenicity in yeast. Mutat. Res. 267(2):193-200.

Brooks, P. G. 1982. Epidemiology and risk factors in breast cancer. Can we change the odds? J. Reprod. Med. 27(11):570-674.

Candreva, E. C., D. J. Keszenman, E. Barrios, U. Gel s, and E. Nunes. 1993. Mutagenicity induced by hyperthermia, hot mat infusion, and hot caffeine in *Saccharomyces cerevisiae*. Cancer Res. 53(23):5750-5753.

Catalano, A., and P. H. T. Huang. 1992. Changes in secondary structure of DNA of rat embryos following treatment with diethylnitrosamine and methylazoxymethanol acetate *in vivo*. Teratogen., Carcinogen., Mutagen. 12:231-241.

Chan, G. L., and J. B. Little. 1982. Dissociated occurrence of single-gene mutation and oncogenic transformation in C3H 10T_ cells exposed to ultraviolet light and caffeine. J. Cell. Physiol. 111:309-314.

Cleaver, J. E. 1989. Caffeine toxicity is inversely related to DNA repair in Simian Virus 40-transformed *Xeroderma pigmentosum* cells irradiated with ultraviolet light. Teratogen., Carcinogen., Mutagen. 9:147-155.

Clegg, J. C. S., H. R. Glatt, and F. Oesch. 1981. Coordinate mutation and transformation of mouse fibroblasts: Induction by nitroquinoline oxide and modulation by caffeine. Carcinogenesis 2(12):1255-1259.

Cohen, S. M., and S. L. Johansson. 1992. Epidemiology and etiology of bladder cancer. Urol. Clin. North Am. 19(3):421-428.

Darragh, A., M. Kenney, R. F. Lambe, and D. A. O Kelly. 1981. Adverse effects of caffeine. Ir. J. Med. Sci 150(2):47-53.

De Flora, S., H. S. Rosenkranz, and G. Klopman. 1994. Structural basis of antimutagenicity of chemicals towards 4-nitroquinoline 1-oxide in *Salmonella typhimurium*. Mutagenesis 9(1):39-45.

DiPaolo, J. A., C. H. Evans., N. C. Popescu, and J. N. Doniger. 1982. Regulation of mammalian cell transformation. Carcinogenesis 7:273-276.

Doniger, J., and J. A. DiPaolo. 1981. Modulation of *in vitro* transformation and the early and late modes of DNA replication of UV-irradiated Syrian hamster cells by caffeine. Rad. Res. 87:565-575.

Eastman, A., and E. Bresnick. 1981. Conversion of 3-nitro-2-propanol (miserotoxin aglycone) to cytotoxic acrolein by alcohol dehydrogenase. Biochem. Pharmacol. 30(9):2719-2720.

el-Bayoumy, K. 1994. Evaluation of chemopreventive agents against breast cancer and proposed strategies for future clinical intervention trials. Carcinogenesis 15(11):2395-2420.

Enomoto, T., N. Martel, Y. Kanno, and H. Yamasaki. 1984. Inhibition of cell communication between Balb/c 3T3 cells by tumor promoters and protection of cAMP. J. Cell. Physiol. 121:323-333.

Fingert, H. J., J. D. Chang, and A. B. Pardee. 1986. Cytotoxic, cell cycle, and chromosomal effects of methylxanthines in human tumor cells treated with alkylating agents. Cancer Res. 46:2463-2467.

Fingert, H. J., R. L. Kindy, and A. B. Pardee. 1984. Enhanced lethality by methylxanthines in human bladder cancer cells treated with thiotepa. J. Urol. 132(3):609-613.

Fingert, H. J., R. J. Boorstein, R. L. Kindy, and C. C. Lau. 1983. Differential enhanced lethality by caffeine in human cancer cells and normal cells treated with alkylating agents. Proc. Am. Assoc. Cancer Res. 24:270. (Abstract)

Fisch, J. D., B. Behr, and M. Conti. 1998. Enhancement of motility and acrosome reaction in human spermatozoa: Differential activation by type-specific phosphodiesterase inhibitors. Hum. Reprod. 13(5):1248-1254.

Fox, M. 1974. The effect of post-treatment with caffeine on survival and uv-induced mutation frequencies in Chinese hamster and mouse lymphoma cells *in vitro*. Mutat. Res. 24(2):187-204.

Gandi, R. K., and K. L. Khanduja. 1992. Action of caffeine in altering the carcinogen-activating and -detoxifying enzymes in mice. J. Clin. Biochem. Nutr. 12:19-26.

Graham, S., P. Correa, M. J. Hill, W. R. Bruce, L. W. Wattenberg, G. N. Stemmermann, and B. S. Reddy. 1981. Epidemiologic tests of hypotheses relating diet and cancer. Banbury Rep. 7:295-408.

Hall, J. G., and D. J. Glover. 1970. Effect of inocula of benzo[a]pyrene-treated sarcoma cells on growth of primary tumors in rate. J. Natl. Cancer Inst. 45:1163-1168.

Handa, S. P. 1979. Analgesic neuropathy and urothelial carcinoma. Can. Med. Assoc. J. 121(7):849-850.

Harvey, A. N., and J. R. K. Savage. 1994. A case of caffeine-mediated cancellation of mitotic delay without enhanced breakage in V79 cells. Mutat. Res. 304:203-209.

Hatayama, T., and M. Yokioka. 1983. Mode of inhibition of DNA replication in neocarzinostatin-treated HeLa cells. Biochim Biophys. Acta 740:291-299.

Heyden, S. 1974. Koffein auf der Anklagebank - Quousque tandem? Nutr. Metabol. 12:261-267.

Hoffmann, D., A. Rivenson, F.-L. Chung, and E. L. Wynder. 1993. Potential inhibitors of tobacco carcinogenesis. Ann. New York Acad. Sci. 686:140-160.

Iliakis, G., and M. N sse. 1983. Evidence that repair and expression of potentially lethal damage cause the variations in cell survival after X irradiation observed through the cell cycle in Ehrlich ascites tumor cells. Rad. Res. 95:87-107.

Iliakis, G., and M. N sse. 1982. Conditions supporting repair of potentially lethal damage cause a significant reduction of ultraviolet light-induced division delay in synchronized and plateau-phase Ehrlich ascites tumor cells. Rad. Res. 91:483-506.

Ishida, Y., Y. Furukawa, J. A. Decaprio, M. Saito, and J. D. Griffin. 1992. Treatment of myeloid leukemic cells with the phosphatase inhibitor okadaic acid induces cell cycle arrest at either G_1/S or G_2/M depending on dose. J. Cell. Physiol. 150:484-492.

Ivankovic, S., J. Seibel, D. Komitowski, B. Spiegelhalder, R. Preussmann, and M. Siddiqi. 1998. Caffeine-derived N-nitroso compounds. V. Carcinogenicity of monomitrosocaffeidine and dinitrosocaffeidine in *bd-ix* rats. Carcinogenesis 19(5):933-937.

Jan, K. Y., R. Y. Huang, and T. C. Lee. 1986. Different modes of action of sodium arsenite, 3-aminobenzamide, and caffeine on the enhancement of ethyl methanesulfonate clastogenicity. Cytogenet. Cell Genet. 41:202-208.

Kihlman, B. A., S. Sturelid, B. Hartley-Asp, and K. Nilsson. 1974. The enhancement by caffeine of the frequencies of chromosomal aberrations induced in plant and animal cells by chemical and physical agents. Mutat. Res. 26:105-122.

Koshiura, R., K. Miyamoto, and F. Sanae. 1980. Combination antitumor effect with central nervous system depressants on rat ascites hepatomas. Gann 71:45-51.

Kunicka, J. E., A. Myc, M. R. Melamed, and Z. Darzynkiewicz. 1990. Caffeine increases sensitivity of DNA to denaturation in chromatin of L1210 cells. Cell Tissue Kinet. 23(1):31-39.

Kuroki, T. 1981. *In vitro* carcinogenesis by 4-nitroquinoline 1-oxide and related compounds. Carcinogenesis 6:93-115.

Kyriazis, A. P., A. A. Kyriazis, and A. Yagoda. 1985. Enhanced therapeutic effect of cis-diaminedichloroplatinum (II) against nude-mouse grown human pancreatic adenocarcinoma when combined with 1-beta-D-arabinofuranosylcytosine and caffeine. Cancer Res. 45(12 pt 1):6083-6087.

Labanowska, J. K. L. Beetham, and L. J. Tolmach. 1988. Caffeine-induced modulation of the lethal action of x-rays on Chinese hamster V79 cells. Rad. Res. 115:176-186.

Lehmann, A. R., S. Kirk-Bell, C. F. Arlett, S. A. Harcourt, E. A. de Weerd-Kastelein, W. Keijzer, and P. Hall-Smith. 1977. Repair of ultraviolet light damage in a variety of human fibroblast cell strains. Cancer Res. 37:904-910.

Lemasters, G. K., G. K. Livingston, J. E. Lockey, D. M. Olsen, R. Shukla, G. New, S. G. Selevan, and J. H. Yiin. 1997. Genotoxic changes after low-level solvent and fuel exposure on aircraft maintenance personnel. Mutagenesis 12(4):237-243.

Lialiaris, T., D. Mourelatos, and J. Dozi-Vassiliades. 1988. Enhancement of cytogenic damage by chloropromazine in human lymphocytes treated with alkylating antineoplastics and caffeine. Mutat. Res. 206:361-365.

Manber, M. 1976. The medical effects of coffee. Med. World News 17(2):63-73.

McKelvey, V. J., and P. G. McKenna. 1986. Enhanced synergism between caffeine and mitomycin C in the induction of cytogenetic aberrations in thymidine kinase-deficient Friend murine erythroleukaemia cells. Mutagenesis 1(3):173-178.

Menz, W., and G. Speit. 1992. Characterization of the genotoxic action of chlorambucil in V79 cells. Mutagenesis 7(2):163. (Abstract)

Mills, L. J., Nelson, S. M., and R. Malcolm. 1994. Effects of selected anti-tumor promoting chemicals on metabolic cooperation between Chinese hamster V79 cells. Tox. Appl. Pharm. 126:338-344.

Mironescu, S. G. D., S. M. Epstein, and J. A. DiPaolo. 1980. Relationship between morphological transformation and [^{3}H]thymidine incorporation stimulated by a chemical carcinogen in postconfluent cultures of hamster embryo cells. Cancer Res. 40(7):2411-2416.

Miyata, Y., A. Hagiwara, T. Nakatsuka, G. Murasaki, M. Arai, and N. Ito. 1980. Effects of caffeine and saccharin on DNA in the bladder epithelium of rats treated with *N*-butyl-*N*-(3-carboxypropyl)-nitrosamine. Chem. Biol. Interact. 29:291-302.

Morrison, R. 1978. Cancer of the urinary bladder Epidemiology and aetiology. Urological Res. 6:183-184.

Mourelatos, D., J. Dozi-Vassiliades, A. Kotsis, and C. Gourtsas. 1988. Enhancement of cytogenetic damage and of antineoplastic effect by caffeine in Ehrlich ascites tumor cells treated with cyclophosphamide *in vivo*. Cancer Res. 48:1129-1131.

Mourelatos, D., J. Dozi-Vassiliades, V. Tsigalidou-Balla, and A. Granitsas. 1983. Enhancement by methylxanthines of sister-chromatid exchange frequency induced by cytostatics in normal and leukemic human lymphocytes. Mutat. Res. 121:147-152.

Mourelatos, D., J. Dozi-Vassiliades, and A. Granitsas. 1982. Anti-tumour alkylating agents act synergistically with methylxanthines on induction of sister-chromatid exchange in human lymphocytes. Mutat. Res. 104:243-247.

Murnane, J. P., J. E. Byfield, C.-T. Chen, and C. H. Wang. 1981. The structure of methylated xanthines in relation to their effects on DNA synthesis and cell lethality in nitrogen mustard-treated cells. Biophys. J. 35(3):665-676.

Nakamura, K., and S. Antoku. 1993. Protein kinase inhibitor, staurospoine, prevents okidaic acid- or caffeine-induced chromosome condensation. *In Vitro* Cell Dev. Biol. 29A(10):760-762.

Nakamura, S., Y. Oda, T. Shimada, I. Oki, and K. Sugimoto. 1987. SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: Examination with 151 chemicals. Mutat. Res. 192:239-246.

Nehlig, A., and G. Debry. 1994. Potential genotoxic, mutagenic and antimutagenic effects of coffee: A review. Mutat. Res. 317:145-162.

Nelson, W. G., and M. B. Kaston. 1994. DNA strand breaks: The DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol. 14(3):815-1823.

Nilsson, K., and A. R. Lehmann. 1975. The effect of methylated oxypurines on the size of newly synthesized DNA and on the production of chromosome aberrations after UV irradiation in Chinese hamster cells. Mutat. Res. 30:255-266.

Niwa, O., T. Seyama, H. Takebe, and T. Sugahara. 1985. High sensitivity of murine teratocarcinoma cells to UV radiation and the effect of post-irradiation treatment with caffeine. Mutat. Res. 145:195-200.

Nomura, T., S. Hata, T. Enomoto, H. Tanaka, and K. Shibata. 1980. Inhibiting effects of antipain on urethane-induced lung neoplasia in mice. Br. J. Cancer 42(2):624-626.

O Gara, R. W., C. W. Lee, J. F. Morton, G. J. Kapadia, and L. J. Dunham. 1974. Sarcoma induced in rats by extracts of plants and by fractionated extracts of *Krameria ixina*. J. Natl. Cancer Inst. 52(2):445-448.

Parsons, P. G. 1984. Dependence on treatment time of melphalan resistance and DNA cross-linking in human melanoma cell lines. Cancer Res. 44:2773-2778.

Petrou, C., D. Mourelatos, J. Dozi-Vassiliades, and P. Catsoulacos. 1990. Synergistic induction of cytogenic damage by the homo-aza-steroidal ester of *p*-bis(2-chloroethyl)aminophenylacetic acid in combination with caffeine in human lymphocytes *in vitro* and in Ehrlich ascites tumour cells *in vivo*. Mutat. Res. 243(2):109-113.

Phelps, H. M., and C. E. Phelps. 1988. Caffeine ingestion and breast cancer. A negative correlation. Cancer 61(5):1051-1054.

Pike, M. C., and L. Bernstein. 1985. Statistical errors invalidate conclusions in Caffeine and unsaturated fat diet significantly promotes DMBA-induced breast cancer in rats. Cancer 55(8):1855-1858.

Pincheira, J., C. Gallo, and J. F. L pez-S ez. 1991. Effects of caffeine and cycloheximide during the G_2 prophase in control and x-ray-irradiated human lymphocytes. Mutat. Res. 251(1):71-77.

Poorman-Allen, P., P. Glover, and D. Clive. 1992. Distinctive aspects of mammalian cell mutagenesis. IV. Implications for genetic toxicology. Environ. Mol. Mutagen. 19(20):50. (Abstract)

Przylepa, K. A., and S. L. Wenger. 1988. Chromosome breaks and fragile sites in leukemic bone marrow cells. Cancer Genet. Cytogenet. 33(1):35-38.

Reich, R., and G. R. Martin. 1996. Identification of arachidonic acid pathways required for the invasive and metastatic activity of malignant tumor cells. Prostaglandins 51:1-17.

Rivedal, E., and T. Sanner. 1985. Caffeine and other phosphodiesterase inhibitors are potent inhibitors of the promotional effect of TPA on morphological transformatio of hamster embryo cells. Cancer Lett. 28:9-17.

Roberts, J. J. 1984. Mechanism of potentiation by caffeine of genotoxic damage induced by physical and chemical agents. Nucleic Acids Symposium Series 13:193-215.

Roberts, J. J., T. Marchbank, V. P. Kotsaki-Kovatsi, M. P. Boland, F. Friedlos, and R. J. Knox. 1989. Caffeine, aminoimidazolecarboxamide and decoumarol, inhibitors of NAD(P)H dehydrogenase (quinone) (DT diaphorase), prevent both the cytotoxicity and DNA interstrand crosslinking produced by 5-(aziridin-1-yl)-2,4-dinitrobenzamide (CB 1954) in Walker cells. Biochem. Pharmacol. 38(22):4137-4143.

Roberts, J. J., and V. P. Kotsaki-Kovatsi. 1986. Potentiation of sulphur mustard or cisplatin-induced toxicity by caffeine in Chinese hamster cells correlates with formation of DNA double-strand breaks during replication on a damaged template. Mutat. Res. 165:207-220.

Rothschild, H., and P. H. Black. 1970. Analysis of SV40-induced transformation of hamster kidney tissue *in vitro*. VII. Induction of SV40 virus from transformed hamster cell clones by various agents. Virology 42:251-256.

Ryan, A. J., S. Squires, H. L. Strutt, A. Evans, and R. T. Johnson. 1994. Different fates of camptothecin-induced replication fork-associated double-strand DNA breaks in mammalian cells. Carcinogenesis 15(5):823-828.

Sasaki, K., S. Rurusawa, and G. Takayanagi. 1981. Effect of prior administration of aminophylline, caffeine or propranolol on the antitumor activity of 6-mercaptopurine or 6-mercaptopurine riboside. Chem. Pharm. Bull. 29(1):276-279.

Sato, K., and N. Hieda. 1980. Mutation induction in a mouse lymphoma cell mutant sensitive to 4-nitroquinoline 1-oxide and ultraviolet radiation. Mutat. Res. 71:233-241.

Sawecka, J., B. Golos, and J. Malec. 1987. Modification by caffeine of acute cytotoxic response of cultured L5178Y cells to hydroxyurea treatment. Neoplasma 34(4):369-377.

Schreiber, G. B., M. Robins, C. E. Maffeo, M. N. Masters, A. P. Bond, and D. Morganstein. 1988. Confounders contributing to the reported associations of coffee or caffeine with disease. Prevent. Med. 17(3):295-309.

Sigler, K., and R. J. Ruch. 1993. Enhancement of gap junctional intercellular communication in tumor promotor-treated cells by components of green tea. Cancer Lett. 69:15-19.
Sivak, A., L. Rudenko, and L. Gaudio Teague. 1982. Variations among species and cell types in the effects of caffeine on mutagen-induced cytotoxicity and postreplication repair of DNA. Environ. Mutagen. 4:143-162.

Stoilov, L. M., L. H. F. Mullenders, and A. T. Natarajan. 1994. Caffeine potentiates or protects against radiation-induced DNA and chromosomal damage in human lymphocytes depending on temperature and concentration. Mutat. Res. 311(2):169-174.

Szumiel, I., D. Wlodek, K. J. Johnson, and S. Sundell-Bergman. 1984. ADP-ribosylation and post-irradiation cellular recovery in two strains of L5178Y cells. Br. J. Cancer 49(Suppl. 6):33-38.

Tanzarella, C., S. Astolfi, and F. Palitti. 1984. Effect of caffeine on induced damage in cells of *Xenopus laevis*. Mutat. Res. 128(1):25-29.

Tashiro, T., and Y. Sato. 1992. Characterization of acquired resistance to *cis*-diamminedichloroplatinum (II) in mouse leukemia cell lines. Jpn. J. Cancer Res. 83:219-225.

Timson, J. 1971. Caffeine and bladder cancer. Lancet 2:212-213.

Tohda, H., J. H. Zhao, and A. Oikawa. 1992. A possible involvement of DNA topoisomerase I in caffeine effect after ultraviolet irradiation. Tohoku J. Exp. Med. 168(2):129-132.

Tohda, H., and A. Oikawa. 1983. Differential features of sister-chromatid exchange responses to ultraviolet radiation and caffeine in *Xeroderma pigmentosum* lymphoblastoid cell lines. Mutat. Res. 107:387-396.

Tomita, K., and H. Tsuchiya. 1989. Caffeine enhancement of the effect of anticancer agents on human sarcoma cells. Jpn. J. Cancer Res. 80(1):83-88.

Tsuchiya, H., K. Tomita, H. Yasutake, Y. Ueda, M. Tanaka, and T. Sasaki. 1989. Growth inhibition and differentiation of murine melanoma B16-BL6 cells caused by the combination of cisplatin and caffeine. Jpn. J. Cancer Res. 80(12):1246-1251.

Tsuji, H., E.-I. Takahashi, S. Tsuji, I. Tobari, T. Shiomi, and K. Sato. 1987. Chromosomal instability in mutagen-sensitive mutants isolated from mouse lymphoma L5178Y cells. Mutat. Res. 178(1):107-116.

Utsumi, H., H. Kitani, C.-M. Chang-Liu, and M. M. Elkind. 1987. 7,12-Dimethylbenz[a] anthracene plus near-u.v. light initiates DNA damage and repair in Chinese hamster cells. Carcinogenesis 8(10):1439-1444.

VanderPloeg, L. C., D. M. Wolfrom, and C. W. Welsch. 1991. Caffeine and the development of benign and carcinomatous mammary tumors in carcinogen-treated female Sprague-Dawley rats. Proc. Am. Assoc. Cancer Res. Annual Meeting 32:145. (Abstract)

Wang, Y., N. Cheong, M. Miura, and G. Iliakis. 1997. Overexpression of insulin-like growth factor (IGF)-1 receptor enhances inhibition of DNA replication in mouse cells exposed to x-rays. Rad. Environ. Biophys. 36:117-123.

Wolff, S., and D. Scott. 1969. Repair of radiation-induced damage to chromosomes. Exp. Cell Res. 55:9-16.

Wun, K. W., and R. H. Shafer. 1982. Structural changes in mammalian cell DNA induced by low-dose x-ray damage and subsequent postirradiation incubation in the presence and absence of caffeine. Rad. Res. 90:310-320.

Yagi, T., and H. Takebe. 1989. Similarity in the effect of caffeine on DNA synthesis after uv irradiation between *Xeroderma pigmentosum* variant cells and mouse cells. Jpn. J. Cancer Res. 80(8):754-758.

Vitek, J. A. 1993. Inhibition of intercellular gap junctional communication by alkyl ethers and its modulation by cAMP. Neoplasma 40(3):167-172.

Vogel, R., G. Laschinski, H. Spielman, W. Ehrlich, B. Drevenstedt, J. Klosa, and H. Kroger. 1991. *In vitro* studies on genotoxicity and cytotoxicity of the anticancer drugs cisplatin and cofplaton, a caffeine-8-ether plus cisplatinum compound. Mutat. Res. 264(4):225-230.

Zamansky, G. B., S. A. Latt, J. C. Kaplan, L. F. Klein-Man, C. Dougherty, and P. H. Black. 1980. The co-induction of sister chromatid exchanges and virus synthesis in mammalian cells. Exp. Cancer Res. 126(2):473-477.

Yasukawa, K., M. Takido, M. Asasu, T. Ikekawa, F. Shimada, Y. Ikeya, H. Mitsuhashi, M. Takeuchi, T. Matsumoto, and S. Nakagawa. 1992. Inhibitory effects of plant constituents on tumor promotion during carcinogenesis. J. Pharmacobio-Dyn. S-3:15. (Abstract)

ACKNOWLEDGEMENTS

Support to the National Toxicology Program for the preparation of Caffeine and Its Modulating Effects Review of Toxicological Literature was provided by Integrated Laboratory Systems, Inc., through NIEHS Contract Number N01-ES-65402. Contributors included: Raymond R. Tice, Ph.D. (Principal Investigator); Brigette D. Brevard, M.A. (Co-Principal Investigator); Kristine Witt, M.S.; Esther Morris, M.S.; and Claudine Gregorio, M.A.

APPENDIX A UNITS AND ABBREVIATIONS

°C = degrees Celsius

µg/L = micrograms per liter

µg/m^3 = micrograms per cubic meter

µg/mL = micrograms per milliliter

µM = micromolar

+ = or more

4NQO = 4-nitroquinoline-1-oxide

5FURA = 5-fluorouracil

ara-C = arabinofurosylcytosine

AT = *Ataxia telangiectasia*

BP = benzo[a]pyrene

CA = chromosome aberrations

CDDP = *cis*-diamminedichloroplatinum(II)

CHD = coronary heart disease

CHM = cyclohexamide

CHO = Chinese hamster ovary

CI = confidence interval

d = day(s)

DACA = *N*-[(2-dimethylamino)ethyl]acridine-4-carboxamide

DAPI = 4',6-diamidino-2-phenyl-indole

DHB = L-threitol-1,4-bismethanesulfonate

DMBA = 7,12-dimethylbenz(a)anthracene

DMH = 1,2-dimethylhydrazine

DMSO = dimethyl sulfoxide

EPA = U.S. Environmental Protection Agency

F = female

FA = Fanconi anemia

FDA = Food and Drug Administration

FISH = fluorescence *in situ* hybridization

FLV = Friend leukemia virus

g = grams

g/mL = grams per milliliter

GC/MS = gas chromatography/mass spectrometry

GRAS = generally recognized as safe

GSH = glutathione

h = hour(s)

Hg = mercury

i.p. = intraperitoneal injection

i.v. = intravenous injection

IQ = 2-amino-3-methylimidazo-[3,4-*f*]quinoline

kg = kilograms

lb = pounds

LC_{50} = lethal concentration for 50% of test animals

LD_{50} = lethal dose for 50% of test animals

L-PAM = L-phenylalanine mustard

M = male

mg/kg = milligrams per kilogram

mg/m^3 = milligrams per cubic meter

mg/mL = milligrams per milliliter

min = minutes

mL/kg = milliliters per kilogram

mm = millimeters

mM = millimolar

mmol = millimoles

mmol/kg = millimoles per kilogram

MN = micronuclei

MNBN = micronucleated binucleated

MNNG = 1-methyl-3-nitro-1-nitrosoguanidine

MNU = methyl nitrosourea

mo = month(s)

MTX = methotrexate

MXN = mitoxantrone

n.p. = not provided

NA = not applicable

NBS = Nijmegen breakage syndrome

ng/g = nanograms/gram

OR = odds ratio

OTC = over-the-counter

PCC = premature chromosome condensation

ppb = parts per billion

ppm = parts per million

RR = relative risk

s.c. = subcutaneous

SCE = sister chromatid exchange

SMART = somatic mutation and recombination test

TNF = tumor necrosis factor

TPA = 12-*O*-tetranodecanoylphorbol-13-acetate

TSCA = Toxic Substances Control Act

UVB = ultraviolet-B

VCL = curvilinear velocity

VL = linear velocity

wk = week(s)

yr = year(s)