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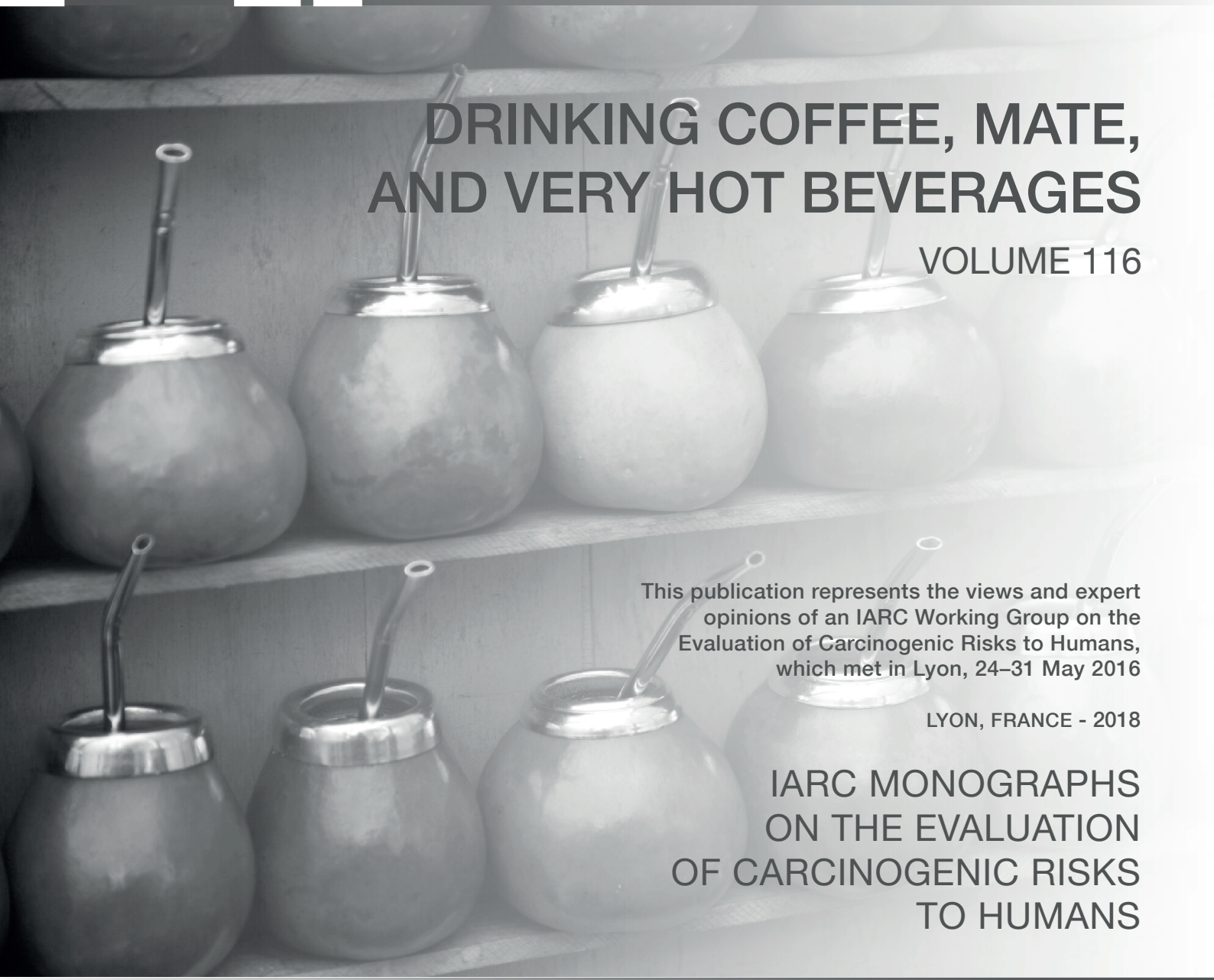

DRINKING COFFEE, MATE, AND VERY HOT BEVERAGES

VOLUME 116

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3. CANCER IN EXPERIMENTAL ANIMALS

In its previous evaluation (*IARC Monographs* Volume 51; IARC, 1991), the Working Group concluded that the results of animal bioassays provided *inadequate evidence* for the carcinogenicity of coffee. This section provides an evaluation of the carcinogenicity, co-carcinogenicity, and initiation–promotion studies reviewed in Volume 51 of the *IARC Monographs* and a review of any studies published since that time.

3.1 Studies of carcinogenicity

See Table 3.1.

3.1.1 Mouse

Bauer et al. (1977) reported the results of a drinking-fluid study in which three cohorts of male C57BL/6J mice were given brewed coffee (55 mice) or boiled water (54 mice) over their lifetime. Mice given coffee demonstrated lower body weights and decreased survival, even though this group had a higher food and fluid intake throughout the study. Since no histopathology was included in the study design, no conclusions could be drawn as to whether the decreased survival was related to cancer incidence. [The Working Group determined that this study was inadequate for evaluation.] Bauer et al. (1977) also mentioned that an identical study was performed with A/J mice, but provided no quantitative data from this study.

Stalder et al. (1990) reported the results of a well-designed and well-conducted 2-year bioassay to determine the possible carcinogenicity of instant coffee (given as a dietary supplement) in Swiss mice. Coffee administration was initiated after mating of parental (F_0 generation) mice and was continued throughout the F_0 and F_1 generations. Beginning after mating and continuing throughout gestation, parturition, and lactation, dams (F_0 generation) were given either basal diet (control dams) or basal diet supplemented with 1% instant coffee (1% coffee was the maximum dietary supplement that did not affect fertility in dams). At weaning, F_1 mice were randomized into groups of 150 per sex and were given diets supplemented with 1%, 2.5%, or 5% instant coffee for 2 years. Controls (born from control dams) were only given basal diet for the same period.

The consumption of a coffee-supplemented diet was associated with a statistically significant, dose-related increase in survival in both sexes. Although food intake in coffee-supplemented groups did not differ from that in sex-matched controls, coffee induced a dose-related suppression of body-weight gain in both sexes. Differences from control body weights were statistically significant in male mice given 2.5% and 5% coffee and in all three groups of female mice given coffee ($P < 0.001$ for all comparisons). [The study authors attributed decreased body weights to increased activity in groups receiving coffee supplements.]

In comparison to female mice in the dietary control group, female mice given coffee

Table 3.1 Studies of carcinogenicity in experimental animals exposed to coffee

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Dose(s) No. animals/group at start No. of surviving animals/group	Results	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) In utero Fetal life + 2 yr Stalder et al. (1990)	In utero + oral (diet) Instant coffee 0 (control), 1%, 2.5%, 5% in F_1 generation diet In utero (dams given 0 (control) or 1% instant coffee in the diet) + continuous exposure (F_1 generation diet) 150, 150, 150, 150/group 32, 48, 57, 76	Liver: hepatocellular adenoma		Principal strengths: large group size (150/group), statistical analysis, in utero exposure Decreased incidences of lymphosarcoma also seen in liver, lung, pancreas, spleen, thymus, lymph nodes, and small intestine
		Tumour incidence: 46/135, 47/140, 26/142, 18/143	Incidences in 2.5% and 5% groups are significantly decreased from control; statistically significant trend towards reduced adenoma incidence with increasing dose	
		Kidney: lymphosarcoma		
		Tumour incidence: 20/135, 11/139, 7/142, 2/143	2.5% and 5% dose: statistically significant negative association; significant negative trend with dose	
		All sites: benign tumours		
		Tumour incidence: 56/136, 59/141, 44/142, 26/143	Statistically significant trend towards lower incidence with increasing dose	
		All sites: malignant tumours		
		Tumour incidence: 54/136, 45/141, 40/142, 26/143	Statistically significant trend towards lower incidence with increasing dose	
		All sites: all tumours		
		Tumour incidence: 96/136, 88/141, 77/142, 49/143	Statistically significant trend towards lower incidence with increasing dose	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Dose(s) No. animals/group at start No. of surviving animals/group	Results	Significance	Comments
Full carcinogenicity Mouse, Swiss (F) In utero Fetal life + 2 yr Stalder et al. (1990)	In utero + oral (diet) Instant coffee 0 (control), 1%, 2.5%, 5% in F_1 generation diet In utero (dams given 0 (control) or 1% instant coffee in the diet) + continuous exposure (F_1 generation diet) 150, 150, 150, 150/group 30, 49, 41, 84	Uterus: leiomyoma		Principal strengths: large group size (150/group), statistical analysis, in utero exposure Decreased incidences of lymphosarcoma also seen in liver, lung, pancreas, spleen, salivary gland, urinary bladder, thymus, lymph nodes, and large intestine
		Tumour incidence: 0/142, 2/146, 0/145, 4/140	Statistically significant trend towards increased incidence ($P < 0.05$) with increasing dose	
		Kidney: lymphosarcoma		
		Tumour incidence: 26/146, 13/146, 16/146, 3/148	Statistically significant decreased incidence in 5% coffee groups; significant negative trend with increasing dose	
		All sites: malignant tumours		
		Tumour incidence: 67/146, 41/147, 46/146, 34/149	Statistically significant trend towards decreased tumour incidence with increasing dose	
		All sites: all tumours		
		Tumour incidence: 83/146, 60/147, 67/146, 54/149	Statistically significant trend towards decreased tumour incidence with increasing dose	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Dose(s) No. animals/group at start No. of surviving animals/group	Results	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) In utero Fetal life + 2 yr Palm et al. (1984)	In utero + drinking fluid Brewed coffee (in tap water) 0, 0, 25, 50, 100% In utero (dams given tap water (control) or 50% coffee as drinking-water) + continuous (F_1 generation drinking-water) exposure 55, 55, 55, 55, 55/group NR	Skin: fibrosarcoma or squamous cell carcinoma (combined) Tumour incidence: 0/55, 2/55, 7/55, 3/55, 0/55 Skin: fibrosarcoma Tumour incidence: 0/55, 1/55, 4/55, 1/55, 0/55 Skin: squamous cell carcinoma Tumour incidence: 0/55, 1/55, 3/55, 2/55, 0/55	Increase was statistically significant only in 25% coffee group ($P < 0.05$; X^2 test, vs pooled controls) NS NS	Principal strengths: in utero exposure, statistics Coffee was analysed chemically; two control groups
Full carcinogenicity Rat, Sprague-Dawley (F) In utero Fetal life + 2 yr Palm et al. (1984)	In utero + drinking fluid Brewed coffee (in tap water) 0, 0, 25%, 50%, 100% In utero (dams given tap water (control) or 50% coffee as drinking-water) + continuous (F_1 generation drinking-water) exposure 55, 55, 55, 55, 55/group NR	Mammary gland: fibroadenoma Tumour incidence: 25/55, 23/55, 23/55, 11/55, 14/55	50% and 100% dose groups: statistically significantly reduced ($P < 0.05$; X^2 test, vs pooled controls)	Principal strengths: in utero exposure, statistics Coffee was analysed chemically; two control groups

F, female; M, male; NR, not reported; NS, not significant; vs, versus; yr, year

demonstrated a statistically significant trend towards increased incidence of leiomyoma of the uterus (0/142, 2/146, 0/145, 4/140). By contrast, statistically significant and dose-related reductions in the incidence of lymphosarcoma were seen in the kidney, liver, lung, pancreas, salivary gland, spleen, thymus, lymph nodes, large intestine, and urinary bladder. In comparison to male mice in the dietary control group, male mice given coffee demonstrated statistically significant and dose-related reductions in the incidence of lymphosarcoma of the kidney, liver, lung, pancreas, thymus, lymph nodes, small intestine, and spleen. In addition, male mice given coffee demonstrated a statistically significant, dose-related reduction in the incidence of hepatocellular adenoma. Statistically significant, dose-related negative associations were seen in both sexes for level of coffee exposure and total tumour incidence, and level of coffee exposure and total incidence of malignant tumours. [The Working Group noted the possibility that the observed reductions in tumour incidence were related to the statistically significant suppression of mean body weights in male and female mice given coffee.]

3.1.2 Rat

Palm et al. (1984) reported the results of a well-designed and well-conducted 2-year bioassay of fresh brewed coffee in Sprague-Dawley rats. The green coffee mix, roast colour, grind, and freshness criteria of the ground coffee used in the study (provided vacuum-packed by the National Coffee Association of the USA) was almost identical to that of the commercial coffee commonly purchased in the USA. Coffee administration was initiated before mating of parental (F_0 generation) rats and was continued throughout the F_0 and F_1 generations. Beginning 5 weeks before mating and continuing throughout gestation, parturition, and lactation, dams (F_0 generation) were given either 50% coffee (the maximum concentration tolerated by dams) or tap water only. When F_1 rats were aged 5–6 weeks, those whose dams were given either 50% coffee or tap water only were randomized into groups (55 F_1 rats per sex per group). F_1 rats from coffee-treated dams were given 100%, 50%, or 25% fresh brewed coffee as their only fluid source for 2 years. F_1 rats from control dams were randomized into two control groups (55 F_1 rats per sex per group) to be given tap water only for 2 years.

No significant differences in mortality were seen in any group of male rats receiving coffee compared with pooled male controls. By contrast, statistically significant decreases in survival were seen in female rats given 50% and 100% coffee as their only fluid source compared with pooled female controls. Despite increases in food and fluid intake, statistically significant reductions in group mean body weight were seen in male rats given 100% coffee as their only fluid source; mean body weights in other groups were not statistically different from controls given tap water only.

When compared with sex-matched controls given water only, no statistically significant increases in the total incidence of primary tumours were seen in any group given coffee at 25%, 50%, or 100% of fluid intake. However, in statistical analyses based on the assumption that tumours were non-lethal (Mantel–Haenszel model), time-to-tumour analyses identified a statistically significant increase in the number of tumour-bearing male rats in the group given 25% coffee. By contrast, no statistically significant differences were seen in male rats given 50% coffee or 100% coffee, or in female rats given coffee at any concentration. [The Working Group noted that the increased number of tumour-bearing male rats was not related to dose.]

In comparison to sex-matched controls, the total incidence of fibrosarcoma or squamous cell carcinoma (combined) of the skin was significantly increased in male rats given 25%

coffee (7/55 vs 2/110 in pooled male controls); however, the incidences in male rats given 50% and 100% coffee (3/55 and 0/55, respectively) were not significantly different from pooled male controls. [The Working Group noted that the increased incidence of skin tumours in male rats given 25% coffee was the result of small increases in the incidences of both epithelial and mesenchymal tumours (squamous cell carcinoma and fibrosarcoma, respectively), neither of which was itself significant.] No significant differences in the incidence of skin tumours in female rats between the control group and any coffee-exposed group were observed. Statistically significant decreases in the incidence of fibroadenoma of the mammary gland were seen in female rats given 50% or 100% coffee (11/55 and 14/55, respectively, vs 48/110 in pooled female controls) (Palm et al., 1984).

Würzner et al. (1977a, b) reported the results of a 2-year study in which groups of 40 male and 40 female Sprague-Dawley rats [age not reported; weight, approximately 100 g] were given a chow diet supplemented with regular instant coffee, decaffeinated instant coffee, or decaffeinated instant coffee + caffeine for 2 years. Both spray-dried and freeze-dried instant coffees were tested; extraction rates of instant coffees given to different groups varied over the range 23.0–50.2%. Instant coffee was given at 6% of the diet, determined to be the maximum tolerated level for rats. The effective numbers of rats were 28–36 for groups of coffee-treated males and 34–39 for groups of coffee-treated females. The effective numbers of rats for the control groups were 31 males and 36 females.

No pair-wise statistical comparisons or trend tests for the effects of coffee on the incidence of specific benign or malignant tumours were performed. In general, rats given caffeinated coffee or decaffeinated coffee + caffeine had fewer tumours than controls; the reduction in the incidence of benign tumours, malignant tumours, or their combination, was significant for three groups of male rats given caffeinated coffee or decaffeinated coffee + caffeine. The only statistically significant difference in female rats was an increase in total malignant tumours in one group given caffeinated coffee; this finding was not seen in a parallel cohort of female rats that were given a comparable level of coffee exposure [interpreted by the Working Group as an isolated and not reproducible finding]. [The Working Group noted that the value of this study is limited by the lack of pair-wise statistical comparisons of tumour incidences at specific sites.]

3.2 Co-carcinogenicity and initiation–promotion studies

Co-carcinogenicity and initiation–promotion studies of coffee were previously reviewed in the *IARC Monographs* (Volume 51; IARC, 1991), where the Working Group reported being aware of various experiments (e.g. Mori & Hirono, 1977; Fujii et al., 1980; Wattenberg & Lam, 1984; Nishikawa et al., 1986) that were part of studies on the modifying effects of coffee on the activity of known carcinogens. These studies were not included in that monograph because their design was considered inadequate for revealing any effect of coffee on tumour production (short duration of exposure and/or limited numbers of animals).

See Table 3.2.

3.2.1 Rat

Mori & Hirono (1977) conducted initiation–promotion studies of coffee by giving four groups of 10 male and 10 female Sprague-Dawley rats either: a solution of brewed Brazilian coffee (2 g/100 mL water) instead of drinking-water for 480 days; a coffee solution for 120 days, a single gavage dose of cycasin at 150 mg/kg bw on day 121 followed by tap drinking-water until day 480; tap water for 120 days, cycasin on day 121, coffee for another 120 days then tap water until day 480;

Table 3.2 Co-carcinogenicity and initiation–promotion studies in experimental animals exposed to coffee

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (M) Age 3 wk 290 days Fujii et al. (1980)	Drinking-water Brewed coffee, Brazilian coffee Tap water Diet containing 0.025% AAF for 8 wk and then fed the basal diet alone, and given concomitantly a solution of coffee instead of drinking-water for 290 days (Group 1); AAF diet and tap water as drinking-water for 8 wk and then fed the basal diet and given coffee solution (Group 2); AAF diet and tap water as drinking-water for the first 8 wk then basal diet and tap water (Group 3); or basal diet and tap water only (Group 4). 10, 10, 10, 10/group 10, 10, 9, 10	Mammary gland: adenocarcinoma Tumour incidence: 2/10, 0/10, 0/9, 0/9	NS	Principal limitations: limited description of experimental details; small number of animals per group
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (F) Age 3 wk 290 days Fujii et al. (1980)	Drinking-water Brewed coffee, Brazilian coffee Tap water Diet containing 0.025% AAF for 8 wk and then fed the basal diet alone, and given concomitantly a solution of coffee instead of drinking-water for 290 days (Group 1); AAF diet and tap water as drinking-water for 8 wk and then fed the basal diet and given coffee solution (Group 2); AAF diet and tap water as drinking-water for the first 8 wk then basal diet and tap water (Group 3); or basal diet and tap water only (Group 4). 10, 10, 10, 10/group 9, 10, 10, 10	Mammary gland: adenocarcinoma Tumour incidence: 4/10, 7/10*, 2/10, 0/9	*P = 0.034 (Fisher exact test, vs Group 3)	Principal limitations: limited description of experimental details; small number of animals per group

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Initiation–promotion (tested as initiator) Rat, Sprague-Dawley (F) Age 34 days 20 wk Wattenberg & Lam (1984)	Feed Green coffee beans Addition of 0, 10, or 20% green coffee beans to the diet for 14 days, 1 day before a single gavage dose of 12 mg DMBA in 1 mL olive oil: experiment 1: 0 or 10% + DMBA; experiment 2: 0 or 20% + DMBA; experiment 3: 0, 10, or 20% + DMBA 16, 16, 16, 16, 32, 16, 16/group 16, 16, 16, 16, 32, 16, 16	Mammary: tumours Tumour incidence: 13/16, 8/16, 16/16, 9/16*, 30/32, 13/16, 9/16* Tumours per rat: 1.9 ± 0.3, 0.9 ± 0.3**, 3.2 ± 0.3, 1.1 ± 0.3**, 2.7 ± 0.2, 1.9 ± 0.3**, 1.2 ± 0.3**	*$P < 0.01$ (decrease) **$P < 0.01$ (decrease)	Principal limitations: contains little experimental details on exact design, clinical observations, body weight gain, or survival In a fourth experiment, 10% green coffee beans tested as promoter significantly decreased the incidence of DMBA-induced mammary tumours
Co-carcinogenicity Rat, Sprague-Dawley (F) Age 4 wk 630 days Nishikawa et al. (1986)	Drinking-water Roasted coffee (Brazil), brewed, 2 g/100 mL Tap water Group 1: diet containing 0.01% aminopyrine and 0.1% sodium nitrite + brewed coffee solution as drinking-water; group 2: diet containing 0.01% aminopyrine and 0.1% sodium nitrite + tap water for drinking-water; group 3: diet containing 0.01% aminopyrine alone + coffee solution as drinking-water; group 4: diet containing 0.01% aminopyrine + tap water for drinking-water; group 5: basal diet + tap water 12, 12, 12, 12, 12/group 9, 9, 7, 8, 10	Liver: tumours Tumour incidence: 2/9* (all adenomas), 7/9 (adenoma, 5/9; carcinoma, 1/9; haemangiosarcoma, 1/9), 0/7, 0/8, 0/10	*$P < 0.03$ (decrease vs group 2; Fisher exact test)	
Initiation–promotion (tested as initiator) Rat, Sprague-Dawley (F) Age 24 days 22.5 wk Welsch et al. (1988)	Drinking-water Brewed coffee, full strength Water (control), full-strength, full-strength decaf., full-strength decaf. + caffeine (860 mg/L), or caffeine (860 mg/L) ad libitum in drinking-water Single i.v. dose of DMBA (2 mg/100 g bw in a lipid emulsion) given at age 53 days; dosing until age 56 days, and held an additional 18 wk 41, 40, 41, 41, 40/group NR	Mammary gland: carcinoma Tumour incidence: 38/41, 31/40, 40/41, 37/41, 36/40 Number of tumours per rat: 6.5, 2.5*, 4.9, 3.3*, 2.7* Total tumours: 266, 99, 199, 137, 109	NS *$P < 0.05$ (decrease)	Principal strengths: well-described and -conducted study Addition of caffeine was also studied

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Initiation–promotion (tested as initiator) Rat, Sprague-Dawley (F) Age 26 days 16.5 wk Welsch et al. (1988)	Drinking-water Brewed coffee, moderate strength Water (control), moderate-strength, or moderate-strength decaf. ad libitum in drinking-water Single i.v. dose of DMBA (2 mg/100 g bw in a lipid emulsion) given at age 55 days; dosing until age 58 days and held an additional 12 wk 40, 41, 41/group NR	Mammary gland: carcinoma Tumour incidence: 37/40, 38/40, 40/41 Number of tumours per rat: 5.5, 3.3*, 6.0 Total tumours: 220, 137, 245	NS *$P < 0.05$ (decrease)	Principal strengths: well-described and -conducted study
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (F) Age 55 days Up to 21 wk Welsch et al. (1988)	Drinking-water Brewed coffee, full strength Water (control), full-strength, or full-strength decaf. Single gavage dose of DMBA (5 mg/rat in sesame oil) given at age 55 days, and then brewed coffee ad libitum as drinking-water starting at age 58 days until termination at 21 wk 82, 80, 81/group NR	Mammary gland: carcinoma Tumour incidence: 39/82, 30/80, 37/81 Number of tumours per rat: 1.0, 0.8, 1.1 Total tumours: 84, 58, 84	NS NS	Principal strengths: well-described and -conducted study
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (F) Age 54 days 18 wk Welsch et al. (1988)	Drinking-water Brewed coffee, moderate strength Water (control), moderate-strength, moderate-strength decaf., or caffeine (430 mg/L) Single gavage dose of DMBA (5 mg/rat in sesame oil) given at age 54 days, and then brewed coffee ad libitum as drinking-water starting at age 57 days until termination at 18 wk 84, 84, 84, 84/group NR	Mammary gland: carcinoma Tumour incidence: 45/84, 46/84, 50/84, 50/84 Number of tumour per rat: 1.5, 1.6, 1.8, 1.8 Total tumours: 127, 129, 147, 149	NS NS	Principal strengths: well-described and -conducted study Addition of caffeine was also studied

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Wistar (M) Age 19 days 15 mo Woutersen et al. (1989)	Drinking-water Brewed coffee Water Low-fat (LF, 5% corn oil) diet, high-fat (HF, 25% corn oil) diet, HF diet + coffee Single i.p. injection of azaserine at 30 mg/kg bw followed or not 6 days later by brewed coffee replacing drinking-water for duration of the study 40, 40, 40/group NR	Pancreas: carcinoma Tumour incidence: Carcinoma in situ: 14/39, 11/37, 10/39 (Micro)carcinoma: 1/39, 8/37, 3/39 Acinar cell carcinoma: 2/39, 7/37, 3/39 Total tumours: 29, 57, 28* Pancreas: adenoma Tumour incidence: 10/39, 6/37, 11/39 Total tumours: 33, 176, 44**	The authors stated that the incidence of carcinomas was slightly lower ($P = 0.076$) in the coffee + HF diet group than in the HF diet group *$P < 0.05$ (decrease vs HF diet group) NS **$P < 0.001$ (decrease vs HF diet group)	Tumour incidence for carcinomas (all) NR
Co-carcinogenicity Rat, Sprague-Dawley (M) Age 24 days 32 wk Gershbein (1994)	Feed Brazilian Arabica green coffee bean oil, pressed/filtered Laboratory chow 0 or 0.10% ad libitum in the feed From day 37, 20 mg/kg bw of 1,2-dimethylhydrazine in buffered water (pH, 7.0) given by gavage 1x/wk for a total of 15 dosages 22, 14/group 15, 5	Colon: adenocarcinoma Tumour incidence: 19/22, 9/14 Total tumours: 132, 43*	[NS] *$P < 0.05$ (decrease)	Principal limitations: study limited by the poor survival

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Co-carcinogenicity Rat, Wistar (M) Newborn 110 days Silva-Oliveira et al. (2010)	Feed Milled roasted coffee (Arabica), lyophilized extract Milled laboratory chow 0, 1.5, 0 + NDEA/AAF, 1.5 + NDEA/AAF (% in diet) Ad libitum, through mothers treated with coffee diet during lactation and then in the feed. At 42 days, chemical hepatocarcinogenesis was induced in 2 groups by means of a single i.p. dose of NDEA (200 mg/kg bw) in saline followed after 17 days by daily gavage doses of AAF (20 mg/kg bw) in propylene glycol for 4 days. A two-thirds partial hepatectomy was then performed on all animals, followed by an additional dose of AAF 2 and 4 days after the hepatectomy. The other two coffee-treated and untreated groups received propylene glycol and saline solution, respectively, rather than NDEA and AAF 10, 10, 10, 10/group NR	Liver: foci and nodules of altered hepatocytes Number persistent lesions/cm^2: NR, NR, 41.52 ± 17.14, 9.14 ± 1.59* Area (mm^2) persistent lesions/section: NR, NR, 1.93 ± 0.51, 0.15 ± 0.08*	 *$P < 0.05$ (decrease) *$P < 0.05$ (decrease)	Milled roasted coffee was extracted using boiled distilled water (6% wt/vol) that was stirred and centrifuged; the supernatant was lyophilized and then stored. Test diets contained 1.5% of the lyophilized coffee extract
Co-carcinogenicity Rat, Wistar (M) Age 6 wk 25 wk Furtado et al. (2014)	Drinking fluid Brewed coffee, 8 g of powder in 140 mL hot water with filtration Water 0, 8 g/140 mL Initial i.p. injection of 200 mg/kg bw NDEA followed 1 wk later by 1×/wk gavage doses of CCl_4 (0.5 mL/kg bw per wk during wk 2–10 followed by 1.0 mL/kg bw per wk during wk 11–24) and either water or brewed coffee (wk 2–25) ad libitum for 5 days/wk 12, 12/group NR	Liver: neoplastic lesions [mainly adenomas] Tumour incidence: 12/12, 11/12 Number of neoplastic lesions/liver area (cm^2): 6.85 ± 1.45, 4.09 ± 0.80	 NS NS	Principal limitations: exposures were for only 5 days/wk; no information on survival An additional group of NDEA/CCl_4-initiated rats received 0.1% caffeine in their drinking-water. The authors reported the mean number of neoplastic lesions per liver area was significantly lower ($P < 0.05$) in the group receiving 0.1% caffeine (1.48 ± 0.36) compared to the group receiving drinking-water

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Co-carcinogenicity Rat, Wistar (M) Age 6 wk 25 wk Furtado et al. (2014)	Drinking fluid Instant coffee, 2% (wt/vol) in hot water Water 0, 2% Initial i.p. injection of 200 mg/kg bw NDEA followed 1 wk later by 1×/wk gavage doses of CCl_4 (0.5 mL/kg bw per wk during wk 2–10 followed by 1.0 mL/kg bw per wk during wk 11–24) and either water or instant coffee (wk 2–25) ad libitum for 5 days/wk 12, 12/group NR	Liver: neoplastic lesions [mainly adenomas] Tumour incidence: 12/12, 11/12 Number of neoplastic lesions/liver area (cm^2): 6.85 ± 1.45, 2.95 ± 0.68*	NS *$P < 0.05$ (decrease);	Principal limitations: exposures were for only 5 days/wk; no information on survival An additional group of NDEA/CCl_4-initiated rats received 0.1% caffeine in their drinking-water. The authors reported the mean number of neoplastic lesions per liver area was significantly lower ($P < 0.05$) in the group receiving 0.1% caffeine (1.48 ± 0.36) compared to the group receiving drinking-water
Co-carcinogenicity Hamster, Syrian golden (F) Age NR 18.5 wk Miller et al. (1988)	Feed Green coffee beans, Colombian Laboratory chow 0 + DMBA, 20 + DMBA, 0, 20 (% in diet) Ad libitum in feed, followed after 2-wk adjustment to diet with painting of right buccal pouch with 0.5% solution of DMBA in heavy mineral oil, 3 × /wk (total of 50 treatments) for 16.5 wk 16, 16, 4, 4/group 12, 9, 4, 4	Buccal pouch: tumours Tumour incidence: 9/12 [mainly carcinomas], 2/9 (carcinomas)*, 0/4, 0/4 Number of tumours per rat: 2.4 ± 0.6, 0.2 ± 0.2**, NR, NR Tumour mass (mm) was 4.5 ± 1.2 for DMBA only treated groups controls vs 0.4 ± 0.3** for coffee + DMBA-treated group	*[$P = 0.03$, Fisher exact test], decrease **$P < 0.01$, decrease	Principal limitations: large number of animals in DMBA+coffee treatment group died before end of study Weight at start, 70 g
Initiation–promotion (tested as promoter) Hamster, Syrian golden (M) Age 6–7 wk 12 mo Woutersen et al. (1989)	Drinking-water Brewed coffee Water LF (5% corn oil) diet, HF (25% corn oil) diet, HF diet + coffee S.c. injection of 20 mg/kg bw BOP at age 6 and 7 wk immediately followed or not by brewed coffee replacing drinking-water for duration of the study 40, 40, 40/group NR	Pancreas: carcinoma Tumour incidence: 17/36, 29/38, 22/34 Total tumours: 23, 37, 30	NS	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested Vehicle Dose(s) No. of animals at start No. surviving animals	Results	Significance	Comments
Initiation–promotion (tested as promoter) Hamster, Syrian golden (M) Age 8–10 wk 14 wk Saroja et al. (2001)	Gavage Black coffee extract (from roasted coffee beans, 8%), store bought Water 0% + DMBA, 8% + DMBA, 0%, 8% Gavage 3×/wk for 14 wk and on alternate days skin application of 0.5% DMBA (0.4 mg) in liquid paraffin on the right buccal pouch, or untreated 10, 10, 10, 10/group NR	Buccal pouch: squamous cell carcinoma Tumour incidence: 10/10, 10/10, 0/10, 0/10 Tumour multiplicity: 9.16, 12.4, 0, 0	[NS] NR	Principal limitations: no statistical analysis provided; no information on survival or body weight

AAF, 2-acetylaminofluorene; BOP, *N*-nitrosobis(2-oxopropyl)amine; bw, body weight; decaf., decaffeinated; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; HF, high-fat; i.p., intraperitoneal; i.v., intravenous; LF, low-fat; M, male; mo, month(s); NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; s.c., subcutaneous; vol, volume; vs, versus; wk, week(s); wt, weight

or tap water for 480 days with cycasin on day 121. A fifth group was given tap water only (controls). The number of rats surviving beyond 200 days was comparable in all groups. At the end of the experiment (480 days), no significant tumour findings were observed. A few single tumours were observed in various organs distributed among the groups. No tumours were observed in the coffee-only group. [The Working Group considered that the study was inadequate for evaluation because of the lack of use of a positive control.]

Fujii et al. (1980) conducted initiation–promotion studies of coffee by giving four groups of 10 male and 10 female Sprague-Dawley rats (age, 3 weeks) one of the following diets the basal diet containing 0.025% 2-acetylaminofluorene (AAF) for 8 weeks from the start of experiment then the basal diet alone, with a solution of brewed Brazilian coffee solution instead of drinking-water for the duration of the experiment (290 days; Group 1); the AAF-containing diet and tap water as drinking-water for the first 8 weeks, and then the basal diet and a coffee solution until termination of the experiment at 290 days (Group 2); the AAF-containing diet for the first 8 weeks and then the basal diet until the end of the experiment, with tap water as drinking-water for the duration (Group 3); or the basal diet and tap water only (Group 4). The number of rats surviving beyond 130 days was comparable in all groups. The incidence of adenocarcinoma of the mammary gland in female rats exposed to AAF followed by coffee (Group 2; 7/10) was significantly higher (P = 0.034, Fisher exact test) compared with that in female rats exposed to AAF only (Group 3; 2/10). The incidence of mammary gland adenocarcinoma was 4/9 in female rats of Group 1. No mammary gland tumours were observed in female rats of Group 4. No significant difference in the incidence of liver tumour was seen between the groups given AAF and coffee solution concurrently (Groups 1 or 2) and the groups given AAF alone (Group 3). [The Working Group noted the limited description of experimental details and the small number of animals per group.]

Wattenberg & Lam (1984) presented data from three experiments (with a similar study design) on the effects on mammary tumour formation in groups of 16–32 female Sprague-Dawley rats (age, 34 days) given green coffee beans at 10% or 20% of diet for 14 days, 1 day before a single gavage dose of 12 mg of 7,12-dimethylbenz[*a*]-anthracene (DMBA) in 1 mL olive oil. The experiments ended 18 weeks after DMBA administration. Limited data were reported on survival or body-weight gain. The consumption of a diet containing green coffee beans resulted in fewer rats with mammary tumours 18 weeks after DMBA administration and fewer tumours per rat. The incidences of mammary tumours for the group given 10% green coffee beans compared with the corresponding DMBA-alone control group was 8/16 (50%) versus 13/16 (81%; not significant) in experiment 1; for the group given 20% green coffee beans compared with the corresponding DMBA-alone control group, incidences of mammary tumours were 9/16 (56%) versus 16/16 (100%; $P < 0.01$, decrease) in experiment 2. In experiment 3, the incidences of mammary tumours were 30/32, 13/16, and 9/16 ($P < 0.01$, decrease) for the DMBA-treated rats given diets containing 0%, 10%, and 20% green coffee beans groups, respectively. In a fourth experiment, a diet with 10% green coffee beans tested as a promoter significantly decreased the incidence of DMBA-induced mammary tumours. [The article contained few experimental details on exact design, body-weight gain, and survival.]

Nishikawa et al. (1986) examined the effect of coffee drinking on hepatocarcinogenesis in rats concurrently administered aminopyrine and sodium nitrite in the diet. Five groups of 12 female Sprague-Dawley rats (age, 4 weeks) were given: a diet containing 0.01% aminopyrine and 0.1% sodium nitrite, and a brewed coffee solution as a drinking fluid (Group 1); a diet containing

0.01% aminopyrine and 0.1% sodium nitrite, and tap water for drinking fluid (Group 2); a diet containing 0.01% aminopyrine alone and the coffee solution as drinking fluid (Group 3); a diet containing 0.01% aminopyrine and tap water for drinking fluid (Group 4); or a basal diet and tap water (Group 5). The study was ended after 630 days. A total of 43 rats survived more than 600 days (17 rats died of pneumonia earlier). The number of rats that survived more than 600 days was considered the effective number of rats. The incidence of liver tumours in the group of rats given coffee in combination with aminopyrine and sodium nitrite (Group 1: 2/9, 22%, both adenomas) was significantly lower than that of the animals receiving aminopyrine and sodium nitrite only (Group 2: 7/9, 78%: 5/9, adenoma; 1/9, carcinoma; and 1/9, haemangiosarcoma) ($P < 0.03$, decrease; Fisher exact test).

Welsch et al. (1988) treated different groups of female Sprague-Dawley rats with regular or decaffeinated coffee in both initiation and promotion phases of DMBA-induced mammary gland tumourigenesis. Groups exposed to caffeine or decaffeinated coffee with added caffeine were also included.

In the initiation studies, groups of 40–41 female rats (age, 24–26 days) were given plain drinking-water (control) or full- or moderate-strength brewed regular or decaffeinated coffee, prepared by using 4.25 or 2.125 cups of coffee and 45 cups of water in a 55-cup coffee maker, ad libitum. There were also two additional groups that received caffeine at 860 mg/L in either the full-strength decaffeinated coffee or their drinking-water. A single intravenous dose of DMBA (2 mg/100 g bw in a lipid emulsion) was given at age 53–55 days. The coffee dosing was stopped at age 56–58 days and the rats were then held for an additional 12–18 weeks. There was no effect on body weight in any of these treated groups. The consumption of full-strength and moderate-strength caffeinated coffee reduced the number of mammary carcinomas per rat by 62% and 40% ($P < 0.05$) compared with control groups, respectively. Full- or moderate-strength decaffeinated coffee did not significantly affect the number of mammary carcinomas per rat. Caffeine alone and addition of caffeine to the full-strength decaffeinated coffee also sharply reduced the number of mammary carcinomas per rat by 58% and 49% ($P < 0.05$), respectively. Coffee and/or caffeine consumption did not significantly affect the percentage of rats with mammary carcinomas or the mean latency period of mammary tumour appearance (Welsch et al., 1988). [These studies were well described and appeared to have been well conducted.] Welsch & DeHoog (1988) conducted the same initiation studies with brewed regular or decaffeinated coffee but used a chemically defined diet containing standard (5%) or high (20%) levels of fat (corn oil) during coffee exposures and observed essentially the same results. [These studies were well described and appeared to have been well conducted.]

In the promotion studies, groups of 80–84 female rats received a single gavage dose of DMBA (5 mg/rat in sesame oil) given at age 54–55 days. At age 57–58 days, rats were given plain drinking-water (control) or full- or moderate-strength brewed regular or decaffeinated coffee, prepared by using 4.25 or 2.125 cups of coffee and 45 cups of water in a 55-cup coffee maker, ad libitum for 18–21 weeks. There was an additional group that received 430 mg/L caffeine in their drinking-water. There was no effect on body weight in any of these treated groups. The consumption of full-strength or moderate-strength caffeinated or decaffeinated coffee did not significantly affect the number of mammary carcinomas per rat. Neither coffee nor caffeine consumption significantly affected the percentage of rats with mammary carcinomas or the mean latency period of mammary tumour appearance (Welsch et al., 1988). [These studies were well described and appeared to have been well conducted.] Welsch & DeHoog (1988)

conducted the same promotion studies with brewed regular or decaffeinated coffee but used a chemically defined diet containing standard (5%) or high (20%) levels of fat (corn oil) during coffee exposures, and observed essentially the same results. [These studies were well described and appeared to have been well conducted.]

In a well-conducted study to investigate the effect of chronic coffee ingestion on pancreatic carcinogenesis promoted by dietary fat (Woutersen et al. 1989), three groups of 40 male Wistar rats (age, 19 days) were given a single intraperitoneal injection of 30 mg azaserine/kg bw in saline followed, or not, by replacement of drinking-water with brewed coffee 6 days later. The coffee was freshly prepared each day of the study by brewing 500 g of ground coffee in 10 L of distilled water. The rats were given either a low-fat (LF) control diet (5% corn oil), a high-fat (HF) diet (25% corn oil), or the HF diet plus coffee (HF+C). Mean body weight of the HF+C group was significantly lower than that of the other two groups ($P < 0.01$) from day 119 onwards. At 15 months, the numbers of pancreatic adenomas and pancreatic carcinomas reported were significantly lower in the HF+C group than in the HF group ($P < 0.001$, decrease and $P < 0.05$, decrease, respectively). [The Working Group noted that the lower body weight in the coffee-treated animals may have contributed to the reduction in pancreatic tumours observed in the treated animals.]

A group of 22 (control) or 14 (treated) male Sprague-Dawley male rats (age, 24 days) were given ad libitum feed containing 0 or 0.10% Brazilian Arabica green coffee bean oil for 32 weeks (Gershbein, 1994). From day 37 of the study, 1,2-dimethylhydrazine was given by weekly gavage at a dose of 20 mg/kg bw to both groups for a total of 15 weeks. Survival in the coffee-treated group was significantly less than that of controls (36% vs 68%). Average body weight was comparable in both groups. There was a significant decrease in the number of adenocarcinomas of the colon observed in the coffee-treated group ($P < 0.05$, decrease) compared with controls (43 vs 132). [The study was limited by the poor survival of the coffee-treated group compared with the controls.]

In a well-conducted study, Silva-Oliveira et al. (2010) investigated the effect of daily coffee ingestion on hepatocarcinogenesis in rats submitted to the resistant hepatocyte (RH) model. Four groups of 10 male newborn Wistar rats were treated with or without milled roasted coffee (*Coffea arabica*) that was extracted by stirring with boiling distilled water (6% wt/vol), centrifuging, and the supernatant lyophilized and then stored. Test diets were prepared with a concentration of 1.5% lyophilized coffee extract. At day 42 of the study, the RH model of chemical hepatocarcinogenesis was induced in one untreated group and one coffee-treated group by means of a single intraperitoneal dose of *N*-nitrosodiethylamine (NDEA, 200 mg/kg bw) in saline, followed 17 days later by daily gavage doses of AAF (20 mg/kg bw) in propylene glycol for 4 days. A two-thirds partial hepatectomy (PH) was then performed on all RH-induced coffee-treated and untreated rats, followed by an additional dose of AAF 2 and 4 days later. The other two coffee-treated and untreated groups received propylene glycol and saline solution, respectively, rather than NDEA and AAF. Coffee consumption and the induction of hepatocarcinogenesis had no effect on body-weight gain, final body weight, liver weight at PH, or on liver regeneration which varied from 108% to 126% in the groups (without statistical differences). The experiment was terminated at 110 days. In the RH model, the rats given the coffee diet had a 78.0% reduction in the total number of preneoplastic lesions, 85.5% in the number of persistent lesions, 70.5% in the number of remodelling lesions, and 92.2% and 92.0% in the total and relative areas occupied by persistent lesions, respectively. [The Working Group felt it appropriate to include this study in the evaluation because it is generally accepted that the foci and

nodules of altered hepatocytes observed in this study are the result of clonal expansion of the initiated hepatocytes and precede the appearance of malignant tumours, acting as potential precursors for subsequent steps in the carcinogenic process.]

Furtado et al. (2014) gave three groups of 12 Wistar male rats (age, 6 weeks) an initial intraperitoneal injection of NDEA at 200 mg/kg bw followed 1 week later by gavage doses of carbon tetrachloride (CCl_4) once per week (0.5 mL/kg bw per week during weeks 2–10 followed by 1.0 mL/kg bw per week during weeks 11–24) and either plain water (control), 2% (wt/vol) instant coffee, or brewed coffee (8 g/140 mL) ad libitum in their drinking-water for 5 days/week for 24 weeks (weeks 2–25). The ingestion of the coffee beverages had no effect on body weight or relative liver weights. At 25 weeks, the incidence of liver neoplastic lesions [mainly hepatocellular adenomas] in both coffee-treated groups was 11/12 (93%) compared with 12/12 (100%) in the control group. The mean number of neoplastic lesions per liver area (per cm^2) was significantly lower (2.95 ± 0.68, $P < 0.05$) in the group receiving the instant coffee in their drinking-water compared with the group receiving plain drinking-water (6.85 ± 1.45). The mean number of neoplastic lesions per liver area (per cm^2) for the brewed coffee group was also lower (4.09 ± 0.80) than controls, but not significantly. The authors reported on an additional group of NDEA/CCl_4-initiated rats that had received 0.1% caffeine in their drinking-water, which also had a significantly lower mean number of neoplastic lesions per liver area (1.48 ± 0.36, $P < 0.05$) compared with the group receiving drinking-water. [The Working Group noted the lack of survival data.]

3.2.2 Hamster

Miller et al. (1988) gave two groups of 16 female Syrian hamsters [age not provided; weight, 70 g] powdered green coffee beans in their feed at 0% or 20% ad libitum. After a 2-week adjustment period to the diet, the right buccal pouch of each group was painted with a 0.5% solution of DMBA in heavy mineral oil three times per week for the remaining 16.5 weeks of the study (a total of 50 treatments). Two other groups of four hamsters were given either the 0% or 20% green coffee diet and were treated three times per week with heavy mineral oil (a total of 50 treatments). Weight gain for the hamsters given coffee + DMBA was less than that of the hamsters given DMBA only throughout the study. There was a significant decrease in survival in all DMBA-treated groups, mostly due to respiratory infections. At 18.5 weeks, the incidence of buccal pouch tumours in the group given coffee + DMBA was 2/9 (22%) (carcinomas) [$P = 0.03$, decrease; Fisher exact test] compared with 9/12 (75%) [mainly carcinomas] in the DMBA-only group. The average number of tumours was 0.2 ± 0.2 versus 2.4 ± 0.6 and the calculated value for tumour mass (number of tumours times the average diameter of the tumours in millimetres) was 0.4 ± 0.3 versus 4.5 ± 1.2 for groups given coffee + DMBA and DMBA only, respectively. Since tumour mass takes into account tumour number and size, the differences in these values are significant ($P < 0.01$). No tumours were seen in the buccal pouches of hamsters given the 0% or 20% green coffee diet and not treated with DMBA. [The Working Group noted the poor survival of hamsters treated with coffee + DMBA.]

In a well-conducted study to investigate the effect of chronic coffee ingestion on dietary fat-promoted pancreatic carcinogenesis, Woutersen et al. (1989) treated three groups of 34–38 male Syrian hamsters (age, 6–7 weeks) with *N*-nitrosobis(2-oxopropyl) amine (BOP) at a dose of 20 mg /kg bw in saline by subcutaneous injection at age 6 and 7 weeks. The hamsters were fed a low-fat (LF) control diet (5% corn oil), a high-fat (HF) diet (25% corn oil), or a HF diet plus coffee (HF+C). For the latter group, drinking-water was replaced by brewed coffee after BOP injection.

The coffee was prepared fresh each day of the study by brewing 500 g of ground coffee in 10 L of distilled water. Body-weight gain of the hamsters maintained on the HF+C diet was comparable to the LF controls, while the mean body weight of the HF group was significantly higher than that of the LF controls. At 12 months, there was no significant difference in the incidence or total number of pancreatic carcinomas between the HF+C group and the HF group.

Saroja et al. (2001) gave two groups of 10 male Syrian hamsters (age, 8–10 weeks) black coffee extract (from roasted coffee beans obtained from a local Indian market) at 0% (untreated) or 8% by gavage three times per week for 14 weeks. On alternate days these groups were given 0.5% DMBA (0.4 mg) in liquid paraffin painted on the right buccal pouch. Two other groups of 10 hamsters were either untreated or given 8% black coffee extract. No information was provided for survival or weight gain or loss in any groups. The incidence of buccal pouch tumours in the DMBA-treated groups and the groups given coffee plus DMBA was 100% (10/10). The mean number of tumours per animal was 12.4 versus 9.16, the mean tumour volume was 300 mm^3 versus 240 mm^3, and the calculated value for mean tumour burden (calculated by multiplying mean tumour volume by mean number of tumours) was 3720 mm^3 versus 2198 mm^3 for coffee+DMBA-treated animals and for DMBA-treated animals, respectively. No tumours were seen in hamsters not given DMBA or in those given only coffee. [The Working Group noted the lack of information on survival or body weight. This study was not suitable for evaluation because no error terms were provided for the mean number of tumours and no statistical analysis was reported.]

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