3. CANCER IN EXPERIMENTAL ANIMALS

No long-term bioassays of full carcinogenicity with red meat or processed meat were available to the Working Group; however, the Working Group considered a variety of animal bioassays.

3.1 Mouse

See Table 3.1

3.1.1 Red meat

Groups of seven to nine male C57Bl/6J-*Apc*^{Min} mice (age, 6–8 weeks), a strain primarily susceptible to spontaneous adenomas of the small intestine, were fed American Institute of Nutrition (AIN-93G)–based diets, either a semisynthetic control diet or a modified diet in which casein, the protein source, was replaced with beef (24%), for 5–6 weeks. The beef was minced and freeze-dried before being added to the diet. [The authors did not specify whether the meat had been cooked before being minced and freeze-dried.] The control diet contained calcium at a concentration of 5.1 g/kg diet, and fat was obtained from sunflower and rapeseed oil. In the modified diet, fat was provided by beef, butter, and sunflower and rapeseed oil. The energy content was similar for both diets. The extent of intestinal neoplasms was determined by light microscopy. Statistical analyses were conducted. Mean body weight (bw) was similar for mice given the control or modified diet. Tumours were observed in the small intestine and colon/caecum. Mice fed the modified diet containing beef had a greater number of tumours in the small intestine compared with mice fed the control diet, with the difference being significant in the distal small intestine (P = 0.009) (Mutanen et al., 2000). [The Working Group noted that the tumour data were confounded by the fact that the beef diet contained considerably more fat (274.8 g/kg diet) than the control diet (70.0 g/kg diet). The control diet contained calcium at a concentration of 5.1 g/kg diet, and did not contain fibre; however, there was no increase in the incidence of tumours of the small intestine in a separate non-fibre, high-fat group.]

Groups of six to eight male and six to eight female C57Bl/6J-*Apc*^{Min} mice (age, 5 weeks) were transferred from a standard rodent chow diet and fed AIN-93G–based diets, either a semisynthetic control diet or a modified diet in which casein was replaced with beef. The control diet contained 40% fat and a fatty acid profile similar to that of a "Western-type" (i.e. enriched in fat and cholesterol) diet. The carbohydrate and protein sources were provided by dextrose and casein, respectively. The beef diet contained freeze-dried, low-fat ground beef instead of casein as the protein source. The other ingredients were adjusted to keep the proportions of energy from carbohydrate, protein, and fat similar to those in the control diet. [The authors did not specify whether the low-fat ground beef had been cooked before being freeze-dried.] The number and size of intestinal adenomas, as determined by light

Table 3.1 Studies of carcinogenicity in mice fed diets containing red meat or processed meat

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, C57Bl/6J-*Apc*^{Min} (M) Age 6–8 wk 5–6 wk Mutanen et al. (2000)	Full carcinogenicity study AIN-93G–based control diet or modified AIN-93G diet in which 24% beef replaced casein as the protein source, fed ad libitum 7–9 mice/group 7, 7	*Small intestine* Total tumours, mean (SD): 35.3 (11.6), 52.8 (13.2) *Colon and caecum* Tumour incidence: 88%, 89% Total tumours, mean (SD): 1.8 (0.9), 3.2 (2.3) *Distal small intestine* Total tumours, mean (SD): 19.6 (6.8), 36.6 (9.4)*	NS NS NS *P = 0.009 (ANOVA with Tukey post hoc test)	Limitations: tumour data were confounded by the fact that the beef diet contained considerably more fat (274.8 g/kg diet) than the control diet (70.0 g/kg diet); histopathological examination not conducted
Mouse, C57Bl/6J-*Apc*^{Min} (F) Age 5 wk 15 wk Kettunen et al. (2003)	Full carcinogenicity study Mice were fed a control diet (AIN-93G diet with 40% fat) or diet containing low-fat ground beef (AIN-93G with 40% fat, 27% beef), fed ad libitum 6–8 mice/group 6, 6	*Small intestine* Adenoma multiplicity, mean (SEM): 72.3 (15.27), 30.9 (4.90)* Total adenomas, mean (SEM): 55.8 (8.46), 28.7 (3.77)* *Small intestine* Medium adenoma, incidence, mean (SEM): 30.1% (3.8), 22.6% (3.3)*	Decrease; *P < 0.01 (multiple linear regression) Decrease; *P < 0.01 (multiple linear regression) Decrease; *P < 0.05 (both sexes combined, multiple linear regression)	Diets were balanced for carbohydrates, protein, and fat
Mouse, C57Bl/6J-*Apc*^{Min} (M) Age 5 wk 15 wk Kettunen et al. (2003)	Full carcinogenicity study Control diet or diet containing beef (g/kg diet), fed ad libitum 6–8 mice/group 8, 8	*Small intestine* Medium adenoma incidence, mean (SEM): 44.3% (2.9), 36.3% (4.6)*	Decrease; *P < 0.05 (both sexes combined, multiple linear regression)	Mice were fed a modified AIN-93G containing 40% fat or low-fat ground beef instead of casein as the protein source. Diets were balanced for carbohydrates, protein, and fat

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, BALB/c (M) Age 5 wk 54 wk Nutter et al. (1983)	Initiation–promotion study Diet containing low milk protein with low fat or low beef protein with low fat, fed ad libitum 100 mice/group 38, 23	*Colon* Tumour incidence: 23/38 (60.5%), 4/23 (17.4%)*	Decrease; *$P \leq 0.05$ (test for the equality of two proportions)	Limitations: tumour incidence in the control groups was not reported; the duration of this experiment (54 wk) was probably not sufficient to determine if the diets by themselves were tumorigenic; histopathological examination not conducted Mice were allocated to six isocaloric diet groups that differed in protein source (milk or beef), protein level (11% or 33%), and fat level (5% or 30%), and injected with DMH (11 weekly injections of 20 mg/kg bw)
	Diet containing low milk protein with high fat or low beef protein with high fat, fed ad libitum 100 mice/group 24, 28	*Colon* Tumour incidence: 19/24 (79.2%), 6/28 (21.4%)*	Decrease; *$P \leq 0.05$ (test for the equality of two proportions)	
	Diet containing high milk protein with low fat or high beef protein with low fat, fed ad libitum 100 mice/group 29, 44	*Colon* Tumour incidence: 19/29 (65.5%), 5/44 (11.4%)*	Decrease; *$P \leq 0.05$ (test for the equality of two proportions)	

*, statistically significant; AIN, American Institute of Nutrition; ANOVA, analysis of variance; DMH, dimethylhydrazine; F, female; M, male; NR, not reported; NS, not significant; SD, standard deviation; SEM, standard error of the mean; wk, week

microscopy, were assessed when the mice were placed on the control and beef diets, and then assessed after they were fed the test diets for 3 weeks or 10 weeks. Statistical analyses were conducted. Male mice fed the control diet gained less weight than male mice fed the beef diet. This difference in body weight was not observed in the female mice. Female mice fed the beef diet had significantly fewer intestinal adenomas ($P < 0.01$) and a significantly lower tumour burden (measured as mm^2; $P < 0.01$) when assessed after 15 weeks of feeding. Mice fed the beef diet also had significantly fewer medium-sized (1.0–1.5 mm) adenomas than did mice fed the control diet when both sexes were combined ($P < 0.05$) (Kettunen et al., 2003).

3.1.2 Red meat with known carcinogens

Groups of 100 male BALB/c mice (age, 5 weeks) were allocated to one of six isocaloric diet groups that differed in protein source (milk or beef), protein level (11% or 33%), and fat level (5% or 30%). [The authors did not state whether the meat had been cooked and/or freeze-dried before being added to the diet. The calcium content of the diet could not be determined.] At age 11 weeks, an unspecified number of mice from each group were given 11 weekly subcutaneous injections of 1,2-dimethylhydrazine (DMH) at a dose of 20 mg/kg bw. [The specific diets may have affected metabolism of the DMH.] The remaining mice in each group were injected with saline to serve as "non–tumour-bearing" control mice. At age 37 weeks and 59 weeks, the subgroups of mice were killed and examined grossly for tumours of the colon. Selected tumours were examined by histopathology, and statistical analyses were conducted. Mice fed diets containing beef protein consumed approximately 20–25% more calories per day than mice fed the diets containing milk protein. When assessed at age 59 weeks, mice fed the diets containing 11% or 33% beef protein with 30% fat weighed significantly more than mice fed the corresponding diets containing milk protein and fat. When assessed at 59 weeks of age, DMH-injected mice fed the beef protein diets had a significantly lower incidence of colon tumours than DMH-injected mice fed the milk protein diets, irrespective of the percentage of protein or fat ($P < 0.05$) (Nutter et al., 1983). [Tumour incidence in the control groups was not reported. The duration of this experiment (54 weeks) was probably not sufficient to determine if the diets by themselves were tumorigenic.]

3.2 Rat

See Table 3.2

3.2.1 Red meat

A study was conducted to investigate the effects of a "complete human diet" prepared under normal household conditions. Male and female Wistar rats (age, 4 weeks) were placed on one of five diets (50 males and 50 females per diet): Diet A, a commercial semisynthetic rodent diet; Diet B, a semisynthetic rodent diet supplemented with fruits and vegetables; Diet C, a complete "human" diet consisting of meat (beef, pork, and chicken), bread, eggs, and margarine, along with other semisynthetic products, including lard, potato flour, sugar, bran, and pectin; Diet D, a diet similar to Diet C, except the food was cooked under "usual household conditions"; and Diet E, a diet similar to Diet D, except supplemented with fruits and vegetables. [The authors did not specify if any of the meats, fruits, or vegetables had been freeze-dried before being added to the diets.] Diets A and B contained 21.6% fat "energy," 26.0% protein "energy," 52.4% carbohydrate "energy," and 10.7% fibre. Diets C, D, and E contained 40.6% fat "energy," 13.2% protein "energy," 46.2% carbohydrate "energy," and 5% fibre. The diets contained calcium at a concentration of 7.5 g/kg diet. The rats were maintained on their respective diets for up to 995 days for males

Table 3.2 Studies of carcinogenicity in rats fed diets containing red meat or processed meat

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Wistar (M) Age 4 wk 142 wk Alink et al. (1989)	Full carcinogenicity study Rats were fed one of five diets, A–E, ad libitum: diet A, a semi-synthetic rodent diet; diet B, diet A supplemented with fruits and vegetables; diet C, a "humanized" diet consisting of meat (including beef), bread, eggs, and margarine, alone with other semi-synthetic products; diet D, cooked diet C; diet E, diet D, supplemented with fruits and vegetables 50 rats/group 48, 46, 48, 48, 48	*Pituitary gland (pars distalis)* Tumour incidence: 26/45 (58%), 28/45 (62%), 33/48 (69%)*, 35/46 (76%)*, 35/48 (73%)* *Thyroid gland* "Light cell" adenoma or carcinoma: Incidence: 3/48 (6%), 1/46 (2%), 3/48 (5%)*, 6/48 (13%)*, 5/46 (11%)*	*P = 0.0016 (human diets, diets C, D, and E, vs rodent diets, diets A and B; two-sided Fisher exact test and IARC method) *P = 0.0014 (human diets, diets C, D, and E, vs rodent diets, diets A and B; two-sided Fisher exact test and IARC method)	Limitations: tumour data were confounded by the fact that the human diets had approximately twofold more fat and 50% less fibre than the rodent diets; rats fed the human diets weighed considerably more than rats fed the rodent diets An equal number of female Wistar rats were also treated; there were no significant differences in tumour incidence
Rat, F344 (F) Age 7 wk 30–34 wk Reddy et al. (1976)	Initiation–promotion study Rats were fed one of four diets: D_1, high soybean protein with high corn oil fat; D_2, low soybean protein with low corn oil fat; D_3, high beef protein with high beef and corn oil fat; D_4, low beef protein with low beef fat; half of the rats in each group (28 rats/group) were initiated with DMH: D_1 with DMH; D_2 with DMH; D_3 with DMH; or D_4 with DMH 28 rats/group 28, 28, 28, 28, 28, 28, 28, 28	*Ear canal* Squamous cell carcinoma: Incidence: 0/28 (0%), 0/28 (0%), 0/28 (0%), 0/28 (0%), 7/28 (25%), 6/28 (21%), 8/28 (29%), 7/28 (25%) *Colon* Tumour incidence: 0/28 (0%), 0/28 (0%), 0/28 (0%), 0/28 (0%), 15/28 (54%), 10/28 (36%), 16/28 (57%), 10/28 (36%) Tumour multiplicity (SEM): 0, 0, 0, 0, 0.90 (0.12)*, 0.44 (0.11), 1.00 (0.19), 0.50 (0.14) Adenocarcinoma: Multiplicity: 0, 0, 0, 0, 0.58 (0.13), 0.23 (0.10), 0.61 (0.14)*, 0.14 (0.06)	NS NS *P < 0.05 (significantly different from D_2) *P < 0.05 (Significantly different from D_4)	DMH-initiated rats had a low incidence (≤ 18%) of kidney mesenchymal tumours and adenocarcinoma of the small intestine. DMH (10 mg/kg bw for 20 wk). [Rats treated with DMH had a significantly increased incidence of ear canal and colon tumours compared with control rats, $P \leq 0.02$, two-tailed Fisher exact test] The duration of this experiment (30–34 wk) was probably not sufficient to determine if the diets by themselves were tumorigenic

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M) Age, weanling 32 wk Clinton et al. (1979)	Initiation–promotion study Rats were fed one of three diets: 20% soy protein with 20% beef tallow, 20% raw beef protein with 20% beef tallow, or 20% charcoal-broiled beef protein with 20% beef tallow, fed ad libitum After 4 wk, all the rats were initiated with DMH (1.25 mg/kg bw for 18 wk) 30 rats/group 29, 30, 28	*Small intestine* Tumour incidence: 9/28 (32%), 12/30 (40%), 8/29 (28%) Tumour multiplicity: 1.1, 1.3, 1.1 *Colon* Tumour incidence: 11/28 (39%), 13/30 (43%), 12/29 (41%) Tumour multiplicity: 1.3, 1.4, 1.4	$P = 0.17$ (number of tumours per tumour-bearing rat, Pearson χ^2) $P = 0.96$ (distribution of colon tumour frequency, Neyman χ^2) Tumour multiplicity was reported as No. of tumours per tumour-bearing rat	Limitations: histopathological examination not conducted
Rat, Wistar (M) Age 4 wk 8 mo Alink et al. (1993)	Initiation–promotion study Rats were fed one of five diets, A–E: diet A, a semisynthetic rodent diet; diet B, diet A supplemented with fruits and vegetables; diet C, a "humanized" diet consisting of meat (beef, pork, and chicken), bread, eggs, and margarine, along with other semisynthetic products; diet D, diet C, that had been cooked; or diet E, diet D, supplemented with fruits and vegetables; fed ad libitum 45 rats/group	*Colon* Adenoma, incidence: 27/43 (63%), 14/36 (39%), 20/42 (48%), 20/43 (47%), 23/43 (53%) Total adenomas: 68, 19, 31, 45, 42 Adenocarcinoma, incidence: 31/43 (72%), 22/36 (61%), 28/42 (67%)*, 34/43 (79%)*, 35/43 (81%)* Total adenocarcinomas: 67, 42, 70, 72, 100	Combined groups C,D, and E significantly higher (*$P < 0.05$; Fisher's exact test) than combined groups A and B Zymbal's gland tumours were also observed, with the incidence being significantly ($P < 0.05$; Fisher's exact test) greater in the combined C, D, and E diet groups compared to the combined A and B groups; specific incidences, NR	All rats were initiated with DMH (10 weekly injections of 50 mg/kg bw)

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, presumably Sprague-Dawley (NR) Age NR NR McIntosh (1993)	Initiation–promotion study Diet containing red meat protein, whey protein, casein protein, soy protein, or fish protein; fed ad libitum 10 rats/group 10, 10, 10, 10, 10	*Intestine* Tumour incidence: 60%, 20%, 40%, 50%, 40% Tumour multiplicity: 1.1, 0.2*, 0.4, 0.9, 0.8	Incidence of intestinal tumours, NS (may be a consequence of the small number of animals per group) Decrease; *$P < 0.05$ (No. of intestinal tumours per rat; whey protein diet vs red meat protein, soy protein, or fish protein diet; statistical test not specified)	Limitations: histopathological examination not conducted Rats were fed one of five diets: 20% protein derived from red meat [type not specified], 20% protein derived from whey, 20% protein derived from casein, 20% protein derived from soy, or 20% protein derived from fish All the rats were initiated with DMH (3 weekly injections of 20 mg/kg bw)
Rat, Sprague-Dawley (M) Age 5 wk 6 mo McIntosh et al. (1995)	Initiation–promotion study Diet containing casein (20.0 g/100 g diet), whey protein concentrate (21.3 g/100 g diet), kangaroo skeletal muscle (22.8 g/100 g diet), or defatted soybean meal (33.3 g/100 g diet), fed ad libitum 20 rats/group Survival: NR	*Large intestine* Total tumours: 6*, 5*, 10, 21 *Intestine* Tumour incidence: 45%, 30%, 50%, 60% Total tumours: 12*, 7*, 21, 26	Decrease; *$P < 0.02$ (tumours per group, casein and whey protein diets vs kangaroo meat and defatted soybean meal diets, regression analysis using Poisson distribution) $P = 0.15$ (No. of surviving rats, NR, all presumed to have survived; χ^2) Decrease; *$P < 0.005$ (tumours per group, casein and whey protein diets vs kangaroo meat and defatted soybean meal diets, regression analysis using Poisson distribution)	Limitations: histopathological examination not conducted All rats were initiated with DMH (3 weekly injections of 15 mg/kg bw)

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M) Age, weanling 27 wk Pence et al. (1995)	Initiation–promotion study Diet containing casein protein with 5% corn oil, casein protein with 20% corn oil, casein protein with 5% beef tallow, casein protein with 20% beef tallow, beef protein with 5% corn oil, beef protein with 20% corn oil, beef protein with 5% beef tallow, or beef protein with 20% beef tallow; fed ad libitum 25 rats/groups Survival: NR	*Small intestine* Adenoma incidence: 4%, 0%, 4%, 0%, 0%, 0%, 0%, 7% Adenocarcinoma, incidence: 48%, 28%, 40%, 24%, 32%, 28%, 48%, NR Adenoma or adenocarcinoma, incidence: 32%, 48%, 28%, 40%, 24%, 32%, 28%, 52% *Colon* Adenoma, incidence: 24%, 20%, 24%, 36%, 16%, 40%, 12%, 29% Adenocarcinoma, incidence: 60%*, 48%*, 32%*, 40%*, 20%, 16%, 28%, 19% Adenoma or adenocarcinoma, incidence: 64%, 52%, 52%, 64%, 28%*, 52%, 36%, 42% *Colon and small intestine* Adenoma or adenocarcinoma, incidence: 72%, 72%, 56%, 80%, 48%, 64%, 44%, 67%	*$P < 0.05$ (casein protein diets vs beef, except 5% beef tallow, diets, irrespective of fat source; $\chi 2$) Decrease; *$P < 0.05$ (beef protein with 5% corn oil diet vs 5% casein protein with 5% corn oil diet, $\chi 2$)	Rats were fed AIN-76A–based test diets using a 2 × 2 × 2 factorial design, with the factors being the protein source (casein or lean beef), fat source (corn oil or beef tallow), and fat level (5% or 20%) Rats were initiated with DMH (10 weekly injections of 20 mg/kg bw) Ten rats per diet group served as vehicle controls; tumour incidence in the controls was NR The duration of this experiment (27 wk) was probably not sufficient to determine if the diets by themselves were tumorigenic

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M) Age, weanling 27 wk Lai et al. (1997)	Initiation–promotion study Rats were fed an AIN-76A–based diet containing casein (protein source) with corn oil or lean ground beef (protein source) with corn oil, fed ad libitum 30 rats/group 28, 28	*Small intestine*		Rats were initiated with DMH (10 weekly injections of 20 mg/kg bw) Five rats per diet group served as vehicle controls; tumour incidence in the controls was NR The duration of this experiment (27 wk) was probably not sufficient to determine if the diets by themselves were tumorigenic
		Adenocarcinoma: Incidence: 15/28 (52%), 18/28 (62%)	NS (χ^2)	
		Multiplicity: 0.66, 0.90	NS (No. of tumours per rat, Student *t* test)	
		Colon Adenocarcinoma:		
		Incidence: 18/28 (62%), 15/28 (52%)	NS (appears that two rats from each group were removed early and not included in the final tumour assessment, χ^2)	
		Multiplicity: 0.86, 0.79	NS (No. of tumours per rat, Student *t* test)	
		Colon or small intestine		
		Adenocarcinoma: Incidence: 23/28 (79%), 24/28 (83%)	NS (χ^2)	
		Multiplicity: 1.52, 1.69	NS (No. of tumours per rat, Student *t* test)	

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M) Age, weanling 27 wk Pence et al. (1998)	Initiation–promotion study Diet containing low fat with low HAAs, then low fat; high fat with high HAAs, then high fat with high HAAs; low fat with low HAAs, then low fat with high HAAs; high fat with low HAAs, then high fat; low fat with high HAAs, then low fat with high HAAs; low fat with high HAAs, then low fat; high fat with high HAAs, then low fat; or high fat with high HAAs, then high fat; fed ad libitum 25 rats/group Survival, NR	*Stomach* Tumour incidence: 4%, 8%, 0%, 4%, 16%, 8%, 8%, 12% Tumour multiplicity: 0.04, 0.08, 0.00, 0.04, 0.16, 0.08, 0.08, 0.12 *Small intestine* Tumour incidence: 20%, 28%, 48%, 46%, 32%, 24%, 16%, 36% Tumour multiplicity: 0.36, 0.28, 0.56, 0.58, 0.56, 0.24, 0.24, 0.40 *Colon* Adenoma or adenocarcinoma: Incidence: 76%, 56%*, 60%, 83%, 88%, 84%, 56%*, 56%* Multiplicity: 1.20, 0.68, 0.96, 1.13, 1.40, 1.04, 0.76, 0.68	NS NS Decrease; *$P < 0.05$ (high-fat with high-HAA diets, incidence, 56%, vs low-fat with high-HAA diets, incidence, 84–88%] during wk 1–12, $\chi 2$)	Rats were fed one of four AIN-76A–based diets: low-fat (5%) with low-HAA (6.6 ng) beef, high-fat (20%) with low-HAA beef, low-fat with high-HAA (85.6 ng) beef, or high-fat with high-HAA beef Rats were initiated with DMH (10 weekly injections of 20 mg/kg bw). Ten rats on the high-fat, high-HAA diet did not receive DMH; these rats did not develop tumours The duration of this experiment (27 wk) was probably not sufficient to determine if the high-fat, high-HAA diet by itself was tumorigenic

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Parnaud et al. (1998)	Initiation–promotion study Diet containing low casein with lard, low casein with olive oil, low beef, low chicken, low bacon, high casein with lard, high casein with olive oil, high beef, high chicken, or high bacon; fed ad libitum 10 rats/group 10, 10, 10, 10, 10, 10, 10, 9, 10, 10	*Colon* No. of crypts per ACF, mean (SD): 3.21 (0.47), 3.11 (0.28), 3.25 (0.44), 3.16 (0.34), 2.84 (0.45), 3.27 (0.38), 2.94 (0.30), 3.15 (0.59), 3.18 (0.32), 2.62 (0.60)* No. of ACF per rat, mean (SD): 65 (34), 83 (30), 69 (23), 76 (37), 86 (47), 75 (44), 61 (43), 71 (25), 98 (30), 72 (37)	The high bacon diet was lower than high casein & lard diet (*$P < 0.001$; ANOVA and Dunnett's test)	Rats were fed low-meat or high-meat diets; fat and protein were provided by beef, chicken, bacon, olive oil, or lard; there were two control diets, where fat was provided by lard or olive oil, and protein provided by casein The rats received a single injection of azoxymethane (20 mg/kg bw)
Rat, F344 (F) Age 5 wk 45 days Parnaud et al. (2000)	Initiation–promotion study AIN-76 diet containing 28% fat (corn oil) and 40% protein (casein) with azoxymethane (20 mg/kg bw); AIN-76 diet containing 28% fat (corn oil) and 40% protein (casein); AIN-76 diets with 60% bacon 5, 10, 10 rats/group 5, 10, 10	*Colon* No. of ACF per colon, mean (range): 9 (7–154), 0, 0		The duration of this experiment (45 days) was probably not sufficient to determine if the diets by themselves were tumorigenic
Rat, F344 (F) Age 5 wk 100 days Parnaud et al. (2000)	Initiation–promotion study Rats were fed one of five AIN-76–based diets containing casein, beef, chicken, pork, or bacon; fed ad libitum 10 rats/group 10, 10, 10, 10, 10	*Colon* No. of aberrant crypts per ACF, mean (SD): 2.9 (0.2), 2.9 (0.3), 2.7 (0.2), 2.7 (0.3), 2.4 (0.2)* No. of ACF per colon, mean (SD): 137 (26), 122 (60), 151 (28), 151 (25), 134 (21) No. of ACF with > 7 crypts per ACF, mean (SD): 19.7 (6.8), 15.6 (9.8), 18.6 (8.1), 18.1 (6.8), 11.1 (4.4)*	Decrease; *$P < 0.01$ (No. of aberrant crypts per ACF, bacon diet vs casein diet, ANOVA and Dunnett test) Decrease; *$P = 0.003$ (No. of ACF with > 7 crypts per ACF, bacon diet vs casein diet, ANOVA and Dunnett test)	All rats were treated with a single injection of azoxymethane (20 mg/kg bw)

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Wistar (NR) Age 13 wk 14 wk Belobrajdic et al. (2003)	Initiation–promotion study Rats were fed one of six AIN-93–modified diets consisting of 8%, 16%, or 32% red meat, 8%, 16%, or 32% whey; fed ad libitum 12 rats/group Survival: NR	*Proximal colon* No. of ACF per rat, mean: 90, 71, 84, 61, 77, 52 No. of single ACF per rat, mean: 32, 33*, 34*, 25, 26, 16	Numbers of ACF estimated from histogram *$P < 0.05$ (No. of single ACF per rat, numbers estimated from histogram; 16% and 32% red meat diets vs 32% whey protein diet; ANOVA and Tukey multiple comparison test)	Red meat was barbecued kangaroo muscle meat Each group presumably consisted of 12 rats, although this was not stated explicitly All rats were treated with two weekly injections of azoxymethane (15 mg/kg bw)
Rat, Sprague-Dawley (M) NR (weight, 50–75 g) 11 wk Khil & Gallaher (2004)	Initiation–promotion study Rats were fed one of four AIN-93G–modified diets using a 2 × 2 factorial design,: casein with soybean oil, beef with soybean oil, casein with tallow, or beef with tallow; fed ad libitum 14 rats/group 14, 14, 14, 14	*Colon* No. of ACF per cm^2, mean (SEM): 2,98 (0.50), 3.45 (0.37), 1.89 (0.39)*, 2.87 (0.44)* No. of aberrant crypts per ACF, mean (SEM): 3.08 (0.19), 2.69 (0.11), 3.56 (0.35), 2.81 (0.08) No. of aberrant crypts per cm^2, mean (SEM): 9.61 (1.98), 9.46 (1.18), 7.02 (1.54), 8.26 (1.45)	Decrease; *$P = 0.043$ (No. of ACF per cm^2, tallow diets vs soybean oil diets, ANOVA and Duncan multiple range test)	Casein and beef were the protein sources, and soybean oil and tallow were the fat sources; the diets were balanced for protein and fat energy content All rats were treated with two injections of DMH (15 mg/kg bw)

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Pierre et al. (2004)	Initiation–promotion study Control AIN-76 diet or modified diet containing skinless chicken meat (600 g/kg diet), beef meat (600 g/kg diet), black pudding (blood sausage, 600 g/kg diet), or powdered bovine haemoglobin (6.3 g/kg diet); fed ad libitum 20, 10, 10, 10, 10 rats/group 20, 10, 10, 10, 10	*Colon* No. of crypts per ACF, mean (SD): 2.7 (0.4), 2.9 (0.4)*, 2.8 (0.2), 3.1 (0.5)*, 2.9 (0.2)*	*$P < 0.05$ (No. of crypts per ACF; chicken, haemoglobin, and black pudding diets vs control diet; ANOVA and Fisher LSD test)	All rats were treated with azoxymethane (20 mg/kg bw) All the diets were balanced for protein, fat, calcium, and iron
		Total ACF crypts per colon, mean (SD): 192 (55), 267 (65)*, 280 (49)*, 285 (78)*, 301 (48)*	*$P < 0.05$ (No. of ACF crypts per colon; chicken, beef, haemoglobin, and black pudding diets vs control diet; ANOVA and Fisher LSD test)	
		No. of ACF per colon, mean (SD): 72 (16), 91 (18)*, 100 (13)*, 93 (24)*, 103 (14)*	*$P < 0.05$ (No. of ACF per colon; chicken, beef, haemoglobin, and black pudding diets vs control diet; ANOVA and Fisher LSD test)	
		No. of crypts per MDF, mean (SD): 4.65 (2.40), 4.92 (1.64), 4.23 (1.15), 4.60 (1.93), 4.29 (0.59)		
		Total MDF crypts per colon, mean (SD): 2.9 (4.0), 6.0 (3.9), 8.5 (6.9)*, 11.5 (9.0)*, 13.1 (6.0)*,**	*$P < 0.05$ (No. of MDF crypts per colon; beef, haemoglobin, and black pudding diets vs control diet; ANOVA and Fisher LSD test) **$P < 0.05$ (black pudding diet vs beef and chicken diets, ANOVA and Fisher LSD test)	

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Pierre et al. (2004) (cont.)		No. of MDF per colon, mean (SD): 0.55 (0.68), 1.20 (0.63), 1.90 (1.37)*, 2.40 (1.50)*, 3.00 (1.24)*,**	*$P < 0.05$ (No. of MDF per colon; beef, haemoglobin, and black pudding diets vs control diet; ANOVA and Fisher LSD test) **$P < 0.05$ (black pudding diet vs beef and chicken diets, ANOVA and Fisher LSD test)	
Rat, F344 (F) Age 5 wk 15 wk Pierre et al. (2008)	Initiation–promotion study The rats were fed one of eight AIN-76–modified diets: a low-calcium control diet; a low-calcium, beef meat diet; a high-calcium control diet; a high-calcium, beef meat diet; an olive oil–fortified control diet; an olive oil–fortified, beef meat diet; an antioxidant-fortified control diet; or an antioxidant-fortified, beef meat diet; fed ad libitum 10 rats/group 10, 10, 10, 10, 10, 10, 10, 10	*Colon* No. of crypts per ACF, mean (SD): 2.3 (0.2), 2.6 (0.2)*, 2.8 (0.2)**, 2.5 (0.2), 2.5 (0.2), 2.4 (0.2), 2.3 (0.2), 2.4 (0.3)	*$P < 0.05$ (No. of crypts per ACF, beef with low-calcium diet vs respective control diet, ANOVA and Fisher LSD test) **$P < 0.05$ (control diet with high calcium vs other control diets, ANOVA and Fisher LSD test)	All rats were treated with a single injection of DMH (190 mg/kg bw) All the diets were balanced for protein, fat, and iron
		No. of ACF per colon, mean (SD): 105 (24), 137 (26)*, 130 (22)**, 106 (24), 104 (25), 125 (20)*, 107 (22), 127 (22)*	*$P < 0.05$ (No. of ACF per colon; beef, except beef with high calcium, diets vs respective control diets; ANOVA and Fisher LSD test) **$P < 0.05$ (control diet with high calcium vs other control diets, ANOVA and Fisher LSD test)	
		No. of ACF crypts per colon, mean (SD): 245 (52), 347 (55)*, 365 (71)**, 265 (74), 258 (71), 299 (60)*, 243 (48), 300 (40)*	*$P < 0.05$ (No. of ACF crypts per colon; beef, except beef with high calcium, diets vs respective control diets; ANOVA and Fisher LSD test) **$P < 0.05$ (control diet with high calcium vs other control diets, ANOVA and Fisher LSD test)	

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Pierre et al. (2008) (cont.)		No. of crypts per MDF, mean (SD): 4.6 (1.7), 5.3 (1.6), 7.6 (2.4)*, 7.8 (3.1), 4.0 (1.2), 4.3 (0.7), 4.4 (1.4), 3.9 (1.4)	*$P < 0.05$ (No. of crypts per MDF, control diet with high calcium vs other control diets, ANOVA and Fisher LSD test)	
		No. of mucin-depleted crypts per colon, mean (SD): 18.2 (15.3), 40.7 (18.9)*, 58.1 (27.5)**, 24.3 (12.6), 15.6 (13.0), 22.5 (5.3), 14.7 (8.8), 22.4 (9.5)	*$P < 0.05$ (No. of mucin-depleted crypts per colon, beef with low-calcium diet vs respective control diet, ANOVA and Fisher LSD test) **$P < 0.05$ (control diet with high calcium vs other control diets, ANOVA and Fisher LSD test)	
		No. of MDF per colon, mean: 3.5 (2.0), 7.4 (2.0)*, 7.6 (3.0)**, 3.4 (1.8), 3.8 (2.5), 5.3 (1.2)*, 3.2 (1.3), 5.6 (1.1)*	*$P < 0.05$ (No. of MDF per colon; beef, except beef with high calcium, diets vs respective control diets; ANOVA and Fisher LSD test) **$P < 0.05$ (control diet with high calcium vs other control diets, ANOVA and Fisher LSD test)	

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Pierre et al. (2010)	Initiation–promotion study AIN-76–modified control diet or diet containing ham (550 g/kg diet), balanced for protein, fat, and iron; fed ad libitum 10 rats/group 10, 10	*Colon* No. of crypts per ACF, mean (SD): 2.3 (0.2), 2.1 (0.1) No. of ACF per colon, mean (SD): 105 (24), 119 (16)* No. of crypts per MDF, mean (SD): 4.6 (1.7), 4.3 (1.2) No. of MDF per colon, mean (SD): 3.5 (2.0), 8.5 (2.2)*	*$P < 0.05$ (No. of ACF per colon, ham diet vs control diet, ANOVA and Tukey multiple comparison test) *$P < 0.05$ (No. of MDF per colon, ham diet vs control diet, ANOVA and Tukey multiple comparison test)	All rats were treated with a single injection of DMH (190 mg/kg bw)
Rat, F344 (F) Age 5 wk 15 wk Santarelli et al. (2010)	Initiation–promotion study The rats were fed a control diet or one of four AIN-76–modified diets: dark cooked meat with nitrite, oxidized; dark cooked meat with nitrite, anaerobic; dark cooked meat, oxidized; or dark raw meat, anaerobic; fed ad libitum 10 rats/group 10, 10, 10, 10, 10	*Colon* No. of ACF per colon, mean (SD): 81 (18), 100 (16)*, 102 (25)*, 106 (21)*, 101 (17)* No. of crypts per MDF, mean (SD): 3.9 (1.5), 4.2 (1.2), 2.7 (1.7)*, 3.5 (1.2), 3.9 (1.9) No. of MDF per colon, mean (SD): 2.9 (1.9), 4.1 (2.9)*, 2.1 (2.0), 2.8 (2.8), 3.4 (2.6)	*$P < 0.05$ (No. of ACF per colon, experimental dark meat diets vs control diet, ANOVA and Fisher LSD test) Decrease; *$P < 0.05$ (No. of crypts per MDF; dark cooked meat with nitrite, anaerobic diet vs control and dark cooked meat, oxidized diets; ANOVA and Fisher LSD test) *$P < 0.05$ (No. of MDF per colon; dark cooked meat with nitrite, oxidized diet vs control diet; Fisher LSD test)	All rats were treated with a single injection of DMH (180 mg/kg bw) The dark meat was pork meat with high haem The diets were balanced for protein and fat

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Santarelli et al. (2010) (cont.)		No. of mucin-depleted crypts per colon, mean (SD): 11 (8), 18 (13)*, 8 (8), 10 (11), 14 (10)	*$P < 0.05$ (No. of mucin-depleted crypts per colon; dark cooked meat with nitrite, oxidized diet vs control; dark cooked meat with nitrite, anaerobic; and dark cooked meat, oxidized diets; ANOVA and Fisher LSD test)	
Rat, F344 (F) 5 wk 15 wk Pierre et al. (2013)	Initiation–promotion study One of three AIN-76–modified diets containing dark cooked meat with nitrite, oxidized by air; dark cooked meat with nitrite, oxidized by air and fortified with α-tocopherol; or dark cooked meat with nitrite, oxidized by air and fortified with $CaCO_3$; fed ad libitum 16, 10, 10 rats/group 16, 10, 10	*Colon* No. of ACF per colon, mean (SD): 126 (20), 125 (15), 124 (24) No. of crypts per MDF, mean (SD): 3.7 (1.3), 2.4 (2.1), 2.5 (1.4) No. of MDF per colon, mean (SD): 2.7 (2.1)*, 1.4 (1.5), 1.3 (1.6)	*$P < 0.05$ (No. of MDF per colon; dark cooked meat treated with nitrite, oxidized by air diet vs dark cooked meat treated with nitrite, oxidized by air and fortified with α-tocopherol or dark cooked meat treated with nitrite, oxidized by air and fortified with $CaCO_3$ diet; Fisher LSD test)	All rats were treated with a single injection of DMH (180 mg/kg bw) The dark meat was pork meat with high haem

Table 3.2 (continued)

Species, strain (sex) Age at start Duration Reference	Dosing regimen Animals/group at start No. surviving animals	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, F344 (F) Age 5 wk 15 wk Santarelli et al. (2013)	Initiation–promotion study Low-calcium AIN-76–modified diets, either 40% hot dog [pork] meat or 50% French saucisson [pork]; the diets were balanced for protein, fat, and iron; fed ad libitum 10 rats/group 10, 10, 10	*Colon* No. of ACF per colon, mean (SD): 110 (17), 108 (32), 102 (25) No. of crypts per MDF, mean (SD): 2.6 (2.4), 4.7 (2.4)*, 3.2 (2.2) No. of MDF per colon, mean (SD): 1.2 (1.4), 3.0 (1.7)*, 2.4 (2.4)	*$P < 0.05$ (No. of crypts per MDF, hot dog with low-calcium diet vs control diet, ANOVA and Fisher LSD test) *$P < 0.05$ (No. of MDF per colon, hot dog with low-calcium diet vs control diet, ANOVA and Fisher LSD test)	All rats were treated with a single injection of DMH (180 mg/kg bw)
Rat, F344 (F) Age 5 wk 15 wk Santarelli et al. (2013)	Initiation–promotion study AIN-76–modified diet containing 40% hot dog [pork] with either low or high calcium (balanced for protein, fat, and iron), fed ad libitum 10 rats/group 10, 10	*Colon* No. of crypts per MDF, mean (SD): 3.5 (0.6), 3.3 (0.4) No. of MDF per colon, mean (SD): 2.3 (1.4)*, 1.2 (1.1) No. of ACF per colon, mean (SD): 136 (25), 118 (19)	*$P < 0.05$ (No. of MDF per colon, hot dog with low-calcium diet vs hot dog with high-calcium diet, ANOVA and Fisher LSD test)	All rats were treated with a single injection of DMH (180 mg/kg bw)

* or **, statistically significant; ACF, aberrant crypt foci; AIN, American Institute of Nutrition; ANOVA, analysis of variance; $CaCO_3$, calcium carbonate; DMH, dimethylhydrazine; F, female; HAA, heterocyclic aromatic amine; LSD, least significant difference; M, male; MDF, mucin-depleted foci; mo, month; NR, not reported; NS, not significant; SD, standard deviation; SEM, standard error of the mean; vs, versus; wk, week

and 997 days for females. All rats were examined grossly, histopathology was conducted, and statistical analyses were conducted. Rats on the human diets (Diets C, D, and E) weighed substantially more than rats on the rodent diets (Diets A and B), probably as a consequence of the greater caloric intake of the rats on the human diets. The maximum difference in weight was 200 g for male rats and 100 g for female rats. At the end of the experiment, approximately 5–22% of the male rats and 7–15% of the female rats survived. Male rats fed the human Diet C had a significantly higher mortality than rats fed the rodent Diets A or B (Alink et al., 1989, 1997).

Male rats on the human diets (Diets C, D, and E) had a significantly greater tumour incidence than male rats fed the rodent diets (Diets A and B; $P < 0.014$). This difference was due to epithelial tumours ($P = 0.0008$), specifically pituitary gland (pars distalis) tumours ($P = 0.0016$) and thyroid gland (light cell adenoma or carcinoma combined) tumours ($P = 0.014$). Stepwise logistic regression analysis indicated that the increased tumour incidence in the tissues of these glands was not associated with the observed increase in body weight. [The linear regression analysis may have been compromised, as the body weights at the early time points did not differ among the groups. At the end of the study, rats fed the human diets weighed considerably more than those fed the rodent diets.] None of the other tumours reported were affected by the diets. [The tumour data were confounded by the fact that the human diets contained approximately twice the amount of fat and half the fibre of the rodent diets. In addition, the increase in tumour incidence could not necessarily be attributed to beef because the diets contained other components typically present in human diets.] There were no significant findings in female rats (Alink et al., 1989, 1997).

3.2.2 Red meat with known carcinogens

Inbred, female F344 rats were randomly divided at weaning into four groups, with 56 rats in each group. One group (designated D_1) was given a high-protein (39%), high-fat (24%) diet, with soybean as the protein source and corn oil as the fat source. Another group (designated D_2) was given a low-protein (19%), low-fat (5.4%) diet, with soybean as the protein source and corn oil as the fat source. A third group (designated D_3) was given a high-protein (40%), high-fat (23%) diet, with freeze-dried ground beef as the protein source and freeze-dried ground beef plus corn oil as the fat source. A fourth group (designated D_4) was given a low-protein (18.5%), low-fat (6.5%) diet, with freeze-dried ground beef as both the protein and fat sources. [The calcium content of the diet could not be determined.] At 7 weeks of age, half the rats in each group were initiated with weekly subcutaneous injections of DMH at a dose of 10 mg/kg bw for 20 weeks. [The specific diets may have affected metabolism of the DMH.] The tumour incidence was assessed 10 weeks after the last injection. Gross and histopathological analyses were conducted. Rats treated with DMH tended to weigh less than the control rats, especially rats fed the soybean and corn oil diets (D_1 and D_2). Rats fed the ground beef plus corn oil diet (D_3) weighed more than the other groups (Reddy et al., 1976).

Tumours were observed in the ear canal, kidney, small intestine, and colon in DMH-treated rats. There were no tumours in the control rats (i.e. those that had not been treated with DMH). [The duration of this experiment (30–34 weeks) was probably not sufficient to determine if the diets by themselves were tumorigenic.] Rats fed the high-protein, high-fat diets (D_1 and D_3) had an increased multiplicity (tumours per animal) of colon tumours ($P < 0.05$) and adenocarcinomas ($P < 0.05$), but not adenomas, compared with rats fed the low-protein, low-fat diets (D_2 and D_4). [The statistical test was not specified,

but was presumably a Student *t* test]. The source of the protein (e.g. soybean or beef) and fat (e.g. corn oil or beef) did not affect the tumour multiplicity. Rats treated with DMH had a significantly increased incidence of ear canal and colon tumours compared with control rats [$P \leq 0.02$, two-tailed Fisher exact test] (Reddy et al., 1976).

In a separate study, groups of 30 weanling, male Sprague-Dawley rats (weight, 50–60 g) were placed on one of three diets: 20% soy protein and 20% beef tallow, 20% raw beef protein (ground beef) and 20% beef tallow, or 20% charcoal-broiled beef protein and 20% beef tallow. [The calcium content of the diets could not be determined.] The charcoal-broiled ground beef was cooked to a "well-done" state, with an internal temperature of approximately 70 °C. Both the raw and cooked ground beef were freeze-dried and ground to a fine powder before being mixed into the diet. After 4 weeks, rats were initiated with intraperitoneal injections of DMH at 1.25 mg/100 g bw per week for 18 weeks. [The specific diets may have affected metabolism of the DMH.] The rats were killed 32 weeks after being placed on the diets. Eighty-seven of the initial 90 rats survived until the end of the experiment. Small intestine and colon tumours were assessed grossly; histopathology was not conducted. Statistical analyses were conducted. The distribution of colon tumour multiplicity was not significantly affected by the diets. Similarly, the number of small intestine tumours per rat did not differ significantly across the diet groups (Clinton et al., 1979).

A study was conducted to investigate the effects of a "complete human diet" prepared under normal household conditions. Male Wistar rats (age, 4 weeks) were placed on one of five diets (45 rats per diet, except Diet B, which had 36 rats): diet A, a semisynthetic rodent diet; diet B, a semisynthetic rodent diet supplemented with fruits and vegetables; diet C, a "humanized" diet consisting of meat (beef, pork, and chicken), bread, eggs, and margarine, along with other semisynthetic products, including lard, potato flour, sugar, bran, and pectin; diet D, a diet similar to diet C, except the food was cooked under "usual household conditions"; and diet E, a diet similar to diet D, except supplemented with fruits and vegetables. All the food items were freeze-dried, homogenized, and pelletized. The pellets were assessed for the presence of heterocyclic aromatic amines (HAAs). [The authors did not present the results of the HAA analyses.] Diets A and B contained 21.6% fat "energy," 26.0% protein "energy," 52.4% carbohydrate "energy," and 10.7% fibre. Diets C, D, and E contained 40.6% fat "energy," 13.2% protein "energy," 46.2% carbohydrate "energy," and 5% fibre. The diets contained calcium at a concentration of 7.5 g/kg diet. Starting at age 8 weeks, all rats were initiated with 10 weekly subcutaneous injections of DMH at a dose of 50 mg/kg bw. [The specific diets may have affected metabolism of the DMH.] The rats were maintained on their respective diets for 8 months. All animals were examined grossly, histopathology was conducted, and statistical analyses were conducted. Of the rats, 18% (range across diet groups, 8.3–28.9%) died or were removed before the scheduled termination; more than 90% of the rats from each group were evaluated for neoplasms. Food consumption was higher in rats fed diets A and B compared with those fed diets C, D, and E, presumably due to the lower caloric density of diets A and B. Body weights did not differ among the groups (Alink et al., 1993, 1997).

For all diet groups, tumours were mainly observed in the colon and small intestine, with a much lower incidence in the caecum, abdominal cavity, and liver. The overall incidence of adenocarcinomas of the colon was significantly ($P < 0.05$) higher in the combined human diet groups (diets C, D, and E) than in the combined rodent diet groups (diets A and B). The incidence of other tumours did not differ between the combined human and rodent diet groups. Zymbal gland tumours were also observed,

with the incidence being significantly ($P < 0.05$) greater in the combined human diet groups than in the combined rodent diet groups; (Alink et al., 1993, 1997). [The Working Group noted that the specific incidences were not reported in the paper. The tumour data were confounded by the fact that the human diets had approximately two-fold more fat and 50% less fibre than the rodent diets. In addition, the increase in tumours could not be necessarily attributed to beef because the diets contained other components typically present in human diets.]

In another study, groups of 10 rats [presumably Sprague-Dawley, and sex and age not specified] were initiated with DMH. Rats were fed diets containing 20% protein, derived from red meat [type not specified], whey, casein, soy, or fish. [The temporal relationship between the DMH treatment and the different diets was not specified; the preparation of the various diets and the duration of feeding were not reported. The calcium content of the diets could not be determined, and there was no indication if histopathology was conducted.] The incidence of intestinal tumours did not differ significantly among the rats fed the different protein diets. [This may have been a consequence of the small number of animals per group.] Rats fed the whey protein diet had significantly fewer intestinal tumours per rat than rats fed the red meat, soy, or fish protein diets ($P < 0.05$) (McIntosh, 1993). [The Working Group noted that the design and results of the study were very poorly reported. Whey protein has been reported to have chemopreventive activity.]

In a separate study, groups of 20 male Sprague-Dawley rats (age, 5 weeks) were fed AIN-76A–based diets formulated with one of four protein sources: casein (20.0 g per 100 g diet), whey protein concentrate (21.3 g per 100 g diet), kangaroo skeletal muscle (22.8 g per 100 g diet), or defatted soybean meal (33.3 g per 100 g diet). The kangaroo meat was dried to a constant low-moisture product at 40 °C, and then ground to a fine meal. The levels of the remaining dietary components (e.g. fat, carbohydrate, and fibre) were adjusted, so the four diets were of comparable composition. The calcium content was 5 g/kg diet. At 9–10 weeks of age, the rats were initiated with three subcutaneous weekly injections of DMH at a dose of 15 mg/kg bw. [The specific diets may have affected metabolism of the DMH.] The rats were maintained on the diets for 5–6 months. The number of rats that survived until the end of the experiment was not specified. Tumours were assessed grossly, and histopathology was conducted in selected instances. Statistical analyses were conducted (McIntosh et al., 1995).

Intestinal tumour incidence was lowest in the whey protein group (30%), followed by the casein group (45%), kangaroo skeletal muscle group (50%), and defatted soybean meal group (65%). However, differences in tumour incidence were not significant. There was a significantly lower intestinal tumour burden (tumours per group) in the two groups (combined) fed the whey protein and casein diets compared with the two groups (combined) fed the kangaroo meat and soybean meal diets ($P < 0.005$). The same was true when only large intestine tumours were considered ($P < 0.02$). The tumour mass index did not differ significantly among the groups (McIntosh et al., 1995; McIntosh & Le Leu, 2001). [Whey protein has been reported to have chemopreventive activity.]

Groups of 25 weanling, male Sprague-Dawley rats were fed AIN-76A–based test diets. The diets contained calcium at a concentration of 5.2 g/kg diet. A 2 × 2 × 2 factorial design was used, with the factors being the protein source (casein or lean ground beef), fat source (corn oil or beef tallow), and fat level (5% or 20%). Ground beef containing 20% fat was cooked in an iron skillet until the meat was no longer pink and then mixed with the remaining dietary components. After a 2-week acclimation period, the rats were initiated with 10 weekly intraperitoneal

injections of DMH at a dose of 20 mg/kg bw. [The specific diets may have affected metabolism of the DMH.] Following the DMH treatment, the rats were maintained on the experimental diets for an additional 15 weeks. An additional 10 rats per diet group served as vehicle controls. Complete necropsies were performed, and all lesions were examined microscopically. Statistical analyses were conducted. Rats fed the 20% fat diets gained more weight than those fed the 5% fat diets, irrespective of the fat source. Rats fed the casein protein diets weighed more than those fed the lean ground beef protein diets (Pence et al., 1995).

Rats fed the casein protein diets had a significantly higher incidence ($P < 0.05$) and multiplicity ($P = 0.0001$) of colon adenocarcinomas than rats fed the lean ground beef protein diets, irrespective of the fat source or fat level. The multiplicity of colon tumours was also higher in the rats fed the casein protein diets ($P = 0.0008$) (Pence et al., 1995). [The tumour incidences in the control groups were not reported. The duration of this experiment (27 weeks) was probably not sufficient to determine if the diets by themselves were tumorigenic.]

A group of 35 weanling male Sprague-Dawley rats was placed on an AIN-76A–based diet containing 17.2% casein (protein source) and 5% corn oil (fat). A second group of weanling, male Sprague-Dawley rats was placed on an AIN-76A–based diet containing 97% lean (3% fat) ground beef at 50% of the total diet (by weight) and 4% corn oil [The diets contained calcium at a concentration of 5.2 g/kg diet.] The lean ground beef was cooked in an iron skillet until the meat was no longer pink and then mixed with the remaining dietary components. Two weeks after being placed on the diets, 30 rats from each group were initiated with intraperitoneal injections of DMH at a dose of 20 mg/kg bw once per week for 10 weeks. [The specific diets may have affected metabolism of the DMH.] Five rats from each group served as vehicle controls. Fifteen weeks after the last DMH injection, the rats were killed to assess the tumour incidence. Complete necropsies were conducted, and all lesions were examined by histopathology. Rats fed the beef diet and initiated with DMH weighed more than those fed the casein diet and initiated with DMH, with the difference (~10%) being significant (as assessed by Student *t* test) towards the end of the experiment (weeks, 17–25). Mean food consumption was similar for both diet groups after correcting for the water content of the beef (Lai et al., 1997). [Although not stated, it appeared that two rats from each group were removed early and not included in the final tumour assessment.]

The only tumours reported were colon adenocarcinomas and small intestine adenocarcinomas, and the incidence and number of tumours per rat did not differ significantly (as assessed by χ^2 test and Student *t* test, respectively) between those fed the casein diet and initiated with DMH and those fed the beef diet and initiated with DMH (Lai et al., 1997). [The tumour incidence in the control groups (those not treated with DMH) was not reported. The duration of this experiment (27 weeks) was probably not sufficient to determine if the diets by themselves were tumorigenic.]

Groups of 25 weanling male Sprague-Dawley rats were fed one of four AIN-76A–based diets: low fat, low HAAs; high fat, low HAAs; low fat, high HAAs; or high fat, high HAAs. The diets contained calcium at a concentration of 5.2 g/kg diet. The fat was provided primarily by beef tallow (4% for low fat and 18.8% for high fat) and corn oil (1% for low fat and 1.2% for high fat), and the HAAs were generated by cooking the beef to give 6.6 and 85.6 ng of HAAs per gram cooked beef for low and high HAAs, respectively. The low-HAA beef was prepared by cooking crumbled beef for 11 minutes in a stainless-steel vessel [the internal temperature was not reported]; the high-HAA beef was prepared by cooking a beef patty for 11 minutes in an iron skillet to an internal temperature of 85 °C. Two weeks after being fed the diets, the rats were initiated with

10 weekly intraperitoneal injections of DMH at a dose of 20 mg/kg bw. One additional group of 10 rats, fed the high-fat, high-HAA diet, was given 10 weekly intraperitoneal injections of the vehicle. Following the last intraperitoneal injection, the rats on the low-fat, low-HAA diet were given either a low-fat AIN-76A diet or a low-fat, high-HAA diet; the rats on the low-fat, high-HAA diet were given either a low-fat AIN-76A diet or a low-fat, high-HAA diet; the rats on the high-fat, low-HAA diet were given a high-fat AIN-76A diet; and the rats on the high-fat, high-HAA diet were given either a low-fat AIN-76A diet, high-fat AIN-76A diet, or a high-fat, high-HAA diet. Twenty-seven weeks after the start of the experiment, the rats were killed to assess tumour incidence. Complete necropsies were performed, and lesions were examined by histopathology. In addition, statistical analyses were conducted (Pence et al., 1998).

Adenocarcinomas were observed in the colon, stomach, and small intestine. These only occurred in rats initiated with DMH. The most consistent observation was a decrease in the incidence of colon tumours ($P < 0.05$) in rats fed the high-fat, high-HAA diet during weeks 1–12 (colon tumour incidence, 56%) compared with those fed the low-fat, high-HAA diet during the same period (colon tumour incidence, 84–88%). (Pence et al., 1998). [The duration of this experiment (27 weeks) was probably not sufficient to determine if the high-fat, high-HAA diet by itself was tumorigenic.]

3.2.3 Red meat and/or processed meat with known carcinogens to give aberrant crypt foci and/or mucin-depleted foci

Groups of 10 female F344 rats (age, 5 weeks) were treated with a single intraperitoneal injection of azoxymethane at a dose of 20 mg/kg bw. One week later, the groups were placed on a low-meat (30%) or high-meat diet (60%). The protein was provided by powdered cooked meat (beef, bacon, or chicken) and casein, and the fat was provided by the meat, lard, chicken fat, olive oil, and corn oil. The high-meat diet contained approximately twice as much fat and protein as the low-meat diet. Each type of meat was cooked in the oven for 15 minutes at 180–185 °C. The estimated HAA content was 1–15 ng/g beef, 15–65 ng/g bacon, and 40 ng/g chicken. [The calcium content of the diets could not be determined.] After cooking, the meats were minced, frozen, and freeze-dried. There were also two control diet groups, where protein was provided by casein, and fat was provided by lard or olive oil. Rats fed the bacon-based diets consumed more drinking-water than rats fed the other diets. The rats were killed 105–107 days after the azoxymethane injection, and the extent of aberrant crypt foci (ACF) formation was determined by light microscopy. Statistical analyses were conducted. The number of ACF per rat did not vary significantly among the diet groups. The multiplicity of ACF was lowest in the bacon-fed rats, and compared with the high-casein, lard-fed group, the multiplicity was reduced by 20% in the high-bacon group ($P < 0.001$) (Parnaud et al., 1998).

An experiment to induce ACF was conducted in a group of five female F344 rats (age, 4 weeks). The rats were treated with a single intraperitoneal injection of azoxymethane at a dose of 5 mg/kg bw and transferred to a high-fat, semisynthetic AIN-76–based diet containing 28% fat (corn oil) and 40% protein (casein). The diet contained calcium at a concentration of 5.2 g/kg diet. A second group of 10 rats was injected with the vehicle and then given the same diet. A third group of 10 rats was injected with 0.9% sodium chloride (NaCl) and transferred to a diet containing 60% bacon, as both the protein and fat sources, and prepared as described in Parnaud et al. (1998). Both diets were identical in terms of protein and fat levels. Thirty days after being fed the bacon diet, the rats were placed on the high-fat control diet for an additional 15 days, after which all rats were assessed

for the formation of colonic ACF (i.e. 45 days after the initial intraperitoneal injection). Body weights were not affected by the diets. ACF was present only in the rats that had been initiated with azoxymethane (Parnaud et al., 2000). [The duration of this experiment (45 days) was probably not sufficient to determine if the diets by themselves were tumorigenic.]

An additional experiment was conducted that focused on the promotion of ACF by various low-fat diets. Female F344 rats (age, 4 weeks) were treated with a single intraperitoneal injection of azoxymethane at a dose of 20 mg/kg bw. One week later, groups of 10 rats were randomly transferred to either an AIN-76–based control diet consisting of 2% corn oil, 5% lard, and 25% casein [the AIN-76A diet contains calcium at a concentration of 5.2 g of calcium per /kg diet] or one of four experimental diets containing 30% low-fat beef (hamburger), pork, lean bacon, or chicken (fillet). Each meat was cooked as described in Parnaud et al. (1998). The meat diets were supplemented with casein to reach 25% protein, and with lard (for bacon and pork diets) or chicken fat (for the chicken diet) to adjust the fat content. Rats fed the low-fat meat diets weighed significantly more (7–8%) than those fed the low-fat control diet. The rats continued on their respective diets for 100 days after azoxymethane initiation and then were assessed for ACF. Statistical analyses were conducted. The number of ACF per rat did not vary significantly among the diet groups. The multiplicity of ACF was lowest in the rats fed the low-fat bacon diet compared with the low-fat control rats: multiplicity was 17% lower ($P < 0.01$), and the number of large ACF (more than seven crypts per focus) was 44% lower ($P = 0.003$). The beef, pork, and chicken diets did not have any effect on the multiplicity of ACF (Parnaud et al., 2000).

Groups of Wistar rats (age, 13 weeks) [sex not reported] were placed on one of six modified AIN-93 diets containing 8%, 16%, or 32% red meat protein or 8%, 16%, or 32% whey protein. The meat for the diets was obtained from barbecued kangaroo muscle, which was dried at 45 °C and milled to give the product 78% protein, 15.3% fat, and 1% moisture. The whey protein concentrate contained 78% protein, 8.3% fat, 4.9% lactose, and 4.2% moisture. Both protein sources were added to the diets at 10.25%, 20.5%, or 41%. The diets were low in calcium (0.1%) and fibre (2%), and fat was adjusted to 20% by the addition of sunflower seed oil. [Each group presumably consisted of 12 rats, although this was not stated explicitly.] Four weeks after being placed on their respective diets, all rats were treated with two weekly subcutaneous injections of azoxymethane at a dose of 15 mg/kg bw. [The specific diets may have affected metabolism of the DMH.] After an additional 8 weeks on the diets, the rats were killed to assess the extent of aberrant crypt formation. Statistical analyses were conducted. The final body weights of the rats fed the 32% whey protein diet were less than the body weights of those fed the 8%, 16%, or 32% red meat protein diet or 8% or 16% whey protein diet. The number of single ACF in the proximal colon was lower in rats fed the 32% whey protein diet ($P < 0.05$) than in those fed the 16% or 32% red meat protein diet (Belobrajdic et al., 2003). [Whey protein has been reported to have chemopreventive activity; as such, it may not have been the proper control for purpose of comparisons. In addition, single ACF may have limited predictive value for colon carcinogenesis (Magnuson et al., 1993).]

Male Sprague-Dawley rats (weight, 50–75 g) were treated by intragastric gavage once per week for 2 weeks with DMH at a dose of 15 mg/kg bw. One week after the second DMH treatment, the rats (14 per group) were placed on one of four modified AIN-93G diets. The diets contained calcium at a concentration of 5.1 g/kg diet. A 2 × 2 factorial design was used, with the factors being the protein source (casein or beef) and fat source (soybean oil or tallow). The beef was cooked in an oven at 93 °C, which is a temperature that minimizes HAA formation, for 2 hours.

It was then freeze-dried, minced, and mixed with the other diet components. All of the diets were balanced with respect to the protein (20%) and fat (15%) content. The extent of colonic ACF formation was assessed 9 weeks after the rats began their respective diets. Statistical analyses were conducted. Rats fed tallow as the fat source had fewer ACF per cm^2 than those fed soybean oil as the fat source ($P = 0.043$). The source of protein (casein or beef) did not affect the extent or multiplicity of ACF (Khil & Gallaher, 2004).

Female F344 rats (age, 5 weeks) were placed on a modified AIN-76 control diet. One week later, all the rats were treated with a single intraperitoneal injection of azoxymethane at a dose of 20 mg/kg bw. One week after the azoxymethane injection, 10 rats per group were transferred to one of four modified AIN-76 diets: a diet containing skinless chicken meat (600 g/kg diet), beef meat (600 g/kg diet), black pudding (blood sausage; 600 g/kg diet), or powdered bovine haemoglobin (6.3 g/kg diet). Each of the meats was freeze-dried before being added to the diets. [The authors did not indicate if the meats had been cooked.] Twenty rats continued on the control diet. All the diets were balanced for protein (50%), fat (20%), calcium (800 mg/kg diet), and iron (140 mg/kg diet, except for the black pudding diet) by the addition of casein, lard, calcium phosphate, and ferric citrate. [The protein content of the diets was approximately three times that typically used in rodent diets.] Ferric citrate was not added to the haemoglobin diet because the iron content was already 950 mg/kg diet. The rats continued on the diets for 100 days, at which time their colons were examined for ACF and mucin-depleted foci (MDF). Statistical analyses were conducted. Rats from the beef-fed group weighed significantly more (~5–10%) than those from the other groups; the body weights of the rats from the other groups did not differ (Pierre et al., 2004).

Rats fed the experimental diets had more ACF per colon ($P < 0.05$) and aberrant crypts per colon ($P < 0.05$) than rats fed the control diet. Rats fed the black pudding diet had more ACF per colon ($P < 0.05$) than rats fed the chicken diet. Rats fed the beef, haemoglobin, and black pudding diets had more MDF per colon and mucin-depleted crypts per colon than rats fed the control diet ($P < 0.05$). Rats fed the black pudding diet also had more MDF per colon and mucin-depeleted crypts per colon than rats fed the chicken or beef diet ($P < 0.05$) (Pierre et al., 2004).

Female F344 rats (age, 5 weeks) were placed on a modified AIN-76 control diet for an unspecified acclimation period before being treated with a single intraperitoneal injection of DMH at a dose of 190 mg/kg bw. One week after the DMH injection, 10 rats continued on the low-calcium control diet, while 10 rats per group were transferred to one of seven modified AIN-76 diets: a low-calcium, beef meat diet; a high-calcium control diet; a high-calcium, beef meat diet; an olive oil–fortified control diet; an olive oil–fortified, beef meat diet; an antioxidant-fortified control diet; and an antioxidant-fortified, beef meat diet. The beef meat diets contained freeze-dried meat (60%) with haem (600 nmol/kg meat). [The authors did not indicate if the meats had been cooked.] The low-calcium diets contained dibasic calcium phosphate at a concentration of 2.7 g/kg diet, and the high-calcium diets contained calcium phosphate at a concentration of 31 g/kg diet. The olive oil diet contained olive oil at a concentration of 50 g/kg diet, and replaced an equal amount of safflower oil contained in the other diets. The antioxidant diet contained rutin at a concentration of 500 mg/kg diet and butylated hydroxyanisole at a concentration of 500 mg/kg diet. All the diets were balanced for protein (50%), fat (20%), and iron (110 mg/kg diet) by the addition of casein, lard, and ferric citrate. [The protein content of the diets was approximately three times that typically used in rodent diets.] The rats continued on the diets for 99–100 days, at which

time their colons were examined for ACF and MDF. Statistical analyses were conducted. Body weights and food intake did not differ among the groups (Pierre et al., 2008).

The total number of aberrant crypt foci per colon, aberrant crypts per colon, mucin-depleted foci per colon, and mucin-depleted crypts per colon was higher in the beef diet groups (except for the beef plus high-calcium group) than in their respective control groups ($P < 0.05$). Furthermore, the number of each of these lesions was significantly higher in the high-calcium control diet group than in the other control diet groups.

Female F344 rats (age, 4 weeks) were fed a modified AIN-76 control diet. One week later, all 20 rats were treated with a single intraperitoneal injection of DMH at a dose of 190 mg/kg bw. One week after the DMH injection, 10 of the rats were transferred to a diet containing freeze-dried ham at a concentration of 550 g/kg diet (11.5% fat), while the remaining rats continued on the control diet. The diets were balanced for protein (50%), fat (21%), calcium (800 mg/kg diet), and iron (140 mg/kg diet) by the addition of casein, lard, calcium phosphate, and ferric citrate. [The protein content of the diets was approximately three times that typically used in rodent diets.] The ham diet provided haem at a concentration of 36 nmol/g diet. The rats continued on the diets for 100 days, at which time their colons were examined for ACF and MDF. Statistical analyses were conducted. Body weights did not differ among the diet groups. Rats fed the ham diet drank more water than rats fed the control diet. Rats fed the ham diet also had significantly more ACF and MDF per colon than rats fed the control diet ($P < 0.05$). There was no difference in the size (crypts per foci) of the ACF or MDF among the diet groups (Pierre et al., 2010).

Female F344 rats (age, 5 weeks) were placed on a standard AIN-76 diet. After a 5-day acclimation period, they were treated with a single intraperitoneal injection of DMH at a dose of 180 mg/kg bw. Seven days after being injected, groups of 10 rats were transferred to one of four experimental diets: dark cooked meat with nitrite, oxidized; dark cooked meat with nitrite, anaerobic; dark cooked meat, oxidized; and dark raw meat, anaerobic. Ten additional rats remained on the AIN-76 control diet. The dark meat was supraspinatus and infraspinatus pig muscle that contained 15–17 mg of haem per 100 g of tissue. The cooked meat was heated at 70 °C, and the raw meat was heated at 50 °C for 1 hour under vacuum to denature the myoglobin and free the haem. The nitrite-treated meat contained 2 g of NaCl (600 mg of sodium nitrite per 100 g of salt) per 100 g of meat. The anaerobic meat was packaged immediately in vacuum-sealed, low–oxygen permeability bags. Each of the diets contained low calcium (calcium phosphate at 2.7 g/kg diet) and contained 5 g of safflower oil per 100 g diet. The diets were balanced for protein (40%) and fat (15%). [The protein content of the diets was approximately twice that typically used in rodent diets.] The rats continued on the diets for 98–99 days, at which time their colons were examined for ACF and MDF. Statistical analyses were conducted. Body weights did not differ among the groups (Santarelli et al., 2010).

Rats fed the meat diets had a significantly increased number of ACF per colon and aberrant crypts per colon compared with rats fed the control diet. [Only the ACF per colon data were presented in the paper.] Rats fed the dark cooked meat with nitrite, oxidized diet had significantly more MDF per colon and mucin-depleted crypts per colon than rats fed the control diet ($P < 0.05$). Rats fed the dark cooked meat with nitrite, oxidized diet had more crypts per MDF and mucin-depleted crypts per colon than rats fed the dark cooked meat with nitrite, anaerobic diet ($P < 0.05$), which suggested that oxidized meat promoted the formation of MDF. Similarly, rats

fed the dark cooked meat with nitrite, oxidized diet had more mucin-depleted crypts per colon than rats fed the dark cooked meat, oxidized diet, which suggested that nitrite promoted the formation of MDF ($P < 0.05$) (Santarelli et al., 2010).

Female F344 rats (age, 5 weeks) were placed on a standard AIN-76 semipurified diet for an unspecified acclimation period before being treated with a single intraperitoneal injection of DMH at a dose of 180 mg/kg bw. One week after the DMH injection, 16 rats were transferred to a modified AIN-76 diet containing 47% (dry weight) moist, cured, dark cooked meat with nitrite, oxidized by air. The meat was prepared from dark red supraspinatus pig muscle (15–17 mg of haem per 100 g of meat) that had been cured with 2 g of salt (600 mg of sodium nitrite per 100 g of salt) per 100 g of meat and 360 mg of sodium erythorbate per 100 g of meat. The meat was heated under vacuum at 70 °C for 1 hour and then exposed in the dark to air at 4 °C for 5 days. An additional 10 rats were fed the same modified AIN-76 diet, but fortified with 0.05% α-tocopherol (added during the curing process), while 10 additional rats were fed the same modified AIN-76 diet, but fortified with 1.5 g of calcium carbonate per 100 g diet, replacing an equivalent amount of casein. The rats continued on the diets for 98–99 days, at which time their colons were examined for ACF and MDF. Statistical analyses were conducted. Body weights and food intake did not differ among the groups (Pierre et al., 2013).

Rats fed the cured, dark cooked meat with nitrite, oxidized by air diet had significantly more MDF than rats fed the same diet fortified with either α-tocopherol or calcium carbonate ($P < 0.05$). Neither the number of ACF per colon nor the size of the MDF was affected by α-tocopherol or calcium carbonate (Pierre et al., 2013). [All rats were fed a diet containing cured, dark cooked meat with oxidized nitrite; thus, the effect of meat on promoting DMH-induced lesions could not be determined.]

Female F344 rats (age, 5 weeks) were fed a standard AIN-76 diet. After a 5-day acclimation period, they were treated with a single intraperitoneal injection of DMH at a dose of 180 mg/kg bw. Seven days after being injected, groups of 10 rats were transferred to one of two experimental diets: a low-calcium (700 mg of calcium phosphate per 100 g diet) modified AIN-76 diet containing 40% hot dog meat or a low-calcium (700 mg of calcium phosphate per 100 g diet) modified AIN-76 diet containing 50% French saucisson (fermented, raw, dry sausage). [Both products were made entirely from pork.] Ten additional rats remained on the AIN-76 control diet. The diets were balanced for protein, fat, and iron by the addition of casein, lard, and ferric citrate. The rats continued on the diets for 98–99 days, at which time their colons were examined for MDF. Body weights did not differ among the groups. Rats fed the low-calcium hot dog diet had more MDF per colon and mucin-depleted crypts per colon compared with rats fed the low-calcium control diet ($P < 0.05$). The number of MDF was not increased in rats fed the saucisson diet. The number of ACF was not altered by either of the experimental diets (Santarelli et al., 2013).

In a second experiment that focused on protection rather than tumour promotion, 10 rats were fed the hot dog diet with 500 mg calcium phosphate per 100 g diet, while an additional 10 rats were fed the hot dog diet fortified with 1.5 g of calcium carbonate per 100 g diet. All other aspects were identical to the first tumour-promotion experiment. Body weights did not differ among the groups. Rats fed the hot dog diet fortified with calcium carbonate had a decrease in the number of MDF compared with rats fed the hot dog diet without calcium carbonate ($P < 0.05$) (Santarelli et al., 2013).

3.3 Haem iron

The promotion of colon carcinogenesis by haem iron was observed in two studies. In the first study, male and female *Apc*$^{Min/+}$mice (age, 4 weeks) were given a diet containing 0% (control) or 2.5% haemoglobin for 49 days. Compared with the control diet, the haemoglobin diet significantly increased the intestinal tumour [not further specified] load (114 ± 47 mm^2 vs 67 ± 39 mm^2; $P = 0.004$), the number of tumours in the jejunum ($P < 0.001$), and the number of tumours with a diameter greater than 1 mm ($P < 0.05$). However, the haemoglobin diet did not produce any tumours in wildtype C57BL/6J *Apc*$^{+/+}$ mice (Bastide et al., 2015). In the second study, F344 female rats (age, 7 weeks) were given 2 mg of *N*-methyl-*N*-nitrosourea intrarectally (six times) during the initial 2 weeks, and then fed a diet containing 0% (control) or 3% haemoglobin for 36 weeks. The number of rats with adenomas or adenocarcinomas (combined) in the colon was significantly higher in rats fed the haemoglobin diet than in those fed the control diet ($P < 0.05$) (Sawa et al., 1998).

In another study, male and female A/J$^{Min/+}$ mice (age, 3 weeks) were fed a low-calcium and low vitamin D, semisynthetic diet containing 0.5 µmol/g of hemin chloride and/or 2.8 µmol/g of sodium nitrite for 8 weeks after weaning. Mice fed the hemin chloride diet (10 males, 11 females) had a lower number of tumours ($P = 0.02$) and tumour load (mm^2 per mouse; $P = 0.019$) in the colon than mice fed the control diet (9 males, 10 females). In the small intestine, dietary hemin chloride increased the tumour size (mm^2 per group; $P < 0.001$). In addition, hemin chloride in combination with sodium nitrite had no effect on tumour development in the colon or small intestine of A/J$^{Min/+}$ mice (Sødring et al., 2015). [The Working Group noted that hemin chloride is a toxic chemical that is not present in food (see Section 4.2.6.]

In a study of male C57Bl/6 mice (age, ~8 weeks) fed a diet containing 0.2 µmol/g of hemin for 18 months, no induction of colon tumours was observed (Winter et al., 2014).

3.4 Overview of cancer bioassays for chemicals related to meat consumption

3.4.1 Heterocyclic aromatic amines

HAAs are foodborne carcinogens formed by pyrolysis of creatine, amino acids, and sugars, which are natural components of protein-rich foods, at normal cooking temperatures (Wakabayashi et al., 1992). More than 20 HAAs have been identified.

Among them, 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethyl-imidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) have been found in cooked red meat and processed meat (Wakabayashi et al., 1992; Johansson & Jägerstad, 1994). With the exception of 4,8-DiMeIQx, which was never evaluated, these HAAs have been evaluated by the *IARC Monographs* as having *sufficient evidence* of carcinogenicity in experimental animals (IARC, 1983, 1986, 1993). Studies reporting the carcinogenicity of these nine HAAs in experimental animals are summarized in this section.

(a) IQ

(i) Mouse

Male and female CDF1 mice (age, 7 weeks) fed a diet containing 0.03% IQ for 96 weeks had a higher incidence of hepatocellular adenoma or carcinoma (combined) compared with mice fed a control diet. In addition, the incidences of adenoma or adenocarcinoma (combined) of the lung, and of papilloma or squamous cell carcinoma (combined) of the forestomach were significantly higher in mice fed the IQ diet than in mice fed the control diet (Ohgaki et al., 1984a; IARC, 1993).

(ii) Rat

Male and female Fischer 344 rats (age, 8 weeks) fed a diet containing 0.03% IQ for either 55 weeks or 72 weeks had significantly increased incidences of hepatocellular carcinoma, Zymbal gland squamous cell carcinoma, colon adenocarcinoma, and small intestine adenocarcinoma. (Takayama et al., 1984; IARC, 1993).

When female Sprague-Dawley rats (age, 6 weeks) were given IQ at a dose of 0.35 mmol/kg bw by gavage three times per week during experimental weeks 1–4, twice per week during weeks 5–8, and once per week during weeks 9–31, and continued without treatment until being killed at week 52, the incidences of adenocarcinoma of the mammary gland, tumours of the liver, and squamous cell carcinoma of the Zymbal gland significantly increased (Tanaka et al., 1985; IARC, 1993).

(iii) Monkey

Male and female cynomolgus monkeys (age, 1 year) were given IQ at doses of 10 mg/kg bw (14 males, 6 females) and 20 mg/kg bw (8 males, 12 females) by gavage five times per week for up to 60 months. Hepatocellular carcinoma was observed in three (3 males) monkeys in the low-dose group and in ten (6 males, 4 females) monkeys in the high-dose group. Metastases in the lung and omentum were also observed. No such tumours occurred in more than 300 monkeys in a colony control (Adamson et al., 1990, 1991; IARC, 1993).

(b) MeIQ

(i) Mouse

In male and female CDF1 mice (age, 6 weeks) fed a diet containing 0.01% or 0.04% MeIQ for 91 weeks, the incidence of hepatocellular adenoma or carcinoma (combined) significantly increased in female mice, and the incidence of forestomach papilloma or carcinoma (combined) significantly increased in both males and females. Many of the mice with squamous cell carcinoma of the forestomach demonstrated metastasis to the liver (Ohgaki et al., 1986; IARC, 1993).

(ii) Rat

In male and female Fischer 344 rats (age, 6 weeks) fed a diet containing 0.03% MeIQ for 40 weeks, the incidence of Zymbal gland tumours (most of these tumours were squamous cell carcinoma) significantly increased. Furthermore, the incidences of oral cavity tumours (squamous cell carcinoma or sebaceous squamous cell carcinoma), colon tumours (adenoma or adenocarcinoma), skin tumours (mainly squamous cell carcinoma), and mammary gland tumours (mostly adenocarcinoma) significantly increased (Kato et al., 1989; IARC, 1993).

In male Wistar rats (age, 6 weeks) given MeIQ at a dose of 10 mg/kg bw by gavage every day for 2 weeks, the incidence of Zymbal gland adenoma or carcinoma (combined) significantly increased after 58 weeks (Kristiansen et al., 1989; IARC, 1993).

(c) MeIQx

(i) Mouse

Male and female CDF1 mice (age, 6 weeks) were fed a diet containing 0% (control) or 0.06% MeIQx for 84 weeks. The incidences of hepatocellular adenoma or carcinoma (combined),

lung adenoma and adenocarcinoma (combined), and lymphoma or leukaemia (combined) significantly increased in mice fed the MeIQx diet compared with mice fed the control diet (Ohgaki et al., 1987; IARC, 1993).

In male *c-myc/λlacZ* mice (at weaning) fed a diet containing 0.06% MeIQx for 40 weeks, the incidence of hepatocellular carcinoma was significantly increased compared with *c-myc/λlacZ* mice fed a control diet and C57B1/*λlacZ* mice fed an MeIQx diet (Ryu et al., 1999).

(ii) Rat

In male and female Fischer 344 rats (age, 7 weeks) fed a diet containing 0.04% MeIQx for 61 weeks, the incidences of tumours of the liver (hepatocellular carcinomas or neoplastic nodules, combined) and Zymbal gland squamous cell carcinoma or papilloma (combined) significantly increased. The incidences of clitoral gland squamous cell carcinoma in females and skin tumours (squamous cell carcinoma, basal cell carcinoma, and squamous cell papilloma in males also significantly increased (Kato et al., 1988; IARC, 1993).

(d) PhIP

(i) Mouse

In male and female CDF1 mice (age, 6 weeks) fed a diet containing 0.04% PhIP for 82 weeks, the incidence of lymphoma significantly increased (Esumi et al., 1989; IARC, 1993).

Male and female Eμ-*pim*-1 transgenic mice (a strain predisposed to the development of T-cell lymphoma) and non-transgenic wildtype littermates (age, 9–12 weeks) were fed a diet containing 0.03% PhIP for 31 weeks. PhIP feeding significantly increased the incidence of lymphoma in the female Eμ-*pim*-1 transgenic mice (Sørensen et al., 1996).

Groups of male and female C57BL/6J-*Min*/+ pups were exposed for 3–6 days to breast milk from dams given eight subcutaneous injections of PhIP at a dose of 50 mg/kg, or were given a single subcutaneous injection of PhIP at a dose of 25 or 50 mg/kg. The mice were killed at age 11 weeks. Untreated pups were used as negative controls. The number of tumours of the small intestine was higher in the female pups exposed to breast milk and in the male and female pups subcutaneously injected with PhIP than in the untreated pups (Andreassen et al., 2001, 2002).

In male and female *Apc*1638N mice (age, 4 weeks) fed a diet containing 0.03% PhIP for 32 weeks, a significantly higher number of small intestine tumours (adenoma or adenocarcinoma, combined) was observed in males (Sørensen et al., 1997).

In male and female *Xpa* knockout mice [which lack a nucleotide excision repair system component] (age, 7–9 weeks) fed a diet containing 0.001% or 0.0025% PhIP for 6 months, and subsequently maintained on a normal diet for another 6 months, the incidences of lymphoma and intestinal tumours (combined) were significantly increased when both sexes were combined (Klein et al., 2001). [The Working Group noted the small number of animals.]

(ii) Rat

In male and female Fischer 344 rats (age, 6 weeks) fed a diet containing 0% (control) or 0.04% PhIP for 52 weeks, the incidence of colon adenocarcinoma was significantly higher in males fed the PhIP diet. In addition, the incidence of mammary gland adenocarcinoma was significantly higher in females, and the incidence of prostate carcinoma was significantly higher in males (Ito et al., 1991; IARC, 1993; Shirai et al., 1997).

Groups of female Sprague-Dawley rats (age, 6 weeks) were given PhIP at a dose of 0 (control) or 100 mg/kg bw by gavage twice per week for 4 weeks. All rats were killed at week 48, and there was an increased incidence of mammary gland carcinoma in the PhIP rats compared with the control rats (Kitamura et al., 2006).

(e) Trp-P-1 and Trp-P-2

(i) Mouse and rat

3-Amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) were tested for carcinogenicity in male and female CDF1 mice, as well as in male and female F344 rats (age, 5–8 weeks). The incidence of liver tumours (mostly hepatocellular carcinoma) increased significantly in female mice and male and female rats after oral administration of 0.01–0.02% Trp-P-1, as well as in female mice and male rats after oral administration of 0.01–0.02% Trp-P-2 for 52–112 weeks. Oral administration of Trp-P-2 also significantly increased the incidence of urinary bladder transitional cell papilloma and carcinoma (combined [mainly papilloma]) in male rats (Matsukura et al., 1981; IARC, 1983; Takayama et al., 1985; Takahashi et al., 1993).

(ii) Hamster

Two groups of female Syrian golden hamsters (age, ~6 weeks) were given a single subcutaneous injection of *N*-nitrosobis(2-oxopropyl)amine (BOP) at a dose of 30 mg/kg, followed by two cycles of augmentation pressure; augmentation pressure consisted of four daily intraperitoneal injections of 500 mg/kg of DL-ethionine, a choline-deficient diet, a single intraperitoneal injection of 800 mg/kg of L-methionine, and a subcutaneous injection of BOP at a dose of 20 mg/kg. One group of hamsters was then fed a diet containing 0.02% Trp-P-1 for 50 days, while the other group was fed a basal diet. The number of invasive pancreatic ductal carcinomas was significantly higher in the Trp-P-1 group than in the control group (Mizumoto et al., 1988; Yoshimoto et al., 1999).

(f) AαC and MeAαC

(i) Mouse

In male and female CDF1 mice (age, not reported) fed a diet containing 0.08% 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) or 0.08% 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) for 73–98 weeks, the incidences of hepatocellular adenoma or carcinoma (combined) and vascular system tumours (primarily haemangioendothelial sarcoma) significantly increased with both test agents in male and female mice (Ohgaki et al., 1984b; IARC, 1986).

(ii) Rat

In groups of male F344 rats (age, 6 weeks) fed a diet containing 0.01–0.02% MeAαC for 100 weeks, the incidences of hepatocellular carcinoma, pancreatic acinar cell adenoma, and fibroma of the subcutis significantly increased (Tamano et al., 1994).

(g) 4,8-DiMeIQx

(i) Hamster

Two groups of female Syrian golden hamsters (age, not reported) were initiated with a single subcutaneous injection of BOP at a dose of 30 mg/kg followed by two cycles of augmentation pressure. The hamsters were then fed a diet containing 0% (control) or 0.06% 4,8-DiMeIQx for 50 days. The number of invasive pancreatic ductal carcinomas was significantly higher in the 4,8-DiMeIQx group than in the control group (Yoshimoto et al., 1999).

(h) Combined treatment with HAAs

Male and female F344 rats (age, 6 weeks) were given diets containing five HAAs – 0.003% Trp-P-1, 0.004% Trp-P-2, 0.006% IQ, 0.01% 2-aminodipyridol[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2), and 0.016% AαC – for 722 days. The incidences of liver tumours (primarily hepatocellular carcinoma), Zymbal gland squamous cell carcinoma, and colon adenocarcinoma in

both sexes; skin squamous cell carcinoma in males; and clitoral gland squamous cell carcinoma in females significantly increased in the HAA group compared with the control group (Takayama et al., 1987).

3.4.2 Polycyclic aromatic hydrocarbons

The Working Group has previously reviewed the evidence for the carcinogenicity of 60 non-heterocyclic polycyclic aromatic hydrocarbons (PAHs) in experimental animals (*IARC Monographs* Volume 92; IARC, 2010a). Most of the data were from studies in mice, rats, or hamsters, and the most common routes of administration were cutaneous application, intraperitoneal injection, or addition to the diet, with sites of tumorigenesis usually dependent on the route of administration. Cutaneous application often resulted in skin tumours, and intraperitoneal injection usually resulted in liver and lung tumours. Benzo[*a*]pyrene (BaP), when administered orally, produced tumours of the oral cavity, gastrointestinal tract, liver, lung, and mammary gland in mice and rats (IARC, 2010a).

The following PAHs have been identified in meat products: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*c*]fluorene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, and 5-methylchrysene (see Section 1).

The *IARC Monographs* Volume 92 Working Group (IARC, 2010a) concluded that, for the following PAHs found in meat products, there was *sufficient evidence* in experimental animals for the carcinogenicity of benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene, and 5-methylchrysene. They further concluded that there was limited evidence in experimental animals for the carcinogenicity of benzo[*c*]fluorene and dibenzo[*a,e*]pyrene (IARC, 2010a). No new data released since this review would lead to changing the evaluation of the carcinogenicity in experimental animals for any of these PAHs with a prior evaluation of *limited evidence* were available to the Working Group.

PAHs that were tested by oral administration in experimental animals and identified in meat products include benz[*a*]anthracene, benzo[*c*]fluorene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and dibenzo[*a,l*]pyrene (IARC, 2010a).

As an example, two recent oral administration studies of benzo[*a*]pyrene (BaP) are summarized below.

Groups of female B6C3F$_1$ mice (age, 5 weeks) were fed diets containing BaP at concentrations of 0, 5, 25, and 100 ppm for 2 years (Culp et al., 1998; IARC, 2012). Statistically significant increases in the incidences of tumours of the forestomach, oesophagus, tongue, and larynx were reported. Tumours at all sites were reported to be papillomas or squamous cell carcinomas.

In another study, BaP was administered to groups of male and female Wistar rats (age, 6 weeks) by gavage five times per week for 98 weeks at doses of 0, 3, 10, or 30 mg/kg bw per day (Wester et al., 2012). [Although the authors reported using statistical analyses in the Methods section, none were described for specific tumour end-points.] Significant increases in the incidences of oral tumours (papilloma and squamous cell carcinoma), forestomach tumours (papilloma and squamous cell carcinoma), hepatocellular adenoma and carcinoma, and auditory canal carcinoma in males and females were reported. The incidences of small intestine (jejunum) adenocarcinoma and kidney cortical adenoma also increased in males.

3.4.3 N-*Nitroso compounds*

Eight *N*-nitroso compounds (NOCs) have been detected in meat: *N*-nitrosodi-*n*-butylamine (NDBA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), *N*-nitrosopiperidine (NPIP), *N*-nitrosoproline (NPRO), *N*-nitrosohydroxyproline (NHPRO), and *N*-nitrosopyrrolidine (NYPR) (see Section 1). Of these, the *IARC Monographs* evaluation of carcinogenicity in experimental animals was that there is *inadequate evidence* for NPRO and NHPRO, while there is *sufficient evidence* for the others (IARC, 1978).

A brief summary of the relevant oral administration studies in experimental animals follows (IARC, 1978).

NDBA has been administered orally in lifetime studies of mice, rats, hamsters, and guinea-pigs. Tumour formation was both species- and strain-dependent, with the most common sites being the stomach, liver, oesophagus, and urinary bladder.

NDEA has been fed to mice, rats, hamsters, guinea-pigs, rabbits, dogs, pigs, and monkeys. It induced tumours in all species when fed at doses of 1–13 mg/kg bw per day for life. Tumours of many different types in various organs were reported, with the most common sites being the liver, oesophagus, forestomach, trachea, and lung.

NDMA has been administered orally to mice, rats, hamsters, guinea-pigs, rabbits, and fish. All species were susceptible to increased tumour formation at doses of 0.4–4 mg/kg bw per day. Tumours of the liver were the most prevalent followed by tumours of the lung.

NMEA has only been tested in rats and administered in drinking-water at 1–2 mg/kg bw, which resulted in 9 of 15 treated animals developing hepatocellular carcinoma over an average induction time of 500 days.

NPIP has been fed to mice at dose of 50 mg/kg diet, and rats at doses of 5 and 20 mg/kg bw per day. Mice fed NPIP developed squamous cell carcinomas of the forestomach (18/24), liver tumours (11/24), and lung tumours. Most rats fed the higher dose (20 mg/kg bw per day) died early without tumours. However, those fed the lower dose (5 mg/kg bw per day) developed oesophageal tumours (9/10) and liver tumours.

NPRO has been tested orally in mice. The mice were exposed to NPRO at a concentration of 0.05% or 0.1% in drinking-water for 26 weeks. NPRO or NHPRO has been tested in rats. The rats were exposed to NPRO or NHPRO at a concentration of 0.015% in drinking-water for 75 weeks. For both species, there was no increase in tumour incidence compared with controls.

NYPR has been given to mice at a concentration of 0.01% in drinking-water; however, most mice died early and no increase in tumour incidence was reported. NYPR has also been given at doses of 0.3–20 mg/kg bw per day in many studies in rats. The majority of the studies found that rats developed hepatocellular carcinoma at doses of 1 mg/kg bw per day or higher. A dose–response study found no increase in the incidence of hepatocellular carcinomas with NYPR at 0.3 mg/kg bw per day. However, with NYPR at 1, 3, or 10 mg/kg bw per day, there were 13/62, 30/38, and 9/24 animals with hepatocellular carcinoma, respectively.

The Working Group has previously evaluated the carcinogenic risks of ingested nitrate and nitrite (*IARC Monographs* Volume 94; IARC, 2010b). The Working Group concluded that there was inadequate evidence of carcinogenicity in mice or rats for nitrate administered in drinking-water or diet. One reviewed study showed that nitrate promoted urinary bladder carcinogenesis in rats previously initiated with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. In most of the reviewed studies, nitrite alone in the drinking-water or diet of rats or mice did

not increase the incidence of tumours compared with untreated controls. However, when nitrite in drinking-water or diet was given along with specific secondary or tertiary amines or amides to either mice or rats, there was an increase in tumour incidence. A similar finding was also reported in hamsters. The Working Group concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of nitrate; there was *sufficient evidence* in experimental animals for the carcinogenicity of nitrite in combination with amines or amides; and there was *limited evidence* in experimental animals for the carcinogenicity of nitrite per se. Target sites with increased tumorigenesis after exposure to nitrite in combination with various amines or amides, which are carcinogens by themselves, included the lung, forestomach, uterus, testicle, and lympho-haematopoietic system.

3.4.4 Others

(a) Advanced glycation end-products

No data were available to the Working Group.

(b) N-Glycolylneuraminic acid (Neu5Gc)

In a single study, CMP-*N*-acetylneuraminic acid (Neu5Ac) hydroxylase gene knockout male mice (*Cmah*$^{-/-}$) of a C57BL/6 background (so that they are unable to produce *N*-glycolylneuraminic acid, Neu5Gc) were immunized against Neu5Gc by injection and were fed Neu5Gc at a dose of 0.25 mg/g food. Neu5Gc derived from porcine submaxillary was fed to these mice for 80–85 weeks. Hepatocellular carcinoma was reported in 8 of the 17 mice in the *Cmah*$^{-/-}$ group immunized against Neu5Gc compared with 1 of the 14 knockout mice immunized against Neu5Ac [$P \leq 0.02$, two-tailed Fisher exact test]. Wildtype mice immunized against Neu5Gc had an incidence of hepatocellular carcinoma of 0/11, and wildtype mice immunized against Neu5Ac had an incidence of hepatocellular carcinoma of 1/11 (Samraj et al., 2015).

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