3. Carcinogenicity Studies in Experimental Animals

3.1 Inhalation exposure: mainstream tobacco smoke

This section summarizes the information available on the effects of exposure to mainstream tobacco smoke and the occurrence of cancer in animals and includes data from the previous *IARC Monographs* on tobacco smoking (IARC, 1986) and subsequent publications to date (see Table 3.1).

Although the evidence for the carcinogenicity of tobacco smoke emerged first in humans, there was a need for an inhalation model in experimental animals in which the carcinogenicity of different types of tobacco and tobacco products could be studied. The availability of animal models also permits the investigation of various modifying factors in the development of cancer.

Early inhalation studies in rodents were reviewed by Wynder and Hoffmann (1967). From about 1960 onwards, the species of animal selected most often for carcinogenicity studies was the Syrian golden hamster, which has a low background incidence rate for spontaneous pulmonary tumours and is prone to few interfering respiratory infections. The discovery that inhalation of tobacco smoke caused carcinomas in the larynx of hamsters enabled the establishment of a model system in which the carcinogenicity of tobacco smoke for hamsters has been repeatedly confirmed (see below). Many bioassays have also been conducted in mice and rats and the results are summarized below.

To enable the responses of animals to tobacco smoke inhalation to be studied, it was necessary to develop methods and equipment to deliver smoke in a standardized, effective way. A number of devices have been employed, some involving whole-body exposure and some 'nose-only' exposure. Usually, in order to simulate human smoking patterns, a 2-s puff from a burning cigarette is diluted with air and forced into a chamber for a short period, followed by an air purge. However, animals that are being forced involuntarily to inhale the smoke suffer avoidance reactions and change their breathing patterns to shallow, hesitant inspirations with reduced minute volumes. This behaviour affects the doses delivered to the different parts of the respiratory system. Because rodents are obligatory nose-breathers and because rodents and dogs have more convoluted and intricate nasal turbinate patterns than humans, the dynamics of particle deposition in the upper respiratory tract might be expected to be different (see Wynder & Hoffmann, 1967;

Table 3.1. Selected studies of carcinogenicity in response to exposure to mainstream tobacco smoke in mouse, rat and hamster

Strain	Sex	No. of treated animals/ group	Route	Type of exposure	Concentration of exposure agent	Exposure duration	Lung burden	Tumour incidence	Reference
Mouse									
C57BL	M	100	Nose-only	Mixture of fresh non-filter cigarette smoke/air (1/39, v/v)	Nicotine,0.1 mg/mL; CO, 0.064% (v/v)	12 min/day, lifetime	Nicotine, 14–17 µg	4/100 (alveologenic AdC) [$p = 0.06$], 0/100 (controls)	Harris & Negroni (1967)
	F	100						4/100 (alveologenic AdC) [$p = 0.06$], 0/100 (controls)	
C57BL	NG	126	Whole-body	Gas phase of 12 cigarettes puffed 2 s/min	NG	90 min/day, lifetime (~27 mo)	NG	7/126 (lung A) [NS], 3/90 (controls)	Otto & Elmenhorst (1967)
BLH	NG	126			NG		NG	40/126 (lung A) [NS], 19/60 (controls)	
Snell	M	160	Whole-body	Whole fresh cigarette smoke	NG	2 puffs, once/day, lifetime (26 mo)	Nicotine, 5 µg	7/107 (lung A), 8/106 (controls); 11/107 (lung AdC), 5/106 (controls)	Leuchtenberger & Leuchtenberger (1970)
	F	118						2/65 (lung A), 1/78 (controls); 5/65 (lung AdC), 3/78 (controls)	
	M	100		Gas phase of fresh cigarette smoke	NG		NG	1/44 (lung A), 8/106 (controls); 10/44 (lung AdC) ($p = 0.005$), 5/106 (controls)	
	F	89						3/44 (lung A), 1/78 (controls); 5/44 (lung AdC), 3/78 (controls)	
C57BL	M	100 (2 ×)	Nose-only	Mixture of fresh flue-cured or air cured cigarette smoke/air (1/39, v/v)	NG	12 min/day on alternate days; lifetime	NG	9/162[a] (flue-cured) [$p = 0.07$], 7/189[a] (air-cured) [$p > 0.05$], 3/160[a] (controls)	Harris *et al.* (1974)
	F	100 (2 ×)						7/164[a] (flue-cured) [$p = 0.04$], 0/173 (air-cured), 1/159[a] (controls)	
	M	100		Gas phase of flue-cured cigarette smoke	NG		NG	3/8[a] [$p > 0.05$], 3/160[a] (controls)	Harris *et al.* (1974)

Table 3.1 (contd)

Strain	Sex	No. of treated animals/ group	Route	Type of exposure	Concentration of exposure agent	Exposure duration	Lung burden	Tumour incidence	Reference
(C57BL/ Cum × C3H/Anf Cum)F_1	F	1014 and 2053	Nose-only	10% smoke from US reference cigarettes	NG	Smoke 20 s/min, 6–8 min/day, 5 days/wk, 110 wks	Particulate deposition, 125–200 µg	19/978 (alveolar AdC), 7/651 (sham-exposed controls); shorter latency of tumour occurrence in smoke-exposed group suggested ($p = 0.10$)	Henry & Kouri (1986)
Rat									
Wistar	F	408	Nose-only	Mixture of cigarette smoke/air (1/5)	NG	15 s/min, 2 × 11 min/day, 5 days/ wk, lifetime (140 wks)	NG	4/408 (1 lung C + 3 lung neoplasms of uncertain malignancy), 0/104 (controls), 0/104 (sham treated controls)	Davis *et al.* (1975a)
Fischer 344	F	80	Nose-only	Mixture of non-filter cigarette smoke/air (1/10)	18.4 mg smoke particulate and 0.89 mg nicotine/cigarette	1 cigarette/h, 7 cigarettes/day, 5 days/wk, 128 wks termination at 160 wks	Particulate deposition, 1.75 mg/day	10/80 (1 nasal AdC, 1 nasal C, 5 pulmonary A, 1 pulmonary C, 2 alveologenic C) ($p < 0.05$), 3/93 (controls) 21/80 (subcutaneous sarcomas at forelimb ulceration sites) ($p < 0.05$), 0/93 (controls)	Dalbey *et al.* (1980)
CDF® (Fischer 344)/CrlBR	M, F	NG	Whole-body	Cigarette smoke/air	100 mg (LCS) or 250 mg (HCS) total particulate matter/m^3	6 h/day, 5 days/ wk, 126 wks		Males: 3/173 (lung tumours, LCS); 7/78 (HCS); 3/119 (filtered air) Females: 4/145 (LCS); 6/83 (HCS) [$p < 0.05$]; 0/113 (filtered air)	Finch *et al.* (1995)
Hamster									
Syrian golden	M + F	Group of 80 + 80	Whole-body	Mixture of German ref. cigarette smoke/air (1/15)	NG	Smoke of 30 cigarettes for 7–10 min: 1, 2 or 3 times/day, 5 days/wk, lifetime	NG	1/160, 17/160 and 9/160 (laryngeal C), 0/800 (controls)	Dontenwill *et al.* (1973)
	M + F	Group of 80 + 80		Mixture of dark air-cured cigarette smoke/air (1/15)	NG	Smoke of 30 cigarettes for 7–10 min: 2 times/day, 5 days/wk, lifetime	NG	2/160 (laryngeal C), 0/800 (controls)	Dontenwill *et al.* (1973)

Table 3.1 (contd)

Strain	Sex	No. of treated animals/ group	Route	Type of exposure	Concentration of exposure agent	Exposure duration	Lung burden	Tumour incidence	Reference
Inbred BIO 15.16	M	102	Whole-body	Mixture of US ref. cigarette smoke/air (1/5)	NG	8 puff cycles, twice/day, 5 days/wk, up to 100 wks	NG	9/84 (laryngeal tumour) and 2/84 (nasopharyngeal tumours), 0/42 (sham exposed controls), 0/40 (controls)	Bernfeld *et al.* (1974)
Inbred BIO 87.20	M	102			NG		NG	2/87 (laryngeal tumour), 0/44 (sham exposed controls), 0/48 (controls)	
Syrian golden	M	80	Whole-body	Mixture of German ref. cigarette smoke/air (1/15)	1.5 mg nicotine, 0.173 mg phenol and 12.7 mL CO/cigarette	Smoke of 30 cigarettes for 7–10 min: 1, 2, 2 or 3 times/day, 5 days/wk, lifetime	NG	0, 4, 6 and 11% (laryngeal C), 0% (controls)	Dontenwill *et al.* (1977a)
	F	80						0, 1, 2 and 7% (laryngeal C), 0% (controls)	Dontenwill et al. (1977a)
Inbred BIO 15.16	M	NG	Whole-body	11 or 22% smoke from commercial British filter cigarettes	NG	2 × 12 min/day, 7 days/wk, up to 74–80 wks	NG	3/44 (laryngeal C; 11% smoke) 27/57 (laryngeal C; 22% smoke) 0/36 (sham exposed controls) 0/50 (controls)	Bernfeld *et al.* (1979)

CO, carbon monoxide; AdC, adenocarcinoma; NG, not given; A, adenoma; NS, not significant; mo, month; wk, week; C, carcinoma; LCS, low cigarette smoke; HCS, high cigarette smoke

[a] Most of these lung tumours are adenomas.

Nagano *et al.*, 1982; Proctor & Chang, 1983; Reznik, 1983). Therefore, these exposure systems are not fully representative of the exposure pattern of smokers.

Under experimental conditions, the smoke in the chamber can be assayed for total particulate matter and carbon monoxide, and animals can be examined at intervals to determine the levels of carboxyhaemoglobin, nicotine or cotinine in their blood, and the deposition and retention of particulate matter in tissues, which gives some indication of the doses administered. In typical experiments, rodents were exposed to the smoke of seven to 10 cigarettes per day on 5–7 days per week (Dalbey *et al.*, 1980; Wehner *et al.*, 1981) and were found to have carboxyhaemoglobin levels of 20–40% (Bernfeld *et al.,* 1974).

3.1.1 *Mouse*

A number of early investigations of the effects of mainstream tobacco smoke on tumour development in mice were conducted using the C57BL strain. This strain is relatively resistant to the development of both spontaneous and chemically induced lung tumours (Shimkin & Stoner, 1975). [Therefore, the lack of tumour response in the first few investigations described below may have been due to the choice of this strain.]

Groups of 100 male and 100 female young adult C57BL mice were exposed (nose only) for 12 min/day to a mixture of fresh cigarette smoke and air (1/39, v/v) every other day for life. [The cigarettes were made from a composite blend of flue-cured tobaccos typical of the major brands of untipped cigarette smoked in the United Kingdom (Harris *et al*., 1974).] The concentration of nicotine in the mixture was about 0.1 mg/mL and that of carbon monoxide, 0.064% (v/v). The nicotine content in the lungs of mice that died during exposure was 14–17 μg. Groups of 100 males and 100 females served as controls. No lung tumours, either adenomas or carcinomas, were found in control mice, but lung tumours (alveologenic adenocarcinomas) were found in 4/100 male and in 4/100 female mice exposed to tobacco smoke [$p = 0.06$]. Some of the adenocarcinomas were transplantable. Mice exposed to smoke often showed emphysema and marked peribronchial and perivascular infiltration with lymphocytes, independent of the presence of tumours (Harris & Negroni, 1967).

A total of 126 C57BL and 126 BLH mice [sex and initial group sizes unspecified] were exposed to the gas phase of 12 cigarettes [type unspecified] puffed for 2 s/min. The gas phase was generated by passing the smoke through a Cambridge filter that retained the particulate matter. The mice were exposed in a 200-L chamber for 90 min daily (approximately the maximal tolerated dose) until death (approximately 27 months). The survival rates for animals that received more than 12 months of treatment were not affected by treatment in either strain of mice [numbers not provided]. The percentages of mice with lung adenomas were 5.5% and 32% (7/126 and 40/126) in exposed C57BL and BLH mice and 3.4% and 22% (3/90 and 19/60) in C57BL and BLH controls, respectively. The percentages of BLH mice with lung adenomas that survived for at least 10 months were 37% of the treated group and 31% of the controls. The difference in incidence of

adenoma between the control and treated groups was stated not to be statistically significant (Otto & Elmenhorst, 1967). [The Working Group noted the incomplete reporting of the experiment.]

A group of 50 male C57BL6/Mil mice, 6 weeks of age, was exposed to cigarette smoke from 85-mm unfiltered cigarettes [type unspecified] in a vacuum chamber for 15 min, five times per week, for 63 weeks. The nicotine content in the lungs of mice that were killed ranged from 14 to 27 μg. When the nasal cavity and respiratory tree were examined microscopically, the incidences of hyperplasia and metaplasia were found to be increased in exposed mice. These conditions were considered to be secondary to inflammation. No tumour was found (Wynder *et al.*, 1968). [The Working Group noted the relatively short duration of the study.]

Groups of Snell's mice, 3–4 months of age at the start of treatment, were exposed to two puffs (15-mL puff volume, 2-s duration, 58-s interval between puffs) of either whole fresh cigarette smoke [type unspecified] (160 males and 118 females) or to the gas phase (100 males and 89 females); 117 males and 83 females served as controls. Animals were exposed in individual containers once a day (except during holidays and weekends) for life. When body weight loss was 4 g or more, animals were withdrawn from exposure treatment for periods varying from 2 days to 2 months. Two popular brands of unfiltered cigarettes were used; each cigarette was smoked to a butt length of 23 mm. The gas phase was produced by passing the smoke through a Cambridge filter. The efficiency of exposure was assessed by determining the carbon monoxide concentration in blood and the nicotine content of the lungs (about 5 μg). Survival after 12 months of age was distinctly reduced by treatment, being about 92% (184/200), 60% (172/278) and 40% (88/189) in the control, whole smoke-treated and gas phase-treated groups, respectively. After 12 months of age, the occurrence of pulmonary tumours was earlier in exposed mice than in controls. At the end of the experimental period (26 months), the numbers of mice that survived 12 months or more that had pulmonary adenomas were 8/106 and 1/78 male and female controls, 7/107 and 2/65, respectively ($p = 0.475$), male and female animals exposed to whole smoke and 1/44 and 3/44, respectively ($p = 0.15$), male and female animals exposed to the gas phase. The proportions of mice with pulmonary adenocarcinomas were 5/106 male and 3/78 female controls, 11/107 ($p = 0.15$) male and 5/65 ($p = 0.35$) female animals exposed to whole smoke and 10/44 ($p = 0.005$) male and 5/44 ($p = 0.15$) female animals exposed to the gas phase (Leuchtenberger & Leuchtenberger, 1970).

Groups of 100 male and 100 female C57BL mice, aged 14–18 weeks, were exposed (nose only) to a mixture of fresh cigarette smoke and air (1/39, v/v) for a 12-min period, generally on alternate days, for life. Groups were exposed to the whole smoke of two different types of cigarette, one made from flue-cured and the other from air-cured tobacco. The latter more closely resembled flue-cured cigarettes than typical air-cured cigarettes which contain, for example, burley tobacco. A further group of animals was exposed to the gas phase only of smoke from the flue-cured cigarette; this was generated by passing the whole smoke through a Cambridge filter to remove the particulate matter. A group of 200 males and 200 females served as controls. The experiments with whole smoke were repeated

under similar conditions with another group of 100 male and 100 female mice. Mean survival time in eight of 10 groups of mice exposed to whole smoke was 4–14 weeks longer than that in controls. The incidences of lung tumours in controls were 3/160 males and 1/159 females; those in mice exposed to the whole smoke of cigarettes made from flue-cured tobacco were 9/162 males and 7/164 females [$p = 0.07$ and 0.04], respectively; the incidences in mice exposed to the gas phase of cigarettes made from flue-cured tobacco were 3/81 males [$p > 0.05$] and 2/88 females [$p > 0.05$]; and those in mice exposed to the whole smoke of cigarettes made from air-cured tobacco were 7/189 males [$p > 0.05$] and 0/173 females. The majority of the lung tumours were adenomas (Harris *et al*., 1974).

A group of 117 female BALB/c mice, 9 weeks of age, was exposed to smoke from 'high-tar'(16 mg tar, 1.1 mg nicotine) cigarettes diluted with air (7:1, air:smoke) for 7–8 min per day on 5 days per week for 95 weeks; the exposure was discontinued for 3 weeks starting between the 48th and 49th week of treatment. An untreated group of 130 females served as controls. Groups of approximately 16 control and 16 exposed mice were killed after 56, 64, 72 and 80 weeks of treatment. Twenty control and 10 exposed mice survived until termination of the experiment after 95 weeks. The incidence of bronchial adenomas was calculated by the authors to be 3.8 times higher in the exposed groups of mice than in the control groups after 83 weeks, but to be similar in the two groups after 95 weeks of exposure. The authors also reported an increase in the incidence of lymphomas after both 83 weeks (2.6 times) and 95 weeks (3.2 times) of exposure (Keast *et al*., 1981). [The Working Group noted that insufficient numerical data were provided in relation to the number of animals at risk.]

Two thousand and fifty-three (C57BL/Cum × C3H/AnfCum)F_1 mice were exposed (nose only) to cigarette smoke from University of Kentucky reference 2R1 cigarettes on 5 days/week for 110 weeks and observed until death. In addition, 1014 mice were sham-exposed and 449 mice were held as cage controls. The deposition of smoke particulates was estimated to be about 125–200 μg total particulate matter/lung/day. The only lung cancers observed were diagnosed as alveolar adenocarcinomas. These adenocarcinomas were observed in 19/978 smoke-exposed mice and 7/651 sham-exposed mice. The difference between the incidence in smoke- and sham-exposed groups was not significant, but the data suggested that the tumours occurred with a shorter latency in the smoke-exposed group ($p = 0.10$). Other changes associated with exposure to smoke were increased incidence of pigmented alveolar macrophages, otitis media and head and neck fibrosarcomas (Henry & Kouri, 1986). [The Working Group noted that both parent strains are relatively resistant to the development of spontaneous and chemically induced lung tumours.]

3.1.2 *Rat*

A group of 408 female Wistar rats, 10 weeks of age, was exposed (nose only) to a mixture of smoke and air in a ratio of 1:5 generated from T29 cigarettes (especially manufactured from a composite blend of flue-cured tobaccos, representing the major untipped cigarette brands smoked in the United Kingdom during 1967–68), for 15 s/min during

11 min, twice a day on 5 days per week for life. After 1 year, the rats received 5% carbon dioxide in air instead of air for 5 min before the start of each smoke exposure session and also for the 45-s/min interval during which smoke was not generated. Measurement of an arsenious sulfate tracer showed that 8.5% and 17.5% of the delivered tar was recovered in the lungs of rats subjected to the air and the air/carbon dioxide regimens, respectively. Two control groups of 102 animals were kept, one sham-exposed and one untreated. The smoke-exposed and sham-exposed animals had lower body weights than the untreated controls. About 25% of the treated rats were still alive at 100 weeks compared with 73% of 102 untreated controls and 66% of 102 sham-exposed controls. All treated animals had died by 140 weeks; the control animals had all died by 160 weeks. Three of the smoke-exposed rats had pulmonary squamous neoplasms of uncertain malignancy, and one had an invasive squamous-cell carcinoma of the lung. No lung tumour was observed in the sham-treated or untreated controls. The incidence of benign mammary tumours was lower in the smoke-exposed rats than in the sham-exposed or untreated controls (Davis *et al*., 1975a). [The Working Group noted that the daily exposure period was very brief.]

A group of 18 female Wistar rats, 13–14 weeks of age, was exposed (nose only) to the gas phase of smoke (produced by passing the smoke through a Cambridge filter) from a standard British reference cigarette diluted 1:5 with air. Exposure took place for 15 s followed by exposure to air for 45 s during 11 min, twice daily on 5 days per week for life. A control group of 16 animals was kept. Average period of survival was 100 weeks for treated animals and 114 weeks for controls. No lung tumour was reported in either the exposed or the control rats (Davis *et al*., 1975b). [The Working Group noted the small number of animals used and the short duration of the period of exposure each day.]

A group of 80 female Fischer 344 rats, 12–14 weeks of age, was exposed (nose only) to a mixture of smoke and air in a ratio of 1:10 for 28–30 s/min followed by air for 30 s during standard smoking of a US reference untipped cigarette (average, 8.4 periods of exposure/cigarette). The mean smoke particulate and nicotine levels recorded during chamber monitoring were 18.4 mg and 0.89 mg per cigarette, respectively. The animals were exposed to 1 cigarette/h, 7 cigarettes per day, on 5 days per week for 128 weeks, followed by observation for a further 6 months. The mean pulmonary particulate deposition during exposure was 0.25 mg/cigarette (total, 1.75 mg/rat per day). A group of 63 rats served as untreated controls and 30 rats were sham-exposed. The length of survival of smoke-treated, sham-treated and untreated controls was not significantly different, but smoke- and sham-treated animals had lower mean body weights. Because the tumour incidences in untreated and sham-treated controls were similar, the two groups were combined (93 animals). One alveologenic carcinoma was observed in one control rat and one pulmonary adenomatoid lesion in two other control rats. Ten respiratory tract tumours were observed in seven smoke-exposed rats: one nasal adenocarcinoma, one nasal squamous-cell carcinoma, five pulmonary adenomas, two alveologenic carcinomas and one squamous-cell carcinoma ($p < 0.05$). Of the smoke-exposed animals, 21 developed subcutaneous sarcomas at sites of ulceration on the forelimbs ($p < 0.05$), four rats developed benign tumours of the oral tissues ($p < 0.1$) and four developed adrenal gland

tumours (one adenoma and three carcinomas) ($p < 0.1$). No such tumours occurred in controls. The incidences of tumours of the pituitary gland, uterus and ovary, haematolymphatic system and mammary gland were lower in smoke-exposed rats than in controls (Dalbey *et al*., 1980).

Three groups of 80 female Fischer 344 rats were exposed to a mixture of smoke and air in a ratio of 1:10. The smoke was generated from three research cigarettes [source not specified] containing 25.4 mg tar, 0.16 mg nicotine; 13.3 mg tar, l.06 mg nicotine; or 25.7 mg tar, 1.91 mg nicotine, respectively (see also Griest *et al*., 1980). Each rat was exposed to smoke for 28 s/min followed by air for 30 s during standard smoking of the cigarettes, to provide 10–11 exposures/cigarette. The animals were exposed to eight cigarettes per day during a 16-h period, on 7 days per week for 96 weeks, at which time all survivors (50–70% of animals) were killed. Groups of 80 animals served as untreated and sham-treated controls. The occurrence of squamous metaplasia of the laryngeal and tracheal epithelium was significantly increased in smoke-exposed rats. One squamous-cell carcinoma of the lung was observed in a rat exposed to 'low-tar medium-nicotine' smoke; the incidences of other tumours were similar in treated and in control animals (Wehner *et al*., 1981). [The Working Group noted that, in contrast to the study described previously, all surviving animals were killed at 104 weeks of age and that the method for sampling for histopathology was not fully reported.]

In an experiment in which combined exposure of CDF®(Fischer 344)/CrlBR rats to mainstream tobacco smoke and $^{239}PuO_2$ was studied, two groups of rats (6 weeks of age) were subjected to whole-body exposure for 6 h/day, 5 days per week, for 126 weeks to mainstream cigarette smoke diluted to concentrations of either 100 or 250 mg total particulate matter/m^3 (LCS and HCS groups). A significant increase in the occurrence of lung tumours was found in females exposed to smoke only but not in males. The incidences of benign and/or malignant lung tumours in female rats were 0/113, 4/145 (3%) and 6/83 (7%; $p < 0.05$; one-sided Yates test) in the groups treated with filtered air alone, and the LCS and HCS groups, respectively. The corresponding tumour incidences for males were 3/119 (2.5%), 3/173 (2%) and 7/78 (8%), respectively (Finch *et al*., 1995).

3.1.3 *Syrian hamster*

In a study involving 4440 hamsters, three groups of 80 male and 80 female Syrian golden hamsters, 8 weeks of age, were exposed to a mixture of smoke and air in a ratio of 1:15. The smoke was generated during a 7–10-min period from 30 German reference cigarettes, once, twice or three times a day on 5 days per week until death. Two groups of 80 males and 80 females were exposed either to the smoke of black (dark air-cured)-tobacco cigarettes twice a day, or to the vapour phase of German reference cigarettes, generated from 30 cigarettes during a 10-min period twice a day. Eight hundred animals served as untreated controls. The average length of survival was 52–65 weeks for treated males and 41–49 weeks for treated females. When compared with untreated controls, a shorter survival time and loss of body weight were noted in animals exposed

to smoke. In all treated groups, 'the most remarkable and severe alterations' were observed in the larynx, and their severity depended on duration of treatment and dosage. The incidence of laryngeal leukoplakias ranged from an average of 11.3% in animals exposed to the reference cigarette for 10 min to 30.6% in those exposed for 30 min; the incidence in animals exposed to smoke from black-tobacco cigarettes was 10%, and slight leukoplakia was observed in 1–5% of control animals. The incidences of laryngeal carcinomas were 0.6–10.6% in animals exposed to smoke from the reference cigarette and 1.25% in animals exposed to smoke from black-tobacco cigarettes. No such tumour was observed in control animals or in those exposed to the vapour phase (Dontenwill *et al*., 1973).

Similar results were obtained in a study involving 2160 hamsters exposed to the smoke from German reference cigarettes or a modification thereof (Dontenwill *et al*., 1977a).

A modification of the method of Dontenwill (above) was used in a study of inbred Syrian golden hamsters. Groups of 102 male BIO® 87.20 and BIO® 15.16 hamsters (13 weeks of age) were exposed to smoke (1:5 in air) from University of Kentucky 1R1 reference cigarettes twice a day on 5 days per week for up to 100 weeks. Groups of 60 animals served as sham-exposed or cage controls for each strain. Exposure to smoke for up to 100 weeks had no effect on length of survival; however, a reduction in body weight was noted. Over 90% of the smoke-exposed animals of both strains showed hyperplastic or neoplastic changes in the larynx; and benign squamous-cell papillomas of the larynx were observed in both strains. Laryngeal cancer occurred nearly five times more frequently in strain BIO® 15.16, however, and two animals of this strain developed nasopharyngeal tumours. The incidences of tumours at locations other than the respiratory tract were similar in the two strains (Bernfeld *et al*., 1974).

Groups of 35–64 male BIO® 15.16 inbred Syrian golden hamsters, 60–70 days of age, were exposed to concentrations of 11% or 22% smoke from commercial British filter-tipped cigarettes or filter-tipped cigarettes composed of tobacco and Cytrel® (a tobacco substitute), or cigarettes made of 100% Cytrel® twice a day (12-min sessions of 27 smoke exposures/min) on 7 days per week for up to 74–80 weeks. Groups of 40 sham-exposed animals and 51 caged animals served as controls. Survival rates were markedly reduced in all groups after 59 weeks of age; a reduction in body weight was noted in treated animals when compared with controls. Laryngeal carcinomas occurred in 47.4% (27/57) of the animals exposed to smoke from the British filter-tipped cigarettes; the first tumours occurred after 59 weeks in the hamsters treated with the 22% smoke concentration. The incidence of this type of tumour was dose-related and decreased as the proportion of Cytrel® in the blends increased. No such tumour was observed in controls. Laryngeal papillomas, laryngeal epithelial hyperplasia and a few tracheal papillomas were also observed in treated animals. Histopathological findings at other sites did not appear to be related to smoke inhalation (Bernfeld *et al*., 1979).

A group of 51 male Syrian golden hamsters, 2 months of age, was exposed three times a day for 10 min, 5 days per week, for life, to smoke from University of Kentucky 1Rl

reference cigarettes. Another group of 51 male hamsters served as sham-exposed controls. The smoke-exposed animals survived longer (mean, 19.6 months) than the sham-exposed animals (mean, 15.3 months). The smoke-exposed animals had significantly higher incidences of epithelial lesions of the larynx than the sham-exposed controls (22% versus 0%, $p < 0.01$) and also had a significantly higher total number of tumours than sham-exposed controls (28% versus 6%; $p < 0.05$). The laryngeal changes in the smoke-exposed group ranged from inflammatory conditions, with growth abnormalities of the epithelium, to squamous-cell papilloma formation (Wehner *et al*., 1974, 1975a,b, 1976).

3.1.4 *Rabbit*

Thirty rabbits were exposed daily in individual compartments to the smoke from 20 cigarettes (2 puffs/min, 2 cigarettes at each treatment period) for up to 66 months. No tumour that could be related to the exposure was observed in any of the exposed rabbits or in 31 controls that survived for 24 months or more (Holland *et al*., 1958, 1963). [The Working Group noted that details of the types of cigarettes, method of smoke generation and doses delivered were not provided.]

3.1.5 *Dog*

Groups of male beagle dogs, 1.7–3.3 years old, trained to inhale cigarette smoke through tracheostomata, were exposed to one of three different treatments: (1) smoke from seven untipped cigarettes containing 34.8 mg tar and 1.85 mg nicotine per day (62 dogs), (2) smoke from seven cigarettes containing the same tobacco, but with filter tips, containing 17.8 mg tar and 1.17 mg nicotine (12 dogs) or (3) smoke from 3.5 untipped cigarettes containing the same tobacco per day (12 dogs). Eight non-exposed tracheotomized dogs served as controls. The body weights of the dogs in the various groups did not differ significantly. Between day 57, when the training period ended, and day 876, when killing of the surviving dogs began, 28 dogs died: 24 in the first group, two in the second, two in the third and none in the control group. Extensive examination of the lungs and bronchial tree at autopsy and microscopically was carried out. Lesions of the lung (reported as tumours), described as 'bronchiolo-alveolar, invasive and noninvasive', were found in 23/62 of dogs in group 1; two of the lesion-bearing dogs in this group also had small bronchial lesions. Non-invasive bronchiolo-alveolar lesions were also found in 4/12 dogs in group 2, 7/12 dogs in group 3 and 2/8 control dogs. The bronchiolo-alveolar lesions tended to be multiple, with as many as 20 per lung lobe, and were found in 41 of the 203 lung lobes from the 29 dogs with such lesions. No distant metastasis was found (Auerbach *et al*., 1970; Hammond *et al*., 1970). [The Working Group noted that no data were given on specific smoking parameters or measures of exposure. They also noted the small number of control dogs used and the unusually high incidence of lung lesions in these animals; these are lesions that rarely occur spontaneously in dogs. The focal inflammatory lesions usually found in the lungs of animals exposed to smoke were not men-

tioned in the report of this study. Examination of the upper respiratory tract and other organ systems was not reported. The authors' interpretation of the photomicrographs as representing neoplasia was not considered to be entirely convincing.]

As part of a study on the combined effects of radon daughters or uranium ore dust and cigarette smoke on the induction of respiratory tumours (see below), 19 male and female beagle dogs, 24–30 months of age, were exposed to cigarette smoke only. The dogs were exposed to the smoke of 10 University of Kentucky 1R1 reference cigarettes per day (three in the morning, four at midday and three in the afternoon) on 7 days per week for 48–60 months; individual masks permitted smoke to be inhaled at every 10th breath. Eight dogs served as sham-exposed controls. Carboxyhaemoglobin levels in smoke-exposed dogs increased with the duration of the experiment, from 2% at 19 months to 4.7% after 50 months; the corresponding mean values for sham-exposed dogs were 1.4% and 1.8%, respectively. Smoke-exposed animals killed after 49 (six dogs), 60 (six dogs), 64 (six dogs) and 65 months (three dogs) were available for analysis. No significant respiratory lesion and no lung tumour was found in either group (Cross *et al*., 1982). [The Working Group noted the small sizes of the groups and the limited evidence of smoke delivery.]

3.2 Inhalation exposure in conjunction with administration of known carcinogens and other agents

3.2.1 *Mouse*

(*a*) *4-(*N-*Nitrosomethylamino)-1-(3-pyridyl)-1-butanone*

A 6-month bioassay in strain A/J mice was conducted to determine whether chronically inhaled mainstream cigarette smoke would either induce lung tumours or promote lung cancer induced by the tobacco-specific nitrosamine, 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK). Groups of 20 female A/J mice were exposed to filtered air or cigarette smoke, injected with NNK, or exposed to both cigarette smoke and NNK. At 7 weeks of age, mice were given a single intraperitoneal injection of NNK at a dose of 100 mg/kg bw and, 3 days later, they were exposed to cigarette smoke for 6 h/day, 5 days per week, for 26 weeks at a mean concentration of 248 mg total particulate matter/m^3. Animals were killed 5 weeks after cessation of exposure for gross and histopathological evaluation of lung lesions. No significant differences in survival between exposure groups were observed. A biologically significant level of exposure to cigarette smoke was achieved as indicated by the reductions in body weight, increases in lung weight and increases in carboxyhaemoglobin levels in blood of about 17%. Tumour incidences were similar between the groups exposed to cigarette smoke and filtered air, and the groups treated with NNK and with cigarette smoke + NNK. Incidences in either of the latter groups were higher than in the groups exposed to cigarette smoke and filtered air. Tumour multiplicity in tumour-bearing animals did not differ significantly between any of the

three groups (filtered air, NNK, cigarette smoke + NNK) in which tumours were observed. Thus, exposure to cigarette smoke neither induced lung tumours nor promoted NNK-induced tumours (Finch *et al*., 1996). [The Working Group noted that the animals were killed 5 weeks after cessation of exposure, which may have been an insufficiently long period to permit tumour development. See also Witschi *et al*. (1997).]

(*b*) *Influenza virus*

The possible interaction between various types of influenza virus and the inhalation of cigarette smoke in the induction of lung tumours in mice has been examined in a series of studies (Harris & Negroni, 1967; Harris *et al*., 1974; Wynder *et al*., 1968). No additive or synergistic effect was observed.

3.2.2 *Rat*

Selected studies are summarized in Table 3.2.

(*a*) *Benzo[*a*]pyrene*

A group of 84 female Wistar rats received a single intratracheal instillation of 2 mg benzo[*a*]pyrene (with infusine and carbon black) followed by exposure to cigarette smoke (cigarette T29, specially manufactured from a composite blend of tobacco representing the major plain cigarette brand smoked in the United Kingdom, diluted 1:5 with air), twice daily, five times per week for life. Three developed squamous-cell carcinomas of the lung. A squamous-cell carcinoma of the lung was observed in one of the 84 rats treated with the benzo[*a*]pyrene mixture alone, and four squamous-cell neoplasms (of which only one was clearly malignant) occurred among 408 rats exposed to cigarette smoke only. The mean survival times were 65 weeks for animals exposed to the smoke plus benzo[*a*]pyrene mixture, 63 weeks for animals exposed to smoke alone and 108 weeks for rats treated with the benzo[*a*]pyrene mixture alone. No lung tumour was observed in untreated or sham-exposed controls (Davis *et al*., 1975a).

Groups of 35 male Wistar rats (weighing 50–70 g) were fed either vitamin A-deficient feed or a conventional diet and were exposed to three weekly intratracheal initiation doses of 20 mg benzo[*a*]pyrene mixed with ferric oxide during the 6th–8th week of the study, or subjected to cigarette smoke from the 2nd to 24th week or the 10th–24th week, or subjected to both types of treatment. The animals were exposed to five cigarettes, 1 h/day in 8.2-L chambers. Animals were killed after the 24th week. Inhalation of cigarette smoke during the initiation and post-initiation phases of carcinogenesis resulted in higher lung tumour multiplicity (2.14 tumours/animal) than that observed in rats exposed during the post-initiation phase only (1.33 tumours/animal) or in the rats treated with benzo[*a*]pyrene only (1.22 tumours/animal). This enhancement of tumour multiplicity was increased further by vitamin A deficiency (2.86 versus 1.67 or 1.83 tumours/animal, respectively). These tumours were classified histopathologically as squamous-cell carcinomas (Gupta *et al*., 1990).

Table 3.2. Selected studies of carcinogenicity in response to exposure to mainstream tobacco smoke in conjunction with exposure to known carcinogens or other agents in rat and hamster

Strain	Sex	No. of treated animals/ group	Agent co-administered	Type of exposure to cigarette smoke	Exposure duration	Tumour incidence	Reference
Rat							
Wistar	F	84–408	Benzo[*a*]pyrene (2 mg) + infusine + carbon black; intratracheally	British reference cigarette smoke/air (1/5)	1 cigarette, twice/day, 5 days/wk, lifetime	3/84 (lung C), 1/84 (lung C; benzo[*a*]pyrene alone), 4/408 (3 A + 1 malignant neoplasm; cigarette smoke only), 0/204 (controls + sham-exposed controls)	Davis *et al.* (1975a)
Wistar	M	35	Benzo[*a*]pyrene (20 mg) + Fe_2O_3; intratracheally (3 weekly doses) during 6th–8th wk of the study	Cigarette smoke (5 cigarettes/8.2 L air)	1 h/day, during 2nd–24th week or 10th–24th week of the study	Conventional diet: 2nd–24th wk, 2.14 lung C/animal; 10th–24th wk, 1.33 lung C/animal; benzo[*a*]pyrene control, 1.22 lung C/animal. Vitamin A-deficient diet: 2nd–24th wk, 2.86 lung C/animal; 10th–24th week, 1.67 lung C/animal; benzo[*a*]pyrene control, 1.83 lung C/animal	Gupta *et al.* (1990)
Sprague-Dawley	NG	28–50	Radon progeny (4000, 500 or 100 WLM)	French reference cigarette smoke (9 cigarettes/ 500 L air)	10–15 min session, 4 days/wk, 1 year	4000 WLM: 34/50 (lung C) [$p = 0.0015$]; 17/50 (lung C; radon progeny alone) 500 WLM: 8/30 (lung C); 2/28 (lung C; radon progeny alone) 100 WLM: 1/30 (lung C); 0/50 (radon progeny alone)	Chameaud *et al.* (1982)
CDF® (Fischer 344)/ CrlBR	M, F	NG	$^{239}PuO_2$ aerosol, 1 wk (6th wk of the study)	Cigarette smoke/air, 100 mg (LCS) or 250 mg (HCS) total particulate matter/m^3	6 h/day, 5 days/wk, 126 wks	49–61% (lung tumours, LCS + $^{239}PuO_2$) 72–74% (HCS + $^{239}PuO_2$) 20–33% ($^{239}PuO_2$) 2–3% (LCS) 7–8% (HCS)	Finch *et al.* (1995)

Table 3.2 (contd)

Strain	Sex	No. of treated animals/ group	Agent co-administered	Type of exposure to cigarette smoke	Exposure duration	Tumour incidence	Reference
Hamster							
Syrian golden/ M + F	M + F	(80 + 80)	DMBA (0.5 mg); intra-tracheally	German ref. cigarette smoke/air (1/15)	Smoke of 30 cigarettes for 7–10 min: twice/day, 5 days/wk, lifetime	32/160 (laryngeal C), 17/160 (laryngeal C; smoke only), 0/160 (DMBA alone)	Dontenwill *et al*. (1973)
Syrian golden	NG	20–40	DMBA (0.24 mg); intra-laryngeally	Cigarette smoke/air (1/7)	Cigarette smoke 2 × 10 min/day, 5 days/wk, 48 wks	3/40 (laryngeal C), 0/20 (smoke only), 0/20 (DMBA alone)	Hoffmann *et al*. (1979)
Syrian golden	M	10–30	NDEA (100 mg/kg bw); subcutaneously	Cigarette smoke/air (1/7)	Smoke of 30 cigarettes for 9 min: twice/day, 5 days/wk, 12 wks	Non-filter cigarettes (2.10 ± 1.74 P+H/animal) [$p < 0.01$] and filter cigarettes (1.93 ± 1.55 P + H/animal) [$p < 0.01$] versus sham-exposed (0.97 ± 1.03 P + H/animal)	Takahashi *et al*. (1992)
Syrian golden	M	30	NDEA (10 mg/hamster); subcutaneously (12 weekly doses)	Non-filter cigarette smoke/air (1/7)	Smoke of 30 cigarettes for 6 min: twice/day, 5 days/wk, 58 wks	14/30 (nasal cavity tumours) ($p < 0.05$), 5/30 (NDEA alone)	Harada *et al.* (1985)

C, carcinoma; A, adenoma; NG, not given; WLM, work-level-months; LCS, low cigarette smoke; HCS, high cigarette smoke; DMBA, 7,12-dimethylbenz[*a*]anthracene; NDEA, *N*-nitrosodiethylamine; P + H, epithelial hyperplasias and papillomas

(*b*) *Chrysotile*

Yoshimura and Takemoto (1991) exposed male Wistar rats intratracheally to 15 mg asbestos (chrysotile) fibres. One month later, the rats were exposed to cigarette smoke in an exposure chamber (smoke from 10 cigarettes/day, 6 days per week, for 1 month). In the group exposed both to asbestos and to cigarette smoke, 4/29 rats developed lung carcinomas, as compared with 1/31 of the rats exposed to asbestos alone. No mesotheliomas were found in the rats treated with asbestos alone, whereas 2/29 rats exposed to a combination of asbestos and cigarette smoke developed mesotheliomas. [The Working Group noted the small numbers of animals per group.]

(*c*) *Radon progeny*

Groups of 28–50 Sprague-Dawley rats were exposed to radon progeny at cumulative doses of 4000, 500 or 100 work-level-months (WLM)[1], with or without concurrent exposure to cigarette smoke by inhalation on 4 days per week for 1 year. Of the 50 rats exposed to 4000 WLM radon progeny without exposure to smoke, 17 developed carcinomas of the lung. In the same group, 34 carcinomas were seen in 50 rats exposed to radon and cigarette smoke. In the groups of rats exposed to 500 WLM radon progeny, two carcinomas of the lung were seen in the 28 rats exposed to radon only as compared with 8/30 rats exposed to radon and cigarette smoke. No lung tumour was observed in any of the 50 rats exposed to 100 WLM radon only, whereas one carcinoma was observed among 30 rats exposed to 100 WLM radon and cigarette smoke. Histopathological evaluation of the lung tumours indicated that approximately 75% were squamous-cell carcinomas, 20% were adenocarcinomas and the remainder were undifferentiated carcinomas (Chameaud *et al*., 1982).

(*d*) *Plutonium oxide*

Beginning at 6 weeks of age, CDF®(Fischer 344)/CrlBR rats were subjected to whole-body exposure for 6 h/day, on 5 days per week, to either filtered air or mainstream cigarette smoke diluted to concentrations of either 100 or 250 mg total particulate matter/m^3 (LCS and HCS groups, respectively). At 12 weeks of age, rats were removed from the smoke exposure chambers and exposed nose-only to either filtered air or plutonium oxide ($^{239}PuO_2$) aerosol, and were returned to the smoke chambers 1 week later for 30 months of continued exposure to either filtered air or cigarette smoke. The incidences of lung tumours in animals exposed to cigarette smoke only are given in Section 3.1.2. In both sexes, there was a pronounced interaction between exposure to smoke and $^{239}PuO_2$ in producing lung tumours. Tumour incidence reached 72–74% in HCS + $^{239}PuO_2$-exposed rats and 49–61% in LCS + $^{239}PuO_2$-exposed rats, whereas the incidence was 20–33%,

[1] WLM is defined as any combination of the short-lived radon daughters in 1 L of air that will result in an ultimate emission of 1.3×10^5 MeV of potential alpha energy in their decay through ^{214}Po. WLM is equivalent to 170 h of exposure to 1 WL.

2–3% and 7–8%, respectively, in rats exposed to $^{239}PuO_2$, LCS or HCS alone. The most prevalent malignant neoplasms were adenocarcinomas, followed by squamous-cell carcinomas and adenosquamous carcinomas. In groups exposed to both agents, there was increased tumour multiplicity, an increased proportion of neoplasms of the squamous phenotype, and several animals with airway-associated lung neoplasms (Finch *et al*., 1995). [The Working Group noted the incomplete reporting of the study.]

3.2.3 *Syrian hamster*

Selected studies are summarized in Table 3.2.

(*a*) *Benzo[*a*]pyrene*

A group of 40 Syrian golden hamsters received a single intratracheal administration of 5 mg benzo[*a*]pyrene mixed with ferric oxide, followed by exposure to cigarette smoke twice for 10 min daily, five times per week, for 48 weeks. Two papillomas of the larynx and three epithelial hyperplasias of the larynx were observed. No such lesion was observed in control groups of 20 hamsters that received benzo[*a*]pyrene plus ferric oxide or smoke alone, and no lung tumour was observed (Hoffmann *et al.*, 1979).

(*b*) *Blue Cape asbestos*

Groups of 80 male and 80 female Syrian golden hamsters were exposed to a single intratracheal instillation of asbestos, followed by exposure to cigarette smoke twice daily, five times per week for life. No significant difference in the occurrence of laryngeal lesions or tumours was observed when compared with another group of hamsters exposed to cigarette smoke alone. No laryngeal tumour occurred in a control group exposed to a single dose of asbestos alone (Dontenwill *et al*., 1973).

(*c*) *7,12-Dimethylbenz[*a*]anthracene*

Three groups of 80 male and 80 female Syrian golden hamsters received 500 μg 7,12-dimethylbenz[*a*]anthracene (DMBA) intratracheally, followed by a 10-min exposure to cigarette smoke twice daily, five times per week for life, or were treated either with cigarette smoke or DMBA only. A total of 32 squamous-cell carcinomas of the larynx were observed in animals treated with both DMBA and cigarette smoke, in comparison with 17 in hamsters exposed to cigarette smoke only and none in hamsters treated with DMBA alone. A similar trend in the incidence of laryngeal leukoplakia was also observed. No increase was observed in the incidences of tumours at other sites in animals treated with both DMBA and cigarette smoke, when compared with animals treated with DMBA alone. Mean survival rates were comparable in all groups (Dontenwill *et al.*, 1973). Similar results were reported from other experiments in which Syrian golden hamsters were exposed to DMBA and cigarette smoke (Kobayashi *et al*., 1974; Hoffmann *et al*., 1979).

(*d*) *4-(*N-*Nitrosomethylamino)-1-(3-pyridyl)-1-butanone*

Groups of 10 male and 9–10 female Syrian golden hamsters received a single subcutaneous injection of NNK at doses ranging from 1 to 10 mg, followed by exposure to University of Kentucky 1R1 reference cigarette smoke twice daily for 69 weeks. Tumours of the respiratory tract were observed in NNK-treated hamsters; their incidence was not affected by exposure to cigarette smoke (Hecht *et al.*, 1983).

(*e*) N-*Nitrosobis(2-oxopropyl)amine*

The influence of tobacco smoke on *N*-nitrosobis(2-oxopropyl)amine (NBOPA)-induced carcinogenesis of the pancreatic duct and respiratory tract was investigated using a two-stage carcinogenesis model in hamsters. Five-week-old male hamsters were divided into five groups. Group 1 ($n = 30$) was injected subcutaneously with NBOPA at a dose of 10 mg/kg bw once a week for 3 weeks for tumour initiation and exposed to cigarette smoke over the same period. Group 2 ($n = 30$) was exposed to cigarette smoke for 26 weeks after the NBOPA initiation. Group 3 ($n = 30$) was given NBOPA initiation alone and group 4 ($n = 10$), 26 weeks of exposure to cigarette smoke alone. Group 5 ($n = 10$) served as a sham-exposed negative control. The experiment was terminated 30 weeks after the first NBOPA injection. The incidence of pancreatic carcinomas was significantly decreased in group 1 (5/29, $p < 0.01$) relative to group 3 (17/30), whereas the incidence in group 2 (12/29) was not significantly less than that in group 3. In contrast, the incidence of proliferative lesions of the larynx and trachea (hyperplasias) was significantly higher in group 2 (11/29, $p < 0.01$) than in group 3 (2/30). The incidence of pulmonary hyperplasias was also higher in group 2 (13/29, $p < 0.05$) than in group 3 (5/30), although that of pulmonary adenomas or adenocarcinomas was lower in group 2 (14/29, $p < 0.01$) than in group 3 (24/30). Exposure to cigarette smoke in the NBOPA-initiation phase (group 1) did not affect the development of respiratory lesions. No animals in groups 4 or 5 developed any tumours of the pancreas or respiratory tract. These results suggest that exposure to cigarette smoke inhibits pancreatic carcinogenesis when given in the initiation phase, whereas it modulates (enhances or suppresses) the development of proliferative lesions in the respiratory tract if applied during the promotion stage to hamsters pre-treated with NBOPA (Nishikawa *et al*., 1994). [The Working Group noted the small size of the experimental groups and the short duration of the study.]

(*f*) N-*Nitrosodiethylamine*

The potential short-term promoting effects of cigarette smoke on the development of tumours in the respiratory system were investigated in male Syrian golden hamsters. A dietary supplement of vitamin C (1%) was given to groups of 30 male Syrian hamsters exposed to cigarette smoke in air (1/7) from 30 unfiltered cigarettes for 6 min, twice a day, 5 days a week and also receiving 12 weekly subcutaneous injections of *N*-nitrosodiethyl-amine (NDEA) (total dose, 10 mg/hamster) to determine whether or not high doses of vitamin C could prevent the development of tumours by NDEA in the respiratory tract.

The experiment was terminated 58 weeks after initiation of the treatments. Treatment with NDEA resulted in the development of both benign and malignant tumours of the respiratory tract, and co-exposure to cigarette smoke potentiated the development of tumours in the nasal cavity (14/30 versus 5/30, $p < 0.05$) (Harada *et al*., 1985).

Three groups (1, 2 and 3) of 30 animals each received a single subcutaneous injection of 100 mg/kg bw NDEA and were then exposed to smoke from untipped cigarettes (NC), from filter-tipped cigarettes (FC) and sham smoke, respectively, from week 1 to week 12. In addition, groups 4, 5 and 6 (10 animals each) did not receive treatment with NDEA, but were exposed to the NC smoke, FC smoke or sham smoke, respectively, for the same time period. In the NDEA-treated groups, epithelial hyperplasias and/or papillomas were induced; in groups 1 and 2, the incidences and numbers of these lesions per animal were significantly higher than in group 3 (2.10 ± 1.74 and 1.93 ± 1.55 versus 0.97 ± 1.03, $p < 0.01$) (Takahashi *et al*., 1992).

3.2.4 *Dog*

Nineteen male and female beagle dogs, 24–30 months old, were exposed by inhalation to a combination of radon (105 nCi/L [3900 Bq/L]), radon progeny (605 WLM) and uranium ore dust (12.9 mg/m^3) for 4 h/day, on 5 days per week, for 54 months. A second group of 19 beagle dogs was exposed to the smoke from 10 cigarettes/day on 7 days per week at intervals between exposures to radon progeny and uranium ore dust. Lifespan was shorter in both groups than in controls. Eight dogs exposed to radon alone had nine respiratory tumours (two nasal carcinomas, six pulmonary carcinomas, one pulmonary fibrosarcoma); and 2/19 dogs in the group that received radon plus cigarette smoke had respiratory tumours (one nasal carcinoma and one pulmonary carcinoma) (Cross *et al*., 1982). [The Working Group noted the small sizes of the groups and the incomplete reporting.]

3.3 Administration of tobacco-smoke condensate[1]

Selected studies are summarized in Table 3.3.

Attempts to induce tumours with tobacco products were reported as early as 1911 (for a summary of early studies, see Wynder & Hoffmann, 1967). In the 1950s, Wynder *et al*. (1953) painted the skin of CAF_1 mice with cigarette-smoke condensate (CSC) suspended in acetone. After 24 months, 48/81 mice had developed skin papillomas and 36/81 had developed skin epidermoid carcinomas at the sites of application.

[1] The terms 'smoke condensate' and 'tar' are often used interchangeably. Cigarette-smoke condensates are produced by passing smoke through cold traps and recovering the material retained within them. This material is often washed from the traps with a volatile solvent, which is later removed, and unknown amounts of the volatile and semivolatile constituents are lost. Total particulate matter (TPM) is that material which is retained in a high-efficiency particulate filter. If this material is washed from the filter and concentrated as above, semivolatiles are lost. In the USA, the term 'tar' as used in official reports of tar yield is equivalent to TPM less nicotine and water.

Table 3.3. Selected studies of carcinogenicity in response to exposure to cigarette-smoke condensate in mouse, rat and rabbit

Strain	Sex	No. of treated animals/ group	Type of exposure	Exposure dosage and duration	Tumour incidence	Reference
Mouse						
CAF1	M + F, 1:1	44–112	Skin painting (dorsal) of CSC	CSC/acetone solution (40 mg CSC/ application), × 3/wk, lifetime	36/81 (skin epidermoid C), 0/30 (acetone controls)	Wynder *et al*. (1953)
ICR Swiss	F	5200	Skin painting (dorsal) of CSC	CSC/acetone solution (150 mg or 300 mg CSC/week), × 6/wk, 78 wks	482/5200 (skin C), 3/800 (acetone controls)[a]	Gargus *et al.* (1976)
ICR Swiss	F	4900	Skin painting (dorsal) of CSC	CSC/acetone solution (25 mg or 50 mg CSC/application), × 6/wk, 78 wks	1157/4900 (skin C), 0/800 (acetone controls)	Gori (1976)
ICR/Ha Swiss	F	100	Topical application with CSC to oral mucosa (lips and oral area)	CSC/acetone (26 mg CSC/application), × 5/wk, 15 months	52/81 (lung tumours)[b] ($p < 0.0001$), 20/89[b] (acetone controls)	DiPaolo & Levin (1965)
Initiation study						
ICR/Ha Swiss	F	30	Skin painting (dorsal) with CSC active fraction with or without subsequent painting of the skin with croton oil	CSC active fraction/acetone (2.5 mg of 0.6% CSC/application), 10 × on alternate days Croton oil (2.5%), × 3/wk, up to 15 months, 10 days after last CSC active fraction application	After 12 and 15 months: 4/30 (skin C), 0/65 (croton oil controls)	Hoffman & Wynder (1971)
Promotion study						
Swiss	F	30–50	Skin painting (dorsal) of CSC with or without initiation by DMBA application	DMBA (75 µg); CSC/acetone (75 mg CSC/application, start: 1 wk after DMBA application), × 2–3/wk, 12 months – animals observed 3 months later	DMBA: 2/30 (skin C) (7 %) 2 × CSC: 1/40 (skin C) (3 %) DMBA + 2 × CSC: 8/30 (skin C) (27 %) 3 × CSC: 11/50 (skin C) (22 %) DMBA + 3 × CSC: 11/30 (skin C) (37 %)	Wynder & Hoffmann (1961)
Other tobacco						
Swiss albino	M	15	Oral gavage of Indian bidi smoke condensate	1 mg bidi smoke condensate/0.1 mg DMSO, 5 days/wk, 55 wks, termination 90 wks	4 hepatic haemangiomas, 1 stomach papilloma and C, and 1 oesophageal C/15 mice; 0/15 (untreated or DMSO-treated controls)	Pakhale *et al*. (1988)

Table 3.3 (contd)

Strain	Sex	No. of treated animals/ group	Type of exposure	Exposure dosage and duration	Tumour incidence	Reference
Rat						
Osborne Mendel	F	NG	Intrapulmonary administration of CSC pellet	CSC/beeswax:tricaprylin (24 mg CSC/injection), up to 107 wks after implantation	14/40[c] (lung squamous-cell C), 0/63[c] (beeswax:tricaprylin controls)	Stanton *et al.* (1972)
OM/NCR	F	120[d]	Intrapulmonary administration of CSC pellet	CSC/beeswax:tricaprylin (5, 10, 20 or 67 mg CSC/injection), 120 wks after implantation	4, 10, 20 and 42% pulmonary C prevalence; 0% C prevalence for 3 control groups of about 190 rats each	Dagle *et al.* (1978)
Rabbit						
Albino New Zealand	M + F	38	Skin painting of CSC (both ears)	CSC/acetone solution (100 mg CSC/ application/ear), × 5/wk, lifetime (4–6 yrs)	4/38 (2 skin C + 1 skin lipo-sarcoma + 1 skin fibrosarcoma), 0/7 (acetone controls)	Graham *et al.* (1957)

CSC, cigarette-smoke condensate; wk, week; C, carcinoma; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; NG, not given; yrs, years

[a] Skin papillomas

[b] Mostly adenomas

[c] Incidence in animals that died 43–107 weeks after injection

[d] 4 × 10 rats/group terminated before 120 weeks

During the next decade, methods of generating CSC and of treating animals were refined and standardized within and between laboratories. Currently, cigarettes are machine smoked, generally using a 35-mL puff drawn for 2 s each minute. The condensate is collected in glass vessels at low temperatures and removed using a volatile solvent, such as acetone, under reduced pressure. In some studies, animals have also been treated with a diluted suspension of CSC in a suitable solvent. Some of the variables that have not been standardized between different laboratories include choice of animal (or strain), dose and frequency of treatment, choice of solvent, conditions of storage of CSC, the puff profile of the smoking machine and the number of cigarettes that are puffed simultaneously.

Nevertheless, when mouse skin has been used as the test tissue in experiments carried out over the last 35 years, the results from various laboratories have been similar with respect to the overall degree of carcinogenic activity of CSC and to the major differences in activity between CSC from cigarettes of different design (Wynder *et al*., 1957; Davies & Day, 1969; Dontenwill *et al.*, 1972; Bernfeld & Homburger, 1976; Gargus *et al.*, 1976; Gori, 1976; Dontenwill *et al*., 1977b; Gori, 1977; Lee *et al*., 1977; Gori, 1980). Subtle differences in smoking technique, CSC storage conditions and procedures for animal exposure do not appear to affect the results critically.

Animal studies conducted before 1964 provided a measure of support for the epidemiological demonstration that cigarette smoke is an important human carcinogen. Since that time, the mouse-skin studies have served primarily to determine whether differences in cigarette design affect the carcinogenic effects of CSC and whether these effects can be correlated with the chemical composition of the condensates. In addition, mouse-skin studies may help to elucidate the mechanisms through which CSC induces tumours in animal tissues. For example, it has been shown that CSC contains tumour initiators (Hoffmann & Wynder, 1971) and that CSC and some of its fractions and components can exhibit tumour-promoting activity (see, for example: Wynder & Hoffmann, 1961; Bock *et al*., 1969; Hoffmann & Wynder, 1971; Lazar *et al*., 1974; Van Duuren & Goldschmidt, 1976).

3.3.1 *Skin application*

(*a*) *Mouse*

CSC produces both benign and malignant tumours on mouse skin. The carcinogenic potency of the CSC depends on tobacco variety, composition of cigarette paper and the presence of additives (see, for example, Wynder *et al*., 1957; Davies & Day, 1969; Dontenwill *et al*., 1972; Gargus *et al.*, 1976; Gori, 1976; Dontenwill *et al*., 1977b; Gori, 1977, 1980). The tumours induced are usually of epidermal origin. Ohmori *et al.* (1981) reported a low, but significant incidence of mastocytomas in a series of experiments in which mouse skin was treated with CSC.

An example of mouse skin studies is given in a series of four publications (see Gargus *et al.*, 1976; Gori, 1976, 1977, 1980) that reported the results of skin-painting experiments

in which more than 100 variables of CSC were tested in female ICR Swiss mice. These data permitted an evaluation of the overall carcinogenicity of CSC and of intralaboratory variation in bioassay results over time, because, in these studies, University of Kentucky 1R1 and SEB cigarettes stored at –20 °C were tested in four series of studies begun in 1970, 1972, 1974 and 1975, and the results (Table 3.4) are representative of the tumour response found in such studies. The purpose of the studies was to investigate possible relationships between biological activity and cigarette design as well as the chemical characteristics of cigarette smoke. The CSC derived from reference cigarettes and specially modified cigarettes was applied generally at two dose levels, usually 25 mg and 50 mg per 0.1-mL application, 6 days a week, for 78 weeks. Acetone was used as the solvent. In some instances, 3-, 6- and 12.5-mg doses were also given, and, for a single type of CSC, doses of 10 mg and 20 mg were given twice daily or five times a week for 78 weeks. Solvent-treated and untreated controls were kept. Test group sizes usually comprised 100 animals, but up to 800 controls were used; 6400–9200 mice were used for each of the four sets of experiments. A skin tumour was observed in 3/800 acetone-treated controls; otherwise, no tumours and no carcinomas were found in control animals. Skin tumours, frequently malignant, were found in every CSC-treated group. [These extensive studies reported differences in the activity of CSCs from cigarettes of different design. The Working Group of the *IARC Monographs* on tobacco smoking (IARC, 1986) noted that, although the trends of the differences were often consistent, different statistical procedures were used to analyse the data, and insufficient data were provided to permit a uniform analysis for quantitative comparison of these data.]

Cigar and pipe smoke condensates have also been tested on mouse skin. In one such study, a 1:1 (w/v) acetone solution of nicotine-free cigar or pipe tar was painted three

Table 3.4. Skin tumour[a] incidences in ICR Swiss mice after skin painting with smoke condensate from reference cigarettes[b,c]

Series	Date of start of study	Reference cigarette				
		1R1	SEBI	SEBII	SEBIII	SEBIV
I	1970	82/200	84/200	–	–	–
II	1972	98/200	115/200	383/800	–	–
III	1974	49/100	51/100	–	46/100	–
IV	1975	95/200	–	–	87/200	326/800

[a] Not corrected for interim deaths; tumours classified as 'papillomas' and 'carcinomas' in series I and II, as 'papillomas' and 'other malignancies' in series III, and as 'tumours' in series IV.

[b] From Gargus *et al.* (1976) and Gori (1976, 1977, 1980)

[c] Age of animals at start: 'approximately' 6 weeks in series I and II; 'at least' 6 weeks in III and IV.

times a week on the skin of female Swiss mice. A 1:1 acetone solution of whole CSC was also employed. At the end of 19 months, skin papillomas were produced in 65% and carcinomas in 41% of 46 mice treated with the nicotine-free cigar tar, papillomas in 69% and carcinomas in 33% of 45 mice treated with the nicotine-free pipe tar, and papillomas in 47% and carcinomas in 33% of 86 mice treated with CSC. No tumour was produced in 23 acetone-treated controls. At 9 months, when the first tumours appeared, 78% of the controls and 70–91% of the tar-treated animals were still alive (Croninger *et al*., 1958). [The Working Group recognized that the nicotine-free cigar and pipe tars were prepared in a manner that would cause unknown changes in their composition.]

(*b*) *Rat*

McGregor (1976) and McGregor and Myers (1982) reported that CSC appeared to act as a co-carcinogen with β-irradiation on rat skin. CSC alone was reported to induce one benign tumour in 72 normal rats and six benign skin tumours in 78 rats in which the skin keratin layer had been removed before painting the skin. [The Working Group noted that the data, as presented, are not adequate to permit evaluation of these reports.]

(*c*) *Syrian hamster*

Bernfeld and Homburger (1983) reported that Syrian golden hamster skin is not responsive to CSC applied alone. [The Working Group noted that only 16 of the 50 animals treated with CSC alone were observed for up to 46–47 weeks.]

(*d*) *Rabbit*

CSC was applied using a brush to the inner surface of the ears of 38 rabbits five times a week as a 50% w/v suspension in acetone. On alternate weeks, the accumulated surface tar was removed using an acetone-soaked cotton ball or forceps. After 4–6 years of treatment, all of the rabbits had developed large papillomas, and four had skin cancer. Of seven control animals treated with acetone, none developed cancer, but five had small papillomas on the ears (Graham *et al*., 1957).

3.3.2 *'Initiation–promotion' skin-painting studies*

Mouse

A number of investigators have found that CSC and its fractions can act as co-carcinogens when applied together with other agents. In one assay for tumour-initiating activity, 50 μL of an acetone solution containing 2.5 mg of a CSC fraction (0.6% of the whole tar) was applied to the dorsal skin of female ICR/Ha Swiss mice during the second telogen phase of hair growth. This procedure was repeated on alternate days until a total of 10 doses had been applied. Ten days after the last initiating dose, the mice were painted three times a week with 2.5% croton oil in acetone. After 12 months, 67% (20/30) of mice had developed papillomas and 13% (4/30) had carcinomas; after 15 months, 73% (22/30)

had papillomas and 13% (4/30) carcinomas The first tumour appeared within 4 months of start of initiating treatment. Sixty-five mice treated with croton oil only developed no tumours (Hoffmann & Wynder, 1971).

Groups of 30 female Swiss mice were painted twice weekly with 75 mg CSC (solution of 50% CSC in acetone) for 12 months. Of animals that had been treated first (one week before) with 75 μg DMBA, 13/30 (43%) developed skin papillomas and eight (27%) had carcinomas 15 months after initiation. In mice that did not receive DMBA treatment, only 4/40 (10%) had papillomas and one (3%) had a carcinoma. Of 50 mice painted three times weekly with CSC, 22 (44%) developed skin papillomas and 11 (22%) had carcinomas. Of 30 animals treated first (one week before) with a single dose of DMBA, 19 (63%) developed skin papillomas and 11 (37%) had carcinomas. Of mice treated with DMBA alone, 3/30 (10%) had skin papillomas and two (7%) had carcinomas. When a 10% solution of the phenolic fraction of CSC in acetone was applied three times weekly to 30 mice pre-treated with DMBA, nine animals (30%) developed papillomas, but no carcinomas. No tumour was seen in mice that had not received DMBA initiation (Wynder & Hoffmann, 1961). [The Working Group noted that the data do not establish that this effect was due to promotion or whether it represented an additive effect of two weak tumorigenic stimuli.]

3.3.3 *Topical application to oral mucosa*

Mouse

The lips and oral areas of groups of 100 ICR/Ha Swiss mice were painted five times a week for 15 months with approximately 26 mg CSC in an acetone suspension**.** Control groups of 100 mice were either untreated or treated with acetone alone. The study was terminated after 18–19 months, at which time 81–90% of the animals were still alive. Of the surviving mice in the experimental group, 64% (52/81, $p < 0.0001$) developed lung tumours, in contrast to 22% (20/89) in the control groups. In addition, 21% of the treated animals developed tumours of other organs, primarily lymphomas, in contrast to 3–8% in the two control groups (DiPaolo & Levin, 1965).

3.3.4 *Intrapulmonary administration*

(*a*) *Rat*

Stanton *et al.* (1972), following up the earlier studies of Blacklock (1961), injected 0.05 mL beeswax:tricaprylin (v/v) containing 24 mg CSC into the lungs of female Osborne Mendel pathogen-free rats after thoracotomy. The pellets thus formed were large enough to entrap bronchioles. The residues of the pellets could be recognized for more than 2 years after treatment. Fourteen of 40 rats that died between 43 and 107 weeks developed squamous-cell carcinomas. The non-polar constituents of CSC appeared to be an important, but not the sole contributor to this activity. Thus, when the beeswax:tricaprylin

mixture contained 12 mg of the heptane-soluble fraction of CSC, squamous-cell carcinomas developed in 5/18 rats. No tumour was seen in any of the 63 rats that died 43–107 weeks after injection with beeswax:tricaprylin alone.

These observations were confirmed by Dagle *et al.* (1978) using CSC from two different types of cigarette. They observed a dose-dependent incidence of lung carcinomas when either condensate was injected in beeswax:tricaprylin. No carcinoma was induced by beeswax:tricaprylin alone. With the highest dose of CSC (67 mg), the prevalence of carcinoma reached 42% in 120 weeks. No difference was observed in tumour response to the two CSCs.

Groups of Wistar rats were injected intratracheally with CSC or CSC fractions without vehicle every 2 weeks for life. There was a dose-dependent increase in the mean grade of squamous metaplasia in the groups treated with CSC and with several fractions of CSC. After treatment with CSC and most fractions, no tumours were observed, but after treatment with a fraction containing most of the polynuclear aromatic hydrocarbons of cigarette smoke, 5/54 rats developed neoplastic lung lesions (Davis *et al*., 1975c). [The Working Group noted that the relatively infrequent dosage employed by Davis *et al.* may have been less adequate as a stimulus than prolonged release of the material from a lipid vehicle that persists at the injection site.]

(*b*) *Hamster*

CSC and the 'nitromethane-soluble' fraction of CSC were found to be weak carcinogens when injected in beeswax directly into hamster lung (Ketkar *et al*., 1979). The method used was similar to that used by Stanton *et al.* (1972) in rats; 0.03 mL of a 1:1 beeswax: tricaprylin mixture was used as the solvent. A group of 31 hamsters injected with 50 mg CSC developed one bronchogenic adenoma; two animals exhibited metaplasia. When 25 mg of the nitromethane fraction of CSC was given to another group of 31 animals, three developed bronchogenic adenomas and nine had metaplasia.

3.3.5 *Gavage study*

Mouse

The carcinogenicity of smoke condensates of Indian bidi [Indian sun-cured tobacco wrapped in *tendu* leaf (*Diospyros melanoxylon* or *Diospyros ebenum)* and smoked] was administered to male Swiss albino mice by oral gavage in suspensions of 1 mg condensate/0.1 mg dimethyl sulfoxide, 5 days per week, for 55 weeks. Animals were killed when moribund or 90 weeks after the beginning of treatment. Lung, liver, stomach and oesophagus were fixed in formalin and processed for histological examination. Four hepatic haemangiomas, one stomach papilloma, one stomach carcinoma and one oesophageal carcinoma were found in 15 mice that received bidi smoke condensate. No tumours were seen in untreated controls (15 mice) or dimethyl sulfoxide controls (15 mice) (Pakhale *et al*., 1988).

References

Auerbach, O., Hammond, E.C., Kirman, D. & Garfinkel, L. (1970) Effects of cigarette smoking on dogs. II. Pulmonary neoplasms. *Arch. environ. Health*, **21**, 754–768

Bernfeld, P. & Homburger, F. (1976) Mouse skin assay of condensate from smoking products other than tobacco. *J. natl Cancer Inst*., **56**, 741–747

Bernfeld, P. & Homburger, F. (1983) Skin painting studies in Syrian hamsters. *Prog. exp. Tumor Res*., **26**, 128–153

Bernfeld, P., Homburger, F. & Russfield, A.B. (1974) Strain differences in the response of inbred Syrian hamsters to cigarette smoke inhalation. *J. natl Cancer Inst*., **53**, 1141–1157

Bernfeld, P., Homburger, F., Soto, E. & Pai, K.J. (1979) Cigarette smoke inhalation studies in inbred Syrian golden hamsters. *J. natl Cancer Inst*., **63**, 675–689

Blacklock, J.W.S. (1961) An experimental study of the pathological effects of cigarette condensate in the lungs with special reference to carcinogenesis. *Br. J. Cancer*, **15**, 745–762

Bock, F.G., Swain, A.P. & Stedman, R.L. (1969) Bioassay of major fractions of cigarette smoke condensate by an accelerated technic. *Cancer Res*., **29**, 584–587

Chameaud, J., Perraud, R., Chrétien, J., Masse, R. & Lafuma, J. (1982) Lung carcinogenesis during in vivo cigarette smoking and radon daughter exposure in rats. *Recent Results Cancer Res*., **82**, 11–20

Croninger, A.B., Graham, E.A. & Wynder, E.L. (1958) Experimental production of carcinoma with tobacco products. V. Carcinoma induction in mice with cigar, pipe and all–tobacco cigarette tar. *Cancer Res*., **18**, 1263–1271

Cross, F.T., Palmer, R.F., Filipy, R.E., Dagle, G.E. & Stuart, B.O. (1982) Carcinogenic effects of radon daughters, uranium ore dust and cigarette smoke in beagle dogs. *Health Phys*., **42**, 33–52

Dalbey, W.E., Nettesheim, P., Griesemer, R., Caton, J.E. & Guerin, M.R. (1980) Chronic inhalation of cigarette smoke by F344 rats. *J. natl Cancer Inst*., **64**, 383–390

Dagle, G.E., McDonald, K.E., Smith, L.G. & Stevens, D.L., Jr (1978) Pulmonary carcinogenesis in rats given implants of cigarette smoke condensate in beeswax pellets. *J. natl Cancer Inst*., **61**, 905–910

Davies, R.F. & Day, T.D. (1969) A study of the comparative carcinogenicity of cigarette and cigar smoke condensate on mouse skin. *Br. J. Cancer*, **23**, 363–368

Davis, B.R., Whitehead, J.K., Gill, M.E., Lee, P.N., Butterworth, A.D. & Roe, F.J.C. (1975a) Response of rat lung to inhaled tobacco smoke with or without prior exposure to 3,4-benzpyrene (BP) given by intratracheal instillation. *Br. J. Cancer*, **31**, 469–484

Davis, B.R., Whitehead, J.K., Gill, M.E., Lee, P.N., Butterworth, A.D. & Roe, F.J.C. (1975b) Response of rat lung to inhaled vapour phase constituents (VP) of tobacco smoke alone or in conjunction with smoke condensate or fractions of smoke condensate given by intratracheal instillation. *Br. J. Cancer*, **31**, 462–468

Davis, B.R., Whitehead, J.K., Gill, M.E., Lee, P.N., Butterworth, A.D. & Roe, F.J.C. (1975c) Response of rat lung to tobacco smoke condensate or fractions derived from it administered repeatedly by intratracheal instillation. *Br. J. Cancer*, **31**, 453–461

DiPaolo, J.A. & Levin, M.L. (1965) Tumor incidence in mice after oral painting with cigarette smoke condensate. *J. natl Cancer Inst*., **34**, 595–600

Dontenwill, W., Chevalier, H.-J., Harke, H.-P., Klimisch, H.-J., Lafrenz, O. & Reckzeh, G. (1972) [Experimental studies on tumorigenic activity of cigarette smoke condensate on mouse skin. IV.

Comparative studies of condensates from different reconstituted tobacco sheets, the effect of $NaNO_3$ as additive to tobacco or reconstituted tobacco sheets, the effect of volatile constituents of smoke, the effect of initial treatment with DMBA.] *Z. Krebsforsch.*, **78**, 236–264 (in German)

Dontenwill, W., Chevalier, H.-J., Harke, H.-P., Lafrenz, U., Reckzeh, G. & Schneider, B. (1973) Investigations on the effects of chronic cigarette-smoke inhalation in Syrian golden hamsters. *J. natl Cancer Inst.*, **51**, 1781–1832

Dontenwill, W., Chevalier, H.-J., Harke, H.-P., Klimisch, H.-J., Kuhnig, C., Reckzeh, G. & Schneider, B. (1977a) [Studies on the effect of chronic cigarette smoke inhalation in Syrian golden hamsters and the importance of vitamin A on morphological alterations after smoke exposure.] *Z. Krebsforsch.*, **89**, 153–180 (in German)

Dontenwill, W., Chevalier, H.-J., Harke, H.-P., Klimisch, H.-J., Reckzeh, G., Fleischmann, B. & Keller, W. (1977b) [Experimental investigations on the tumorigenic activity of cigarette smoke condensate on mouse skin. VII. Comparative studies of condensates from different modified cigarettes.] *Z. Krebsforsch.*, **89**, 145–151 (in German)

Finch, G.L., Nikula, K.J., Barr, E.B., Bechtold, W.E., Chen, B.T., Griffith, W.C., Hahn, F.F., Hobbs, C.H., Hoover, M.D., Lundgren, D.L. & Mauderly, J.L. (1995) Effects of combined exposure of F344 rats to radiation and chronically inhaled cigarette smoke. In: Bice, D.E., Hahn, F.F., Hoover, M.D., Neft, R.E., Thornton-Manning, J.R. & Bradley, P.L., eds, *Annual Report of the Inhalation Toxicology Research Institute* (ITRI-146), Albuquerque, NM, Inhalation Toxicology Research Institute, pp. 77–79

Finch, G.L., Nikula, K.J., Belinsky, S.A., Barr, E.B., Stoner, G.D. & Lechner, J.F. (1996) Failure of cigarette smoke to induce or promote lung cancer in the A/J mouse. *Cancer Lett.*, **99**, 161–167

Gargus, J.L., Powers, M.B., Habermann, R.T. & Everly, J.R. (1976) Mouse dermal bioassays of cigarette smoke condensates. In: Gori, G.B., ed., *Report No. 1. Toward Less Hazardous Cigarettes. The First Set of Experimental Cigarettes* (DHEW Publ. No. (NIH) 76-905), Washington DC, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, pp. 85–94

Gori, G.B. (1976) Report on the second set of experimental cigarettes. In: Gori, G.B., ed., *Report No. 2. Toward Less Hazardous Cigarettes. The Second Set of Experimental Cigarettes* (DHEW Publ. No. (NIH) 76-1111), Washington DC, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, pp. 4–15

Gori, G.B. (1977) Report on the third set of experimental cigarettes. In: Gori, G.B., ed., *Report No. 3. Toward Less Hazardous Cigarettes. The Third Set of Experimental Cigarettes* (DHEW Publ. No. (NIH) 77-1280), Washington DC, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, pp. 3–24

Gori, G.B. (1980) Report on the fourth set of experimental cigarettes. In: Gori, G.B., ed., *Report No. 4. Toward Less Hazardous Cigarettes. The Fourth Set of Experimental Cigarettes*, Washington DC, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, pp. 5–40

Graham, E.A., Croninger, A.B. & Wynder, E.L. (1957) Experimental production of carcinoma with cigarette tar. IV. Successful experiments with rabbits. *Cancer Res.*, **17**, 1058–1066

Griest, W.H., Guerin, M.R., Quincy, R.B., Jenkins, R.A. & Kubota, H. (1980) Chemical characterization of experimental cigarettes and cigarette smoke condensates in the fourth cigarette experiment. In: Gori, G.B., ed., Report No. 4. *Toward Less Hazardous Cigarettes. The Fourth Set of*

Experimental Cigarettes, Washington DC, Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, pp. 65–97

Gupta, M.P., Khanduja, K.L., Koul, I.B. & Sharma, R.R. (1990) Effect of cigarette smoke inhalation on benzo[*a*]pyrene-induced lung carcinogenesis in vitamin A deficiency in the rat. *Cancer Lett.*, **55**, 83–88

Hammond, E.C., Auerbach, O., Kirman, D. & Garfinkel, L. (1970) Effects of cigarette smoking on dogs. I. Design of experiment, mortality, and findings in lung parenchyma. *Arch. environ. Health*, **21**, 740–753

Harada, T., Kitazawa, T., Maita, K. & Shirasu, Y. (1985) Effects of vitamin C on tumor induction by diethylnitrosamine in the respiratory tract of hamsters exposed to cigarette smoke. *Cancer Lett.*, **25**, 163–169

Harris, R.J.C. & Negroni, G. (1967) Production of lung carcinomas in C57BL mice exposed to a cigarette smoke and air mixture. *Br. med. J.*, **iv**, 637–641

Harris, R.J.C., Negroni, G., Ludgate, S., Pick, C.R., Chesterman, F.C. & Maidment, B.J. (1974) The incidence of lung tumours in C57BL mice exposed to cigarette smoke:air mixtures for prolonged periods. *Int. J. Cancer*, **14**, 130–136

Hecht, S.S., Adams, J.D., Numoto, S. & Hoffmann, D. (1983) Induction of respiratory tract tumors in Syrian golden hamsters by a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and the effect of smoke inhalation. *Carcinogenesis*, **4**, 1287–1290

Henry, C.J. & Kouri, R.E. (1986) Chronic inhalation studies in mice. II. Effects of long-term exposure to 2R1 cigarette smoke on (C57BL/Cum × C3H/AnfCum)F_1 mice. *J. natl Cancer Inst.*, **77**, 203–212

Hoffmann, D. & Wynder, E.L. (1971) A study of tobacco carcinogenesis. XI. Tumor initiators, tumor accelerators, and tumor promoting activity of condensate fractions. *Cancer*, **27**, 848–864

Hoffmann, D., Rivenson, A., Hecht, S.S., Hilfrich, J., Kobayashi, N. & Wynder, E.L. (1979) Model studies in tobacco carcinogenesis with the Syrian golden hamster. *Prog. exp. Tumor Res.*, **24**, 370–390

Holland, R.H., Wilson, R.H., Morris, D., McCall, M.S. & Lanz, H. (1958) The effect of cigarette smoke on the respiratory system of the rabbit. A preliminary report. *Cancer*, **11**, 709–712

Holland, R.H., Kozlowski, E.J. & Booker, L. (1963) The effect of cigarette smoke on the respiratory system of the rabbit. A final report. *Cancer*, **16**, 612–615

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 38, *Tobacco Smoking*, Lyon, IARC*Press*

Keast, D., Ayre, D.J. & Papadimitriou, J.M. (1981) A survey of pathological changes associated with long-term high tar tobacco smoke exposure in a murine model. *J. Pathol.*, **135**, 249–257

Ketkar, M.B., Haas, H. & Althoff, J. (1979) Implantation of cigarette smoke condensate in the lungs of Syrian golden hamsters. *J. Cancer Res. clin. Oncol.*, **94**, 111–114

Kobayashi, N., Hoffmann, D. & Wynder, E.L. (1974) A study of tobacco carcinogenesis. XII. Epithelial changes induced in the upper respiratory tracts of Syrian golden hamsters by cigarette smoke. *J. natl Cancer Inst.*, **53**, 1085–1089

Lazar, P., Chouroulinkov, I., Izard, C., Moree-Testa, P. & Hemon, D. (1974) Bioassays of carcinogenicity after fractionation of cigarette smoke condensate. *Biomedicine*, **20**, 214–222

Lee, P.N., Rothwell, K. & Whitehead, J.K. (1977) Fractionation of mouse skin carcinogens in cigarette smoke condensate. *Br. J. Cancer*, **35**, 730–742

Leuchtenberger, C. & Leuchtenberger, R. (1970) Effects of chronic inhalation of whole fresh cigarette smoke and of its gas phase on pulmonary tumorigenesis in Snell's mice. In: Nettesheim, P., Hanna, M.G., Jr & Deathevage, J.W., Jr, eds, *Morphology of Experimental Respiratory Carcinogenesis. Proceedings of a Biology Division, Oak Ridge National Laboratory Conference*, US Atomic Energy Commission, pp. 329–346

McGregor, J.F. (1976) Tumor-promoting activity of cigarette tar in rat skin exposed to irradiation. *J. natl Cancer Inst*., **56**, 429–430

McGregor, J.F. & Myers, D.K. (1982) Enhancement of skin tumorigenesis by cigarette smoke condensate following β-irradiation in rats. *J. natl Cancer Inst*., **68**, 605–611

Nagano, K., Miyakawa, Y., Takahashi, M., Furukawa, F., Kokubo, T. & Hayashi, Y. (1982) Comparative studies on anatomical and histological structures of nasal cavity in rodents. *Natl public Health Inst. Jpn*, **100**, 101–109

Nishikawa, A., Furukawa, F., Imazawa, T., Yoshimura, H., Ikezaki, S., Hayashi, Y. & Takahashi, M. (1994) Effects of cigarette smoke on *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic and respiratory tumorigenesis in hamsters. *Jpn. J. Cancer Res.*, **85**, 1000–1004

Ohmori, T., Mori, H. & Rivenson, A. (1981) A study of tobacco carcinogenesis. XX. Mastocytoma induction in mice by cigarette smoke particulates ('cigarette tar'). *Am. J. Pathol.*, **102**, 381–387

Otto, H. & Elmenhorst, H. (1967) [Experimental studies on tumour induction with the gas phase of cigarette smoke.] *Z. Krebsforsch.*, **70**, 45–47 (in German)

Pakhale, S.S., Sarkar, S., Jayant, K. & Bhide, S.V. (1988) Carcinogenicity of Indian bidi and cigarette smoke condensate in Swiss albino mice. *Cancer Res. clin. Oncol.*, **114**, 647– 649

Proctor, D.F. & Chang, J.C.F. (1983) Comparative anatomy and physiology of the nasal cavity. In: Reznik, G. & Stinson, S.F., eds, *Nasal Tumors in Animals and Man*, Vol. 1, *Anatomy, Physiology and Epidemiology*, Boca Raton, FL, CRC Press, pp. 1–33

Reznik, G. (1983) Comparative anatomy and histomorphology of the nasal and paranasal cavities in rodents. In: Reznik, G. & Stinson, S.F., eds, *Nasal Tumors in Animals and Man*, Vol. 1, *Anatomy, Physiology and Epidemiology*, Boca Raton, FL, CRC Press, pp. 35–44

Stanton, M.F., Miller, E., Wrench, C. & Blackwell, R. (1972) Experimental induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate. *J. natl Cancer Inst.*, **49**, 867–877

Shimkin, M.D. & Stoner, G.D. (1975) The genetic basis of susceptibility to lung tumours in mice. *Adv. Cancer Res.*, **21**, 1–56

Takahashi, M., Imaida, K., Mitsumori, K., Okamiya, H., Shinoda, K., Yoshimura, H., Furukawa, F. & Hayashi, Y. (1992) Promoting effects of cigarette smoke on the respiratory tract carcinogenesis of Syrian golden hamsters treated with diethylnitrosamine. *Carcinogenesis*, **13**, 569–572

Van Duuren, B.L. & Goldschmidt, B.M. (1976) Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J. natl Cancer Inst.*, **56**, 1237–1242

Wehner, A.P., Busch, R.H. & Olson, R.J. (1974) Effect of chronic exposure to cigarette smoke on tumor incidence in the Syrian golden hamster. In: Karbe, E. & Park, J.F., eds, *Experimental Lung Cancer. Carcinogenesis and Bioassays*, New York, Springer, pp. 360–368

Wehner, A.P., Busch, R.H., Olson, R.J. & Craig, D.K. (1975a) Chronic inhalation of nickel oxide and cigarette smoke by hamsters. *Am. ind. Hyg. Assoc. J.*, **36**, 801–810

Wehner, A.P., Busch, R.H., Olson, R.J. & Craig, D.K. (1975b) Chronic inhalation of asbestos and cigarette smoke by hamsters. *Environ. Res*., **10**, 368–383

Wehner, A.P., Olson, R.J. & Busch, R.H. (1976) Increased life span and decreased weight in hamsters exposed to cigarette smoke. *Arch. environ. Health*, **31**, 146–153

Wehner, A.P., Dagle, G.E., Milliman, E.M., Phelps, D.W., Carr, D.B., Decker, J.R. & Filipy, R.E. (1981) Inhalation bioassay of cigarette smoke in rats. *Toxicol. appl. Pharmacol*., **61**, 1–17

Witschi, H., Espiritu, I., Peake, J.L., Wu, K., Maronpot, R.R. & Pinkerton, K.E. (1997) The carcinogenicity of environmental tobacco smoke. *Carcinogenesis*, **18**, 575–586

Wynder, E.L. & Hoffmann, D. (1961) A study of tobacco carcinogenesis. VIII. The role of the acidic fractions as promoters. *Cancer*, **14**, 1306–1315

Wynder, E.L. & Hoffmann, D. (1967) *Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis*, New York, Academic Press, pp. 225–231

Wynder, E.L., Graham, E.A. & Croninger, A.B. (1953) Experimental production of carcinoma with cigarette tar. *Cancer Res*., **13**, 855–864

Wynder, E.L., Gottlieb, S. & Wright, G. (1957) A study of tobacco carcinogenesis. IV. Different tobacco types. *Cancer*, **10**, 1206–1209

Wynder, E.L., Taguchi, K.T., Baden, V. & Hoffmann, D. (1968) Tobacco carcinogenesis. IX. Effect of cigarette smoke on respiratory tract of mice after passive inhalation. *Cancer*, **21**, 134–153

Yoshimura, H. & Takemoto, K. (1991) Effect of cigarette smoking and/or *N*-bis(2-hydroxypropyl)-nitrosamine (DHPN) on the development of lung and pleural tumors in rats induced by administration of asbestos. *Jpn. J. ind. Health*, **33**, 81–93