3. Studies of Cancer in Experimental Animals

3.1 Inhalation

3.1.1 *Mouse*

Groups of 42–60 C3H mice [sex and age unspecified] were exposed to concentrations of 0, 50, 100 or 200 mg/m^3 formaldehyde (US Pharmacopeia grade) vapour for 1 h per day, three times a week, ostensibly for 35 weeks. Treatment of mice with the highest concentration was discontinued after the 11th exposure because of severe toxicity, and 36 of the mice exposed to 50 mg/m^3 for 35 weeks were subsequently exposed to 150 mg/m^3 for a further 29 weeks. Surviving animals in the initial groups were killed at 35 weeks and those on extended treatment at 68 weeks. The nasal epithelium was not examined, either grossly or microscopically. There was no evidence of induction of pulmonary tumours at any dose. Basal-cell hyperplasia, squamous-cell metaplasia and atypical metaplasia were seen in the trachea and bronchi of most of the exposed mice but not in untreated controls (Horton *et al*., 1963). [The Working Group noted the high doses used, the short intervals of exposure, the short duration of the experiment and the lack of pathological examination of the nose.]

Groups of 119–120 male and 120–121 female B6C3F$_1$ mice, 6 weeks of age, were exposed to 0, 2.0, 5.6 or 14.3 ppm [0, 2.5, 6.9 or 17.6 mg/m^3] formaldehyde (> 97.5% pure) vapour by whole-body exposure for 6 h per day on 5 days per week for up to 24 months, followed by a 6-month observation period with no further exposure. Ten males and 10 females from each group were killed at 6 and 12 months, no or one male and 19–20

females at 18 months, 17–41 of each sex at 24 months and 9–16 females at 27 months. Between 0 and 24 months, 78 male and 30 female controls, 77 males and 34 females exposed to 2 ppm formaldehyde vapour, 81 males and 19 females exposed to 5.6 ppm and 82 males and 34 females exposed to 14.3 ppm died; all animals that died or were killed were examined grossly. Thorough histopathological examinations were performed on control and high-dose mice, on multiple sections of the nasal cavity and on all lesions that were identified grossly in the other two groups. Squamous-cell carcinomas occurred in the nasal cavities of 2/17 male mice in the high-dose group that were killed at 24 months. No nasal cavity tumours developed in male mice treated with lower doses of formaldehyde, in females at any dose or among 21 male or 31 female control mice killed at 24 months ($p > 0.05$). A variety of non-neoplastic lesions (such as squamous-cell hyperplasia, squamous-cell metaplasia and dysplasia) were commonly found in the nasal cavities of mice exposed to formaldehyde, particularly at 14.3 ppm (Kerns *et al*., 1983a,b; Gibson, 1984).

3.1.2 *Rat*

Groups of 119–120 male and 120 female Fischer 344 rats, 7 weeks of age, were exposed to 0, 2.0, 5.6 or 14.3 ppm [0, 2.5, 6.9 or 17.6 mg/m^3] formaldehyde (> 97.5% pure) vapour by whole-body exposure for 6 h per day on 5 days per week for up to 24 months and were then observed for 6 months with no further exposure. Ten males and 10 females from each group were killed at 6 and 12 months, 19–20 of each sex at 18 months, 13–54 at 24 months, 0–10 at 27 months and 0–6 at 30 months. Between 0 and 24 months, six males and 13 females in the control group, 10 males and 16 females exposed to 2 ppm, 19 of each sex exposed to 5.6 ppm and 57 males and 67 females exposed to 14.3 ppm died; all animals that died or were killed were examined grossly. Histopathological examinations were performed on multiple sections of the nasal cavity, on all lesions that were identified grossly and on all major tissues of each organ system (approximately 40 per animal) from control and high-dose rats. The findings for the nasal cavity are summarized in Table 24. While no nasal cavity malignancies were found in rats exposed to 0 or 2.0 ppm formaldehyde, two squamous-cell carcinomas (1/119 males and 1/116 females examined) occurred in the group exposed to 5.6 ppm and 107 (51/117 males and 52/115 females examined) in those exposed to 14.3 ppm ($p < 0.001$). Five additional nasal cavity tumours (classified as carcinoma, undifferentiated carcinoma/sarcoma and carcinosarcoma) were identified in rats exposed to 14.3 ppm; two of these tumours were found in rats that also had squamous-cell carcinomas of the nasal cavity. There was a significant overall increase in the incidence of polypoid adenomas in treated animals (males and females combined) when compared with controls ($p = 0.02$, Fisher's exact test). The incidences of polypoid adenomas were marginally significantly elevated in females at the low dose ($p = 0.07$, Fisher's exact test) and in males at the middle dose ($p = 0.06$, Fisher's exact test) (see also Table 24). A variety of non-neoplastic lesions were commonly found in the nasal cavities of rats exposed to formaldehyde, particularly at 14.3 ppm (Swenberg *et al*., 1980; Kerns *et al*., 1983a,b; Gibson, 1984). More

Table 24. Neoplastic lesions in the nasal cavities of Fischer 344 rats exposed to formaldehyde vapour

Lesion	Exposure (ppm)							
	0		2.0		5.6		14.3	
	M	F	M	F	M	F	M	F
No. of nasal cavities examined	118	114	118	118	119	116	117	115
Squamous-cell carcinoma	0	0	0	0	1	1	51[a]	52[a]
Nasal carcinoma	0	0	0	0	0	0	1[b]	1
Undifferentiated carcinoma or sarcoma	0	0	0	0	0	0	2[b]	0
Carcinosarcoma	0	0	0	0	0	0	1	0
Osteochondroma	1	0	0	0	0	0	0	0
Polypoid adenoma	1	0	{ 4	4[c]	6[d]	0	4	1 }[e]

From Kerns *et al.* (1983a)
[a] $p < 0.001$, pair-wise comparisons
[b] One animal in this group also had a squamous-cell carcinoma.
[c] [$p = 0.07$, Fisher's exact test in comparison with female controls]
[d] [$p = 0.06$, Fisher's exact test in comparison with male controls]
[e] [$p = 0.02$, Fisher's exact test in comparison of all treated rats with controls]

than half (57%) of the squamous-cell carcinomas in rats exposed to 14.3 ppm formaldehyde were observed on the anterior portion of the lateral side of the nasoturbinate and the adjacent lateral wall, 25% were located on the midventral nasal septum, 10% on the dorsal septum and roof of the dorsal meatus and a small number (3%) on the maxilloturbinate (Morgan *et al.*, 1986a).

In a study to investigate the carcinogenicity of bis(chloromethyl)ether formed *in situ* in inhalation chambers by mixing formaldehyde and hydrogen chloride gas at high concentrations before introduction into the chamber in order to maximize its formation, 99 male Sprague–Dawley rats, 8 weeks of age, were exposed to a mixture of 14.7 ppm [18.1 mg/m^3] formaldehyde [purity unspecified] vapour and 10.6 ppm [15.8 mg/m^3] hydrogen chloride gas for 6 h per day on 5 days per week for life. The average level of bis(chloromethyl)ether was 1 ppb [4.7 μg/m^3]. Groups of 50 rats were sham-exposed to air or were untreated. The animals were allowed to die naturally and were then necropsied. Histological sections of nasal cavities, respiratory tract, major organs and gross lesions were prepared and examined microscopically. No nasal cancers were found in the controls, but 28 of the treated rats developed tumours of the nasal cavity, 25 of which were squamous-cell carcinomas [$p < 0.001$, Fisher's exact test] and three of which were papillomas. Mortality was greater in the treated group than in controls throughout the experiment; about 50% of the exposed rats were still alive at 223 days, when the first nasal carcinoma was observed. About two-thirds of the exposed rats showed squamous-cell metaplasia of the nasal mucosa; these lesions were not seen in controls (Albert *et al*., 1982).

In a follow-up study, groups of 99–100 male Sprague-Dawley rats, 9 weeks of age, were exposed for 6 h per day on 5 days per week for life to: (1) 14.3 ppm [17.6 mg/m^3] formaldehyde [purity unspecified] and 10 ppm [14.9 mg/m^3] hydrogen chloride gas mixed before dilution in the exposure chamber to maximize formation of bis(chloromethyl)ether; (2) 14.1 ppm [17.3 mg/m^3] formaldehyde and 9.5 ppm [14.2 mg/m^3] hydrogen chloride gas not mixed before introduction into the exposure chamber; (3) 14.2 ppm [17.5 mg/m^3] formaldehyde vapour alone; (4) 10.2 ppm [15.2 mg/m^3] hydrogen chloride gas alone; or (5) air (sham-exposed controls). A control group of 99 rats was also available. The findings in the nasal cavity are summarized in Table 25. At the end of the experiment, 38 squamous-cell carcinomas of the nasal cavities and 10 papillomas or polyps were observed in rats exposed to formaldehyde alone; none were seen in the controls ($p \leq 0.001$, Fisher's exact test). No differences were reported between groups in the incidences of tumours outside the nasal cavity (Albert *et al*., 1982; Sellakumar *et al*., 1985).

Table 25. Neoplastic lesions in the nasal cavities of male Sprague-Dawley rats exposed to formaldehyde (HCHO) and/or hydrogen chloride (HCl) vapour

Lesion	Group 1: Premixed HCl (10 ppm) and HCHO (14.3 ppm)	Group 2: Non-premixed HCl (9.5 ppm) and HCHO (14.1 ppm)	Group 3: HCHO (14.2 ppm)	Group 4: HCl (10.2 ppm)	Group 5: Air controls	Colony controls
No. of rats examined	100	100	100	99	99	99
Squamous-cell carcinoma	45	27	38	0	0	0
Adenocarcinoma	1	2	0	0	0	0
Mixed carcinoma	0	0	1	0	0	0
Fibrosarcoma	1	0	1	0	0	0
Aesthesioneuroepithelioma	1	0	0	0	0	0
Papillomas or polyps	13	11	10	0	0	0
Tumours in organs outside the respiratory tract	22	12	10	19	25	24

From Sellakumar *et al.* (1985)

Nine groups of 45 male Wistar rats [age unspecified], initially weighing 80 g, were exposed to 0, 10 or 20 ppm [0, 12.3 or 25 mg/m^3] formaldehyde [purity unspecified] vapour beginning 1 week after acclimatization. Whole-body exposures for 6 h per day on 5 days per week were continued for 4, 8 or 13 weeks; thereafter, the rats were observed during recovery periods of 126, 122 or 117 weeks, respectively, after which all survivors were killed. All rats were autopsied and examined by gross pathology; histological examination was limited to six cross-sections of the nose of each rat. Hyperplasia and metaplasia of the nasal epithelium were found to persist in rats exposed to formaldehyde. Significant tumour incidences are presented in Table 26. In control rats, the only nasal tumours reported were two squamous-cell carcinomas among 45 rats that were exposed to air for 8 weeks: one was a small tumour found at 130 weeks which appeared to involve a nasolachrymal duct; the second was a large squamous-cell carcinoma in a rat killed at

Table 26. Nasal tumours in male Wistar rats exposed to formaldehyde for 4, 8 or 13 weeks followed by observation up to 126 weeks

Exposure time; no. of rats	Tumour	Dose (ppm [mg/m^2])		
		0	10 [12.3]	20 [25]
4 weeks				
No. of rats		44	44	45
	Polypoid adenoma	0	0	1[a]
	Squamous-cell carcinoma	0	0	1
8 weeks				
No. of rats		45	44	43
	Polypoid adenoma	0	0	1[a]
	Squamous-cell carcinoma	2	1	1
13 weeks				
No. of rats		45	44	44
	Squamous-cell carcinoma	0	1	3[a]
	Cystic squamous-cell carcinoma	0	0	1
	Carcinoma *in situ*	0	0	1[a]
	Ameloblastoma	0	0	1

From Feron *et al*. (1988)
[a] Considered by the authors to be related to exposure to formaldehyde

week 94, which formed a large mass outside the nasal cavity and was thought to have arisen in a nasolachrymal duct or maxillary sinus. The tumours were considered by the authors not to resemble those observed in the rats exposed to formaldehyde. Rats exposed to 10 ppm formaldehyde also had two squamous-cell carcinomas: one was reported to be a small nasolachrymal-duct tumour in a survivor at 130 weeks, and the second occurred largely outside the nasal cavity in association with an abnormal incisor tooth in a rat killed at week 82. Rats exposed to 20 ppm formaldehyde had 10 tumours: polypoid adenomas of the nasal cavity were found in one rat exposed for 4 weeks and killed at 100 weeks and in another rat exposed for 8 weeks and killed at 110 weeks; six were squamous-cell carcinomas, two of which were thought to originate in the nasolachrymal ducts, one of which appeared to be derived from the palate and the three others, all in the group exposed for 13 weeks, appeared to arise from the naso- or maxillo-turbinates and formed large tumours that invaded the bone and subcutaneous tissues. The other two neoplasms observed in treated animals were an ameloblastoma found at week 73 and an exophytic tumour of the nasal septum of doubtful malignancy, which was designated a carcinoma *in situ*, in a rat that died at 81 weeks. The authors concluded that the nasal tumours were induced by formaldehyde only at 20 ppm and at an incidence of 4.5% (six tumours/132 rats) [$p = 0.01$, Fisher's exact test] (Feron *et al*., 1988). [The Working Group noted that positive findings were made in spite of the short duration of exposure.]

A total of 720 male specific pathogen-free Wistar rats [age unspecified], initially weighing 30–50 g, were acclimatized for 1 week, and then the nasal mucosa of 480 of the rats was severely injured bilaterally by electrocoagulation. One week later, groups of

180 rats were exposed to 0, 0.1, 1.0 or 10 ppm [0, 0.123, 1.23 or 12.3 mg/m^3] formaldehyde [purity unspecified] vapour by whole-body exposure for 6 h per day on 5 days per week. Half of the animals (30 undamaged and 60 damaged rats) were exposed for 28 months, and the other half (30 undamaged and 60 damaged rats) were exposed for only 3 months and then allowed to recover for 25 months with no further treatment. All surviving rats were killed at 29 months, autopsied and examined grossly; histological examination was restricted to six cross-sections of the nose of each rat. The neoplastic lesions found in the nasal cavity are summarized in Table 27. A high incidence of nasal tumours (17/58) was found in rats that had damaged noses and were exposed to 10 ppm formaldehyde for 28 months; only one was found in 54 controls [$p < 0.001$; Fisher's exact test]; and only one of the 26 rats that had undamaged noses and were exposed to 10 ppm formaldehyde for 28 months developed a nasal tumour. The tumour incidences in the other groups were low (0–4%). Eight additional squamous-cell carcinomas found in this study that appeared to be derived from the nasolachrymal ducts were excluded from the analysis (Woutersen *et al*., 1989).

Table 27. Nasal tumours in male Wistar rats that had damaged or undamaged noses and were exposed to formaldehyde vapour for 28 months or 3 months followed by a 25-month recovery period

Exposure time; no. of rats	Tumour	Exposure (ppm [mg/m^3])							
		0		0.1 [0.123]		1.0 [1.23]		10.0 [12.3]	
		U	D	U	D	U	D	U	D
28 months									
Effective number		26	54	26	58	28	56	26	58
	Squamous-cell carcinoma	0	1	1	1	1	0	1	15
	Adenosquamous carcinoma	0	0	0	0	0	0	0	1
	Adenocarcinoma	0	0	0	0	0	0	0	1
3 months									
Effective number		26	57	30	57	29	53	26	54
	Squamous-cell carcinoma	0	0	0	2	0	2	1	1
	Carcinoma *in situ*	0	0	0	0	0	0	0	1
	Polypoid adenoma	0	0	0	0	0	0	1	0

From Woutersen *et al.* (1989)
U, undamaged nose; D, damaged nose

In a study to explore the interaction between formaldehyde and wood dust, two groups of 16 female Sprague-Dawley rats, 11 weeks of age, were exposed either to air or to formaldehyde [purity unspecified] at an average concentration of 12.4 ppm [15.3 mg/m^3]. Exposures were for 6 h per day for 5 days a week for a total of 104 weeks. At the end of the experiment, surviving animals were killed, and histological sections were prepared from five cross-sections of the nose of each rat. Pronounced squamous-cell metaplasia or metaplasia with dysplasia was observed in 10/16 rats exposed to formaldehyde and in

0/15 controls. One exposed rat developed a squamous-cell carcinoma. Neither the frequency nor the latent periods of induction of tumours outside the nasal cavity differed from those in controls (Holmström *et al*., 1989b). [The Working Group noted the small numbers of animals used in the study.]

To study the correlation of indices of regional cell proliferation with the sites of formaldehyde-induced nasal squamous-cell carcinomas, five groups of 90 and one (high-dose) group of 147 male Fischer 344 (CDF(F344)CrlBr) rats, 8–9 weeks of age, were exposed to 0, 0.69, 2.05, 6.01, 9.93 or 14.96 ppm [0, 0.84, 2.4, 7.2, 12 or 18 mg/m^3] formaldehyde vapour (produced by thermal depolymerization of paraformaldehyde) by whole-body exposure for 6 h per day on 5 days per week for up to 24 months; six rats per group were killed at 3, 6, 12 and 18 months for interim observation. Histopathological examination of the nasal cavities was performed on all rats. The distribution of the nasal tumours was recorded on diagrams of the nasal passages at 30 selected levels that were designed to permit accurate localization of nasal lesions. In the high-dose group, survival was significantly decreased relative to that in the control group (18.8% versus 35.7%; $p < 0.001$, life-table analysis using the product-limit procedure of Kaplan and Meier; Cox's method for pairwise comparisons). Survival in the other exposure groups was similar to that in controls. According to the authors, formaldehyde induced nasal squamous-cell carcinomas in a highly non-linear fashion: no such tumours were observed after exposure to 2 ppm or lower or in controls; the incidences in the groups exposed to 6.01, 9.93 and 14.96 ppm were 1/90 [1%], 20/90 (22%) and 69/147 (45%) [47% according to the Working Group], respectively. The single nasal tumour found in the 6.01-ppm group was located in the anterior lateral meatus, a region that was predicted to receive a relatively high dose of formaldehyde. The time-to-tumour appearance of nasal squamous-cell carcinomas was 622, 555 and 350 days in the 6.01-, 9.93- and 14.96-ppm groups, respectively. No other type of nasal tumour was found among controls or among animals exposed to the two lowest concentrations. Polypoid adenomas (5/90 (5.6%) and 14/147 (9.5%)), rhabdomyosarcomas (1/90 [1%] and 1/47 [1%]) and adenocarcinomas (1/90 [1%] and 1/147 [1%]) were observed in the nasal cavities of rats exposed to 9.93 and 14.96 ppm, respectively. Formaldehyde-induced non-neoplastic nasal lesions were primarily confined to the transitional and respiratory epithelium of the anterior passages, were only found in the groups exposed to the three highest concentrations and were most severe in the groups exposed to 9.93 and 14.96 ppm. These lesions mainly comprised epithelial hypertrophy, hyperplasia and metaplasia, mixed inflammatory cell infiltrates, turbinate adhesions and, in many high-dose animals, significant distortion and destruction of the nasoturbinate architecture. In rats exposed to 6.01 ppm, non-neoplastic lesions were minimal or absent, and were limited to focal squamous metaplasia in the anterior regions (Monticello *et al.*, 1996).

Four groups of 32 male Fisher 344 rats (F-344/DuCrj), 5 weeks of age, were exposed to 0, 0.3, 2.17 and 14.85 ppm [0, 0.36, 2.6 and 17.8 mg/m^3] formaldehyde [purity unspecified] vapour by whole-body exposure for 6 h per day on 5 days per week for up to 28 months. A group of 32 rats served as unexposed room controls. The formaldehyde vapour was produced from a 37% aqueous formaldehyde solution containing 10%

methanol as an anti-polymerization agent. Rats in the 0-ppm control group were exposed to the same concentration of methanol (4.2 ppm) as those of the high-dose group. At 12, 18 and 24 months, five (randomly selected) animals per group (in the high-dose group, only two animals were alive at 24 months) were killed and examined grossly. At 28 months, all survivors were killed and necropsied. Animals that were found dead or killed *in extremis* also underwent necropsy. Histopathological examinations were performed on five anatomically specified cross-sections of the nose from all animals, on all lesions identified grossly and on other major organs (approximately 23 per animal) [probably all control and all exposed animals, although this was not mentioned specifically]. The total number of animals that died or were killed in moribund condition were 11 room controls and eight, six, 10 and 20 0-ppm control, low-, mid- and high-dose rats, respectively. Mortality rates (calculated by the life-table technique) at 28 months were 59.6% of room controls, 45.5% of 0-ppm controls, 31.8% of the 0.3-ppm group, 55.9% of the 2.17-ppm group and 88.3% of the 14.85-ppm group ($p \leq 0.01$ compared with the 0-ppm group; Fisher's exact test). Gross and microscopic pathological changes attributable to exposure to formaldehyde were found only in the nose. Except for an unclassified sarcoma found in one room control, nasal tumours were seen only in the high-dose group, and included three squamous-cell papillomas, 13 squamous-cell carcinomas ($p \leq 0.01$; Fisher's exact test) and one sarcoma. Grossly, the first nasal tumour was observed in the high-dose group after 13 weeks of exposure. Most of the nasal tumours were located in the incisor teeth and maxillary turbinate regions. Large tumours invaded the subcutis through the nasal bones. Hyperplasia with squamous-cell metaplasia of the nasal epithelium [not further specified] was found only in rats exposed to formaldehyde, the incidences (combined for all animals examined at interim and terminal sacrifices and found dead or killed *in extremis*) of which were 0/32, 0/32, 4/32, 7/32 ($p \leq 0.01$; Fisher's exact test) and 29/32 ($p \leq 0.01$; Fisher's exact test) for the room-control, 0-, 0.3-, 2.17- and 14.96-ppm groups, respectively. Hyperkeratosis of the nasal epithelium was found in 1/32 rats exposed to 2.17 ppm and in 26/32 rats exposed to 14.96 ppm ($p \leq 0.01$; Fisher's exact test); papillary hyperplasia of the nasal epithelium was seen in 2/32 rats in the high-dose group (Kamata *et al*., 1997).

3.1.3 *Hamster*

A group of 88 male Syrian golden hamsters [age unspecified] was exposed to 10 ppm [12.3 mg/m^3] formaldehyde [purity unspecified] for 5 h a day on 5 days a week for life; 132 untreated controls were available. At necropsy, all major tissues were preserved, and histological sections were prepared from two transverse sections of the nasal turbinates of each animal; longitudinal sections were taken of the larynx and trachea, and all lung lobes were cut through the major bronchus. No tumours of the nasal cavities or respiratory tract were found in either controls or animals exposed to formaldehyde.

In a second study in the same report, 50 male Syrian golden hamsters [age unspecified] were exposed to 30 ppm [36.9 mg/m^3] formaldehyde [purity unspecified] for 5 h once a week for life. A group of 50 untreated hamster served as controls. When the animals died,

their respiratory tract tissues were preserved, stained with Wright's stain, rendered semitransparent and evaluated for 'subgross' evidence of tumours. Areas of dense staining of 1 mm or more were scored as tumours. Multiple transverse sections of the nasal turbinates were evaluated similarly. No nasal tumours were observed in control or treated hamsters (Dalbey, 1982).

3.2 Oral administration

Rat

In a study to evaluate the effects of formaldehyde on gastric carcinogenesis induced by oral administration of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (see Section 3.4.2), two groups of 10 male Wistar rats, 7 weeks of age, received tap-water for the first 8 weeks of the study. During weeks 8–40, one group was continued on tap-water and the other group received 0.5% formaldehyde [purity unspecified] in the drinking-water. Animals that were still alive at 40 weeks were killed; rats that survived beyond 30 weeks were considered as effective animals for the study. Necropsy was performed on most animals that died and all animals that were killed, and the stomach and other abdominal organs were examined grossly and histologically. Eight of 10 animals that had received formaldehyde in the drinking-water and none of the controls developed forestomach papillomas ($p < 0.01$, Fisher's exact test) (Takahashi *et al.*, 1986).

Wistar rats, obtained at 5 weeks of age and acclimatized for 9 days, were divided into four groups of 70 males and 70 females and were treated for up to 24 months with drinking-water that contained formaldehyde generated from 95% pure paraformaldehyde and 5% water. The mean doses of formaldehyde were 0, 1.2, 15 or 82 mg/kg bw per day for males and 0, 1.8, 21 or 109 mg/kg bw per day for females. Selected animals were killed at 53 and 79 weeks, and all surviving animals were killed at 105 weeks. Thorough necropsies were performed on all animals. Extensive histological examinations were made of animals in the control and high-dose groups; somewhat less extensive examinations were made of animals that received the low and middle doses, but the liver, lung, stomach and nose were examined in each case. Treatment-related atrophy, ulceration and hyperplastic lesions were found in the forestomachs and glandular stomachs, but the incidence of tumours did not vary notably between groups. Two benign gastric papillomas were observed (one in a male at the low dose and the other in a female control). The authors noted that the other tumours observed were common in this strain of rat and that there was no indication of a treatment-related tumour response (Til *et al*., 1989).

Two groups of male and female Sprague-Dawley breeder rats, 25 weeks of age, were given 0 (20 males and 20 females) or 2500 (18 males and 18 females) ppm formaldehyde [purity unspecified] in the drinking-water for life. The offspring of these breeders were initially exposed transplacentally to 0 (59 males and 49 females) or 2500 ppm (36 males and 37 females) formaldehyde via their mothers beginning on day 13 of gestation and then received the same levels in the drinking-water for life. [The Working Group noted the lack

of information on selection of the offspring.] All animals were necropsied and subjected to histopathological examination. The survival rates in the treated groups were similar to those of controls. According to the authors, the preliminary results of this study showed a slight, statistically non-significant increase in the incidence of leukaemias (including haemolymphoreticular lymphomas) in male and female breeders exposed to formaldehyde. A statistically non-significant increase in the incidence of leukaemias was also reported in the exposed male, but not in the exposed female, offspring (see Table 28). The authors also reported a variety of malignant and benign tumours of the stomach and intestines in the treated animals (see Table 28). Leiomyosarcoma was the most frequent malignant tumour. [The incidence of leiomysarcomas in the intestines was statistically significantly increased in the exposed offspring, both in females and in males and females combined; $p \leq 0.01$, χ^2 test.] This gastrointestinal tumour was exceptionally rare in the historical controls of this laboratory (Soffritti *et al.*, 1989). Concerns about the results by Soffritti *et al.* (1989) and their interpretation were published by Feron *et al*. (1990). One concern was that incidences of leukaemia in untreated Sprague-Dawley rats vary widely and that incidences similar to those seen in the group that received formaldehyde have been reported previously among controls in the same laboratory and in others.

Seven groups of 50 male and 50 female Sprague-Dawley rats, 7 weeks of age, received 10, 50, 100, 500, 1000 or 1500 mg/L formaldehyde (purity > 99.0%; 0.3% methanol as stabilizer; impurities: 0.6 mg/L iron, 0.1 mg/L lead, < 5.0 mg/L sulfur and < 5.0 mg/L chlorine) or 15 mg/L methanol in the drinking-water for 104 weeks. A control group of 100 males and 100 females received tap-water alone. By week 163, all animals had died. The consumption of treated drinking-water decreased in males of the high-dose group and in females of the three highest-dose groups (approximately 30% according to Soffritti *et al*., 1989). No differences between treated and control animals were observed in food consumption, body weight or survival. Moreover, no treatment-related non-neoplastic changes were detected by gross or histopathological examination. Percentages of animals that had malignant tumours and incidences of major tumours are presented in Table 29. Tumour incidences in the treated groups were compared with those in the untreated control group and statistically significantly increases were found in the number of males that had malignant tumours in the high-dose group, and in the incidences of malignant mammary gland tumours in females in the 1500-mg/L group, of testicular interstitial-cell adenomas in the 1000-mg/L group and of haemolymphoreticular tumours in males in the four highest- and in females in the two highest-dose groups. According to the authors, there was a dose–response relationship [no statistics given] for the increased incidences of haemolymphoreticular tumours. Gastrointestinal leiomyomas and leiomyosarcomas, which are very rare tumours in the strain of rats used, occurred sporadically in some formaldehyde-treated animals but not in controls (Soffritti *et al*., 2002). [The Working Group performed statistical analyses for trend and incidence in comparison with the methanol-treated group and found that the only statistically significant increases were in the total number of animals that had malignant tumours ($p < 0.01$) and in the incidences of haemolymphoreticular tumours ($p < 0.01$) in high-dose males and of testicular interstitial-cell adenomas ($p < 0.01$)

Table 28. Incidences (%) of leukaemia and tumours (benign and malignant) of the gastrointestinal tract after administration of formaldehyde in the drinking-water to Sprague-Dawley breeder rats and their offspring

Treatment	No. of rats	Leukaemias				Stomach			Intestine		
		Lymphoblastic leukaemias and lymphosarcomas	Immunoblastic lymphosarcomas	Others	Total	Papilloma/ acanthoma/ adenoma	Adeno-carcinoma/ squamous-cell carcinoma	Leiomyo-sarcoma	Adenoma	Adeno-carcinoma	Leiomyo-sarcoma
Breeders (25 weeks old)											
0 ppm											
Male	20	–	–	–	–	–	–	–	–	–	–
Female	20	5.0	–	–	5.0	–	–	–	–	–	–
Male + female	40	2.5	–	–	2.5	–	–	–	–	–	–
2500 ppm											
Male	18	–	5.6	5.6	11.1	–	5.6	–	–	–	–
Female	18	5.6	5.6	–	11.1	5.6	–	–	–	–	–
Male + female	36	2.8	5.6	2.8	11.1	2.8	2.8	–	–	–	–
Offspring											
0 ppm											
Male	59	–	3.4	1.7	5.1	–	–	–	–	–	–
Female	49	–	4.1	2.0	6.1	–	–	–	–	–	–
Male + female	108	–	3.7	1.8	5.5	–	–	–	–	–	–
2500 ppm											
Male	36	2.8	8.2	–	11.1	2.8	2.8	2.8	2.8	2.8	–
Female	37	–	–	–	–	–	2.7	2.7	–	2.7	13.5[a]
Male + female	73	1.4	4.1	–	5.5	1.4	2.8	2.7	1.4	2.7	6.8[a]

From Soffritti *et al.* (1989)

[a] [$p \leq 0.01$; χ^2 test, calculated by the Working Group]

Table 29. Percentage of animals that had malignant tumours and incidence of selected types of tumour in a number of organs of Sprague-Dawley rats after administration of formaldehyde in the drinking-water for up to 24 months

Site and type of tumour	Formaldehyde dose (mg/L)															
	Males								Females							
	0	0[a]	10	50	100	500	1000	1500	0	0[a]	10	50	100	500	1000	1500
No. of animals	100	50	50	50	50	50	50	50	100	50	50	50	50	50	50	50
Animals bearing malignant tumours (%)	38	42	28	24	44	48	46	72[b,c]	43	46	40	40	50	38	58	54
Mammary gland																
Adenocarcinoma	1	0	0	0	0	0	0	2	11	14	4	8	16	6	18	22
Fibrosarcoma	0	2	0	0	0	0	0	0	0	0	2	0	4	2	2	0
Liposarcoma	0	0	0	0	2	0	0	0	0	2	0	2	0	4	0	2
Angiosarcoma	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Total	1	2	0	0	2	2	0	2	11	16	6	10	20	12	20	24[d]
Forestomach																
Leiomyosarcoma	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Glandular stomach																
Leiomyosarcoma	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Intestine																
Leiomyoma	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	6
Leiomyosarcoma	0	0	0	0	0	0	0	4	0	0	0	2	0	0	0	0
Testes																
Interstitial-cell adenoma	10	6	6	12	12	20	24[d,e]	18								
Haemolymphoreticular tissues[f]																
Lymphomas and leukaemias	8	20	8	20	26[b]	24[d]	22[d]	46[b,c,g]	7	10	10	14	16	14	22[d]	20[d]

From Soffritti *et al.* (2002)

[a] 15 mg/L methanol

[b] $p < 0.01$, χ^2 test, versus untreated controls

[c] [$p < 0.01$, χ^2 test, versus methanol group; calculated by the Working Group]

[d] $p < 0.05$, χ^2 test, versus untreated controls [the Working Group noted that this category is an aggregate of tumours of different cellular origin]

[e] [$p < 0.01$, 2-tail Fisher exact test, versus methanol group; calculated by the Working Group]

[f] Including thymus, spleen and subcutaneous, mesenteric and pancreatic lymph nodes

[g] [$p < 0.01$ for trend, Cochran Armitage; calculated by the Working Group]

in the 1000-mg/L group. There was a statistically significant dose–response relationship for the increased incidences of haemolymphoreticular tumours ($p < 0.01$) in males. The Working Group noted the 'pooling' of lymphomas and leukaemias (designated as haemolymphoreticular neoplasias), the lack of reporting of non-neoplastic lesions and the absence of information on incidences of haemolymphoreticular tumours in historical controls.] Preliminary results of this study were published by Soffritti *et al*. (1989). [The Working Group noted that, in spite of the extensive histopathological examinations on which the preliminary data on tumours presented by Soffritti *et al*. (1989) were stated to be based, the reported total number of animals that had haemolymphoreticular neoplasias increased from 79 (Soffritti *et al.*, 1989) to 150 (Soffritti *et al*., 2002).]

3.3 Dermal application

Mouse

In a study to evaluate the effects of formaldehyde on skin carcinogenesis induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) (see Section 3.4.1), two groups of 16 male and 16 female Oslo hairless mice [age unspecified] received topical applications of 200 μL aqueous solution of 1 or 10% formaldehyde on the skin of the back twice a week for 60 weeks. Animals were observed weekly for skin tumours. All of the animals treated with 10% formaldehyde were necropsied and the brain, lungs, nasal cavities and all tumours of the skin and other organs were examined histologically. Virtually no changes were found in the mice treated with 1% formaldehyde. The 10% dose induced slight epidermal hyperplasia and a few skin ulcers. No benign or malignant skin tumours or tumours in other organs were observed in either group (Iversen, 1986). [The Working Group noted that no group treated with water only was included.]

3.4 Administration with known carcinogens and other modifying factors

3.4.1 *Mouse*

Oslo hairless mice [initial number and age unspecified] received a single topical application of 51.2 μg DMBA in 100 μL reagent-grade acetone on the skin of the back. Nine days later, a group of 16 male and 16 female mice received twice-weekly applications of 200 μL 10% formaldehyde in water (technical-grade formalin) on the skin of the back. Another group of 176 mice [sex unspecified] was given no further treatment. Animals were observed weekly for skin tumours for 60 weeks (first group) or 80 weeks (second group). All of the animals treated with 10% formaldehyde were necropsied, and the brain, lungs, nasal cavities and all tumours of the skin and other organs were examined histologically. In the first group, 3/32 mice had lung adenomas and 11/32 (34%) had 25 skin tumours, including three squamous-cell carcinomas and 22 papillomas. In mice that received DMBA alone, 225 skin tumours (including six squamous-cell carcinomas) occurred in 85/176 (48%) animals. Statistical analysis of the results for these two groups

showed that formaldehyde significantly enhanced the rate of skin tumour induction ($p = 0.01$, Peto's test), and reduced the latency period for the tumours (Iversen, 1986). [The Working Group noted the incomplete reporting of the tumours in the group of rats treated with DMBA alone.]

3.4.2 *Rat*

Two groups of 30 and 21 male Wistar rats, 7 weeks of age, received 100 mg/L MNNG in the drinking-water and a standard diet that contained 10% sodium chloride for 8 weeks. Thereafter, the rats received the standard diet and 0 or 0.5% formaldehyde in the drinking-water for a further 32 weeks. Animals still alive at 40 weeks were killed, and rats that survived 30 weeks or more were considered as effective animals for the study. Necropsies were performed on most animals that died and on all animals that were killed at week 40. Malignant tumours of the stomach and duodenum were found in 4/30 (13%) rats that received MNNG and in 5/17 (29%) rats that received both MNNG and formaldehyde [not significant]. Adenocarcinomas of the glandular stomach were found in 4/17 (23.5%) rats that received the combined treatment and in 1/30 rats that received MNNG alone ($p < 0.05$, Fisher's exact test). Papillomas of the forestomach were found in 15/17 rats that received the combined treatment and in 0/30 that received MNNG alone ($p < 0.01$, Fisher's exact test). The incidence of adenomatous hyperplasia of the fundus of the glandular stomach was significantly greater in the group that received the combined treatment (15/17) than in those that received MNNG alone (0/30) ($p < 0.01$, Fisher's exact test) (Takahashi *et al.*, 1986).

Groups of 50 female white non-inbred rats [strain and age not specified] received intratracheal injections of one of three doses of benzo[*a*]pyrene as a suspension in 5% albumin in saline once every 2 weeks for 20 weeks (total doses, 0, 0.02, 0.1 or 5.0 mg/animal). One group of 50 female rats served as an untreated control. All benzo[*a*]pyrene-treated groups were then exposed by inhalation to 0, 0.003, 0.03 or 0.3 mg/m^3 formaldehyde for 7 h per day on 5 days per week for 12 months. All animals were maintained until natural death, and organs and tissues that were suspected to have developed a tumour were subjected to histological examination. Tumours developed in rats of all 16 groups. Two of 39 effective (survived to the time of the first tumour development) rats in the control group developed reticulosarcomas of the lung and two developed fibroadenomas of the mammary gland. Similar tumours were observed at almost the same incidence in rats exposed to the three doses of formaldehyde alone. The incidence of total tumours in benzo[*a*]pyrene-treated rats was dose-dependent and varied from 13 to 28%. The incidence of lung tumours varied from 9 to 19%. In the mid- and high-dose groups, squamous-cell cysts and carcinomas of the lung were observed as well as lymphatic leukaemia. The tumour response to the combined treatment with benzo[*a*]pyrene and formaldehyde was also dose-dependent. The most prominent and statistically significant increase in the incidence of lung tumours (43%) and all tumours (69%) occurred in rats that were treated with the highest doses of benzo[*a*]pyrene and formaldehyde (5.0 mg and 0.3 mg/m^3, respectively). In addition,

tumours developed earlier in this group and had greater multiplicity than those in animals that were exposed to benzo[*a*]pyrene or formaldehyde alone. The authors concluded that the combined treatment of rats with benzo[*a*]pyrene and formaldehyde leads to an increase in tumour response which is manifested as an increased incidence of tumours, a reduction in the latency period of tumour development and a broader spectrum of tumours (Yanysheva *et al*., 1998). [The Working Group noted the very low level of exposure to formaldehyde compared with levels used in other experiments in rats.]

3.4.3 *Hamster*

Groups of male Syrian golden hamsters [age unspecified] were treated in various ways: 50 were exposed by inhalation to 30 ppm [36.9 mg/m^3] formaldehyde [purity unspecified] for 5 h per day once a week for life; 100 hamsters were injected subcutaneously with 0.5 mg *N*-nitrosodiethylamine (NDEA) once a week for 10 weeks and then given no further treatment; 50 hamsters were injected with NDEA once a week for 10 weeks, exposed to 30 ppm formaldehyde for 5 h, 48 h before each injection of NDEA, and then received weekly exposure to 30 ppm formaldehyde for life; and the fifth group of [presumably] 50 hamsters was injected with NDEA once a week for 10 weeks and then exposed to 30 ppm formaldehyde for 5 h per day once a week for life, beginning 2 weeks after the last injection of NDEA. A group of 50 animals served as untreated controls. After the animals had died, the respiratory tract tissues were removed, stained with Wright's stain, rendered semitransparent and evaluated for 'subgross' evidence of tumours. Areas of dense staining greater than 1 mm in 2–3-mm transverse-step sections of nasal turbinates were scored as tumours. No tumours were observed in untreated hamsters or those exposed only to formaldehyde, but 77% of hamsters treated with NDEA alone had tumours at one or more sites in the respiratory tract. Ten or more such lesions from each tissue were examined histologically, and all were found to be adenomas. Lifetime exposure of NDEA-treated hamsters to formaldehyde did not increase the number of tumour-bearing animals. The incidences of nasal tumours in NDEA-treated groups were low (0–2%). The only significant increase was in the multiplicity of tracheal tumours in the group that received formaldehyde concurrently with and subsequent to injections of NDEA compared with that in animals that received NDEA alone ($p < 0.05$, Kolmogorov–Smirnoff test) (Dalbey, 1982).