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 started on a regimen of exposure to formaldehyde (USP grade) vapour at concentrations of 0, 0.05, 0.1 or 0.20 mg/L [0, 50, 100 or 200 mg/m^3] for 1 h per day, three times a week, ostensibly for 35 weeks. Treatment of mice with the highest concentration was discontinued after the eleventh exposure because of severe toxicity, and 36 of the mice exposed to 0.05 mg/L for 35 weeks were subsequently exposed to 0.15 mg/L [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 B6C3F1 mice, six weeks of age, were exposed to 0, 2.0, 5.6 or 14.3 ppm [0, 2.5, 6.9, 17.6 mg/m^3] formaldehyde (> 97.5% pure) vapour by whole-body exposure for 6 h per day on five days per week, for up to 24 months, followed by a six-month observation period with no further exposure. Ten males and 10 females

Table 14. Aggregated risk ratios (RR), 95% confidence intervals (95% CI) and observed (O) and expected (E) frequencies of respiratory cancers in the meta-analyses of Blair *et al.* (1990a) and Partanen (1993)

Site	Level or duration of exposure to formaldehyde											
	Any				Low/medium				Substantial			
	Blair *et al.*		Partanen		Blair *et al.*		Partanen		Blair *et al.*		Partanen	
	O/E	RR (95% CI)	O/E	RR (95% CI)	O/E	RR (95% CI)	O/E	RR (95% CI)	O/E	RR (95% CI)	O/E	RR (95% CI)
Lung												
Medical professions[a]	29/89	0.3 (0.2-0.5)	54/160	0.3 (0.3-0.4)								
Nonmedical professions[b]	490/520	0.9 (0.9-1.0)	474/486	1.0 (0.9-1.1)								
Industrial workers	1181/1097	1.1 (1.1-1.1)	833/752	1.1 (1.0-1.2)	514/422	1.2 [1.1-1.3]	518/425	1.2 (1.1-1.3)	250/240	1.0 [0.9-1.2]	233/216	1.1 (1.0-1.2)
Nose and nasal sinuses	60/56	1.1 (0.8-1.4)	93/78	1.1 (0.8-1.5)	38/46	0.8 (0.6-1.1)	33/30	1.1 (0.7-1.8)	30/28	1.1 (0.7-1.5)	36/21	1.7 (1.0-2.8)
Nasopharynx	31/25	1.2 (0.8-1.7)	36/21	2.0 (1.4-2.9)	30/27	1.1 (0.7-1.6)	23/16	1.6 (1.0-2.7)	13/6	2.1 (1.1-3.5)	11/4	2.7 (1.4-5.6)
Other respiratory			69/57	1.2 (0.9-1.6)			52/48	1.1 (0.7-1.5)			23/20	1.2 (0.6-2.1)

Blair *et al.* (1990a) included the following studies in their analysis: Harrington & Shannon (1975), Petersen & Milham (1980), Jensen & Andersen (1982), Fayerweather *et al.* (1983), Friedman & Ury (1983), Marsh (1983), Milham (1983), Walrath & Fraumeni (1983), Wong (1983), Acheson *et al.* (1984a,c), Coggon *et al.* (1984), Harrington & Oakes (1984), Levine *et al.* (1984), Liebling *et al.* (1984), Malker & Weiner (1984), Olsen *et al.* (1984), Walrath & Fraumeni (1984), Stayner *et al.* (1985), Partanen *et al.* (1985), Walrath *et al.* (1985), Bertazzi *et al.* (1986), Blair *et al.* (1986), Bond *et al.* (1986), Gallagher *et al.* (1986), Hayes *et al.* (1986), Logue *et al.* (1986), Stroup *et al.* (1986), Vaughan *et al.* (1986a,b), Blair *et al.* (1987), Roush *et al.* (1987), Stayner *et al.* (1988), Bertazzi *et al.* (1989), Gérin *et al.* (1989), Blair *et al.* (1990b), Hayes *et al.* (1990)

Partanen (1993) included in his analysis both the above studies and: Brinton *et al.* (1984), Partanen *et al.* (1990), Merletti *et al.* (1991)

[a] Anatomists, pathologists, forensic medicine specialists

[b] Funeral directors, embalmers, undertakers, medicinal drug users

from each group were killed at 6 and 12 months, 0–20 of each sex at 18 months, 17–41 at 24 months and 0–16 at 27 months. Between 0 and 24 months, 78 male and 30 female controls, 77 and 34 exposed to 2 ppm formaldehyde vapour, 81 and 19 exposed to 5.6 ppm and 82 and 34 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 identified grossly in the other two groups. Squamous-cell carcinomas occurred in the nasal cavities of 2/17 male mice at the high dose killed at 24 months. There were no nasal cavity tumours in males mice treated with the 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, seven weeks of age, were exposed to 0, 2.0, 5.6 or 14.3 ppm [0, 2.5, 6.9, 17.6 mg/m^3] formaldehyde (> 97.5% pure) vapour by whole-body exposure for 6 h per day on five days per week for up to 24 months and were then observed for six 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, 6 males and 13 females in the control group, 10 and 16 exposed to 2 ppm, 19 of each sex exposed to 5.6 ppm and 57 and 67 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 identified grossly and on all major tissues of each organ system (approximately 40/animal) from control and high-dose rats. The findings for the nasal cavity are summarized in Table 15. While no nasal cavity malignancies were found in rats exposed to 0 or 2.0 ppm formaldehyde, two squamous-cell carcinomas (one among 119 males and one among 116 females examined) occurred in the group exposed to 5.6 ppm and 107 (51 among 117 males and 52 among 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 and in males at the middle dose (see also Table 15). A variety of non-neoplastic lesions were commonly found in the nasal cavities of rats exposed to formaldehyde, particularly at 14.3 ppm (Kerns *et al.*, 1983a,b; Gibson, 1984). More 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, 26% 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).

Table 15. 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[e]	1	0	{ 4	4[c]	6[d]	0	4	1 }[e]

From Morgan *et al.* (1986a)

[a] $p < 0.001$, pair-wise comparisons

[b] One animal 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]

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 formation of bis(chloromethyl)ether, 99 male Sprague–Dawley rats, eight weeks of age, were exposed to a mixture of 14.7 ppm [18.1 mg/m^3] formaldehyde vapour [purity unspecified] and 10.6 ppm [15.8 mg/m^3] hydrogen chloride gas for 6 h per day on five 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. 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 the sames series of experiments, groups of 99–100 male Sprague-Dawley rats, nine weeks of age, were exposed for 6 h per day on five 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 16. At the end of the experiment, 38 squamous-cell carcinomas of the nasal cavities and 10 polyps or papillomas were observed in rats exposed to formaldehyde alone; none were seen in the controls (p = 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 16. Neoplastic lesions in the nasal cavities of 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
Polyps or papillomas	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] of formaldehyde vapour [purity unspecified] starting one week after acclimatization. Whole-body exposures for 6 h per day on five days per week were continued for four, eight or 13 weeks; thereafter, the rats were observed during recovery periods of 126, 122 or 117 weeks, respectively, when 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 17. In control rats, the only nasal tumours reported were two squamous-cell carcinomas among 45 rats that were exposed to air for eight 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 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 four weeks and killed at 100 weeks and in another rat exposed for eight weeks and killed at 110 weeks; and there were six 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, at an incidence of 4.5% (6 tumors/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.]

Table 17. Nasal tumours in rats exposed to formaldehyde for various periods 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 causally related to exposure to formaldehyde

A total of 720 male specific pathogen-free Wistar rats initially weighing 30–50 g were acclimatized for one 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] of formaldehyde [purity unspecified] vapour by whole-body exposure for 6 h per day on five days per week. One-half of the animals (30 undamaged, 60 damaged rats) were exposed for 28 months, and the other half (30 undamaged, 60 damaged) were exposed for only three 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 18. A high incidence of nasal tumours (17/58) was found in rats with damaged noses and 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 with undamaged noses that 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 18. Nasal tumours in male Wistar rats with damaged or undamaged noses and exposed to formaldehyde vapour for 28 or three 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; D, damaged nose

In a study to explore the interaction between formaldehyde and wood dust (see also p. 165 of the monograph on 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 five 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 [not significant]. Neither the frequency nor the latent periods of induction of tumours outside the nasal cavity differed from those in controls (Holmström *et al.*, 1989a). [The Working Group noted the small numbers of animals used in the study.]

3.1.3 Hamster

A group of 88 male Syrian golden hamsters [age unspecified] were exposed to 10 ppm [12.3 mg/m^3] formaldehyde [purity unspecified] for 5 h a day on five 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 the controls or the animals exposed to formaldehyde (Dalbey, 1982).

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 per 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 lifetime study, formaldehyde was administered in drinking-water to male and female Sprague-Dawley rats beginning at various ages. Groups of 50 male and 50 female rats received 10, 50, 100, 500, 1000 or 1500 ppm [mg/L] formaldehyde from seven weeks of age for life; two control groups of 50 males and 50 females and 100 males and 100 females received 15 mg/L (ppm) methanol or nothing, respectively, in their drinking water. Two groups of 18–20 male and female breeder rats, 25 weeks old, were given formaldehyde at 0 or 2500 ppm for life. The offspring of these breeders, 36–59 males and 37–49 females, were initially exposed to 0 or 2500 ppm formaldehyde via their mothers starting on day 13 of gestation and then received these levels in the drinking-water for life. The survival rates in the treated groups were similar to those of controls. All animals were necropsied, and extensive histological examinations were performed. The authors reported an increased, dose-related incidence of leukaemias in the treated groups (see Table 19). They also observed a variety of malignant and benign tumours of the stomach and intestines in the treated animals. Although the incidences of intestinal tract tumours were low, there were no comparable tumours in the control groups in this study, and some of these tumours were reported to be uncommon among historical controls (Soffritti *et al.*, 1989).

Table 19. Incidences of leukaemia and gastrointestinal tract tumours after administration of formaldehyde to rats in drinking-water (males and females combined)

Treatment	No. of tumours (benign and malignant)		
	Leukaemia	Gastrointestinal tract	
		Stomach	Intestine
7 weeks old			
(0 ppm + 15 ppm methanol)	8/100	0	0
0 ppm	7/200	0	0
10 ppm	3/100	2/100	1/100
50 ppm	9/100	0	2/100
100 ppm	9/100	0	0
500 ppm	12/100 *a*	0	0 *a*
1000 ppm	13/100	1/100	1/100
1500 ppm	18/100	2/100	6/100
25 weeks old (breeders)			
0 ppm	1/40	0/40	0/40
2500 ppm	4/36	2/36	0/36
Offspring			
0 ppm	6/108 *b*	0/108 *b*	0/108 *b*
2500 ppm	4/73	5/73	8/73

From Soffritti *et al.* (1989)

[a][Significant linear dose–response relationship when formaldehyde-treated groups are compared with water controls, $p < 0.001$, or water-methanol controls, $p < 0.01$, Cochran–Mantel-Haenszel test]

[b][$p = 0.01$; Fisher's exact test]

Concerns about the results in this study and their interpretation have been published by Feron *et al.* (1990). They noted that leukaemia incidences in untreated Sprague-Dawley rats vary widely and that incidences similar to those seen in the group receiving the highest dose of formaldehyde have been reported previously among controls in the same laboratory and others. The Working Group, however, noted the absence of gastric or intestinal tumours among the 300 control animals, while, on the basis of the authors' report of historical incidences, three gastric and one intestinal tumour would have been expected. Furthermore, the reporting of the data was limited. The Group subjected the available data from this study to statistical analysis, despite the above reservations. The groups treated at seven weeks were found to differ significantly with regard to both leukaemia and intestinal tumour incidence from the 300 combined controls [$p < 0.05$, Fisher's exact test]. The incidence of leukaemia in the treated groups also differed significantly [$p < 0.001$, Fisher's exact test] from that in the untreated controls; however, the difference in intestinal tumours was only marginally significant

[$p = 0.055$, Fisher's exact test]. When the groups treated at seven weeks were compared with the controls given methanol, the differences were not significant. A significant, linear dose–response relationship was found for the incidences of both leukaemia and intestinal tumours, in comparison with either the untreated [$p < 0.01$] or the methanol controls [$p < 0.01$] [Cochran–Mantel Haenszel test].

Wistar rats, obtained at five weeks of age and acclimatized for nine days, were divided into four groups of 70 males and 70 females and were treated for up to 24 months with drinking-water containing 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 102 weeks. Thorough necropsies were done 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 receiving the low and middle doses, but the liver, lung, stomach and nose were examined in each case. Treatment-related hyperplastic lesions, ulceration and atrophy were found in the 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 response (Til *et al.*, 1989).

Four groups of 20 male and 20 female Wistar rats, four weeks of age, were given formaldehyde (prepared from 80% pure paraformaldehyde) in their drinking-water at concentrations of 0, 0.02, 0.1 or 0.5% for up to 24 months. Six rats were chosen at random from each group and killed after 12 and 18 months of treatment; surviving animals were killed at 24 months and necropsied, and histological examinations were performed on major organs. [The Working Group noted that gross or microscopic examination of the nasal cavities was not mentioned specifically.] Rats given the high dose had reduced body weight gain and high mortality. Non-neoplastic lesions, such as squamous- and basal-cell hyperplasia, erosion and ulceration, were seen in the stomachs and forestomachs of rats given 0.5% at 12 months. The incidences of tumours in all groups were similar to those occurring spontaneously in this strain of rat. The authors reported that there were no significant differences in the incidences of any tumours from those in the control groups (Tobe *et al.*, 1989). [The Working Group noted the lack of detailed reporting of tumours and the small numbers of animals used.]

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 below), two groups of 10 male Wistar rats, seven weeks of age, received tap water for the first eight weeks of the study. During weeks 8–40, one group then received pure water and the other group received 0.5% formaldehyde in the drinking-water. Animals still alive at 40 weeks were killed, rats surviving beyond 30 weeks being 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 drinking-water and none of the controls developed forestomach papillomas ($p < 0.01$, Fisher's exact test) (Takahashi *et al.*, 1986).

3.3 Skin application

Mouse: Two groups of 16 male and 16 female Oslo hairless mice [age unspecified] received topical applications of 200 µl of 1 or 10% formaldehyde in water on the skin of the back twice a week for 60 weeks. 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 higher dose induced slight epidermal hyperplasia and a few skin ulcers. There were no benign or malignant skin tumours or tumours in other organs in either group (Iversen, 1986). [The Working Group noted the incomplete reporting of the data.]

3.4 Subcutaneous injection

Rat: In a study reported as an abstract, 10 rats [strain, age and sex unspecified] were injected subcutaneously once a week for 15 months with 1 ml of a 0.4% aqueous solution of formaldehyde and then observed for life. Spindle-cell sarcomas were found in three rats: two in the skin at the injection site and one in the peritoneal cavity (Watanabe *et al.*, 1954). [The Working Group noted the lack of controls.]

3.5 Administration with known carcinogens and other modifying factors

3.5.1 Mouse

Two groups of 50 female CBA × C57Bl6 mice, weighing 10–12 g, received drinking-water containing *N*-nitrosodimethylamine (NDMA) at a concentration of 10 mg/L and formaldehyde at a concentration of 0.5 mg/L for 26 or 39 weeks. Other groups of mice were treated either with NDMA alone for 26 and 39 weeks or formaldehyde alone for 39 weeks. Animals were killed after completion of treatment and necropsied, and the liver, kidney, lung, spleen and all gross lesions were examined histologically. No tumours were observed in the group receiving formaldehyde alone for 39 weeks. Combined administration of NDMA and formaldehyde increased the proportions of surviving mice bearing tumours in the liver, kidney and/or lung in the groups treated for 26 weeks and for 39 weeks, as compared with mice treated with NDMA alone (11/15 versus 17/30 and 19/19 versus 20/25) ($p = 0.049$, Fisher's exact test). The effect was not associated with obvious changes in the relative incidence of tumours at any site (Litvinov *et al.*, 1984).

Oslo hairless mice [age unspecified] each received a single application of 51.2 µg 7,12-dimethylbenz[*a*]anthracene (DMBA) in 100 µl of reagent-grade acetone on the skin of the back. Nine days later, the first group of 16 male and 16 females mice received twice weekly applications of 200 µl 10% formaldehyde in water (technical-grade formalin) on the skin of the back. A second group of 16 males and 16 females received 17 nmol 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on the skin of the back twice a week. A third group, of 176 mice [sex unspecified], was given no further treatment. Animals were observed weekly for 60 weeks (groups 1 and 2) or 80 weeks (group 3). 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 group 1, 3/32 mice had lung adenomas and 11/32 (34%) had 25 neoplasms of the skin, including three squamous-cell carcinomas and 22 papillomas. In mice receiving 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 was reported by the authors to show no significant effect of formaldehyde on the skin tumour yield initiated by DMBA ($p > 0.30$, Gail test), but formaldehyde significantly enhanced the rate of skin tumour induction ($p = 0.01$, Peto's test), thus reducing the latent period for the tumours (Iversen, 1986). [The Working Group noted the incomplete reporting of the tumours.]

3.5.2 Rat

Two groups of 30 and 21 male Wistar rats, seven weeks of age, received MNNG in the drinking-water at a concentration of 100 mg/L and a standard diet containing 10% sodium chloride for eight weeks. Thereafter, the rats received the standard diet with 0 or 0.5% formaldehyde in the drinking-water for a further 32 weeks. Animals still alive at 40 weeks were killed, rats surviving 30 weeks or more being considered effective animals for the study. Necropsies were performed on most animals that died and on all animals killed at week 40. Malignant tumours of the stomach and duodenum were found in 5/17 (29%) rats that received both MNNG and formaldehyde and in 4/30 (13%) rats that received MNNG [not significant]. Adenocarcinomas of the glandular stomach were found in 4/17 (23.5%) rats given the combined treatment and in 1/30 rats given MNNG alone ($p < 0.05$, Fisher's exact test). Papillomas of the forestomach were found in 15/17 rats given the combined treatment, in 0/30 given MNNG alone ($p < 0.01$, Fisher's exact test) and in 8/10 given formaldehyde alone (not significant; see section 3.2). The incidence of adenomatous hyperplasia of the fundus of the glandular stomach was significantly greater in the group given the combined treatment (15/17) than in those given MNNG alone (0/3) ($p < 0.01$, Fisher's exact test) (Takahashi *et al.*, 1986).

3.5.3 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 two weeks after the last NDEA injection. A group of 50 animals were untreated. 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 receiving formaldehyde concurrently with and subsequent to NDEA injection as compared with that in animals receiving NDEA alone ($p < 0.05$, Kolmogorov–Smirnoff test) (Dalbey, 1982).