3. Studies of Cancer in Experimental Animals

3.1 Continuous glass filament

3.1.1 *Intraperitoneal injection* (see Table 59)

Rat: Groups of 28–50 female Wistar rats, 12–15 weeks old, were treated with one of three test fibres (ES3, ES5 or ES7) [chemical composition presumed to be E-glass] derived from continuous filaments and administered as a single (10 mg) or two weekly (2 × 20 mg) intraperitoneal injections in 2 mL saline or by intraperitoneal laparotomy (50-mg and 250-mg doses). The median dimensions and doses of the glass filament test fibres were as follows: ES3, 16.5 μm × 3.7 μm, 50 and 250 mg/rat; ES5, 39 μm × 5.5 μm, 10, 40 and 250 mg/rat; ES7, 46 μm × 7.4 μm, 40 mg/rat. Groups of control animals were similarly treated with saline only, ground glass (40, 50 or 250 mg) or UICC/A chrysotile asbestos (6 or 25 mg). Animals were observed for life. Median survival times were 111, 107, 121 and 119 weeks for the groups given 10 mg ES5, 40 mg ES5, 40 mg ES7 and 40 mg ground glass, respectively. The corresponding mean survival times of animals with tumours were 106, 119, 126 and 129 weeks, respectively. No statistically significant increase in the incidence of abdominal tumours was observed in the groups treated intraperitoneally with ES3, ES5 or ES7 compared with controls treated with saline or ground glass. The tumour incidences were (from low to high dose): for ES3, 6% and 9%; for ES5, 4%, 11% and 7%; for ES7, 2%; for ground glass, 4–8%; for saline, 4–6%; and for chrysotile, 77–81% (Pott *et al.*, 1987). [The Working Group noted that administration by laparotomy shortened the survival times and that the number of fibres injected was much smaller in these studies (as these fibres were relatively coarse (i.e. > 3 μm in diameter)) than in studies on glass wool carried out in the same laboratory.]

3.2 Glass wool

3.2.1 *Inhalation exposure* (see Table 60)

(*a*) *Rat*

Groups of 46 young adult male Sprague-Dawley rats [age at outset not stated] were exposed by whole-body inhalation to ball-milled glass fibre [chemical composition not

Table 59. Study of carcinogenicity of glass filaments administered by intraperitoneal injection in female Wistar rats

Fibre type	Median diameter (μm)	Median length (μm)	Mass (mg)	No. of tumours	No. of rats	Percentage of tumours	Age in weeks
E-glass, continuous filament (ES3)	3.7	16.5	50	3	48	6	15
	3.7	16.5	250	4	46	9	
E-glass, continuous filament (ES5)	5.5	39	10	2	50	4	12
	5.5	39	40	5	46	11	
	5.5	39	250	2	28	7	
E-glass, continuous filament (ES7)	7.4	46	40	1	47	2	
Saline (control)			1 mL	2	32	6	5
			4 mL	2	45	4	15
Ground glass (control)			40	2	45	4	12
			50	4	48	8	15
			250	4	48	8	
Chrysotile (positive control)	0.15	9	6	26	34	77	12
	0.15	9	25	25	31	81	

From Pott *et al.* (1987)

given] (700 fibres > 5 μm/cm^3; 24% with diameters < 3 μm) or to amosite asbestos (3100 fibres > 5 μm/cm^3; 38% with diameters < 3 μm) for 5 h per day on five days per week for three months followed by observation for 21 months. [The aerosol concentrations estimated as WHO fibres/cm^3 were approximately 168 for glass fibre and 1178 for amosite.] A group of 46 untreated rats served as negative controls. Four to 10 rats per exposure group were killed at 20 and 50 days; 3, 6, 12 and 18 months; and the remainder at 24 months. No pulmonary tumours were observed in any of the rats that died or were killed prior to 24 months. The incidences of tumours observed in rats killed at 24 months were not significantly above the level typical for this species: glass fibre, 2/11 (adenomas); amosite asbestos, 3/11 (two adenomas and one carcinoma); controls, 0/13 (Lee *et al*., 1981). [The Working Group noted the short period of exposure and the small number of animals involved. No fibres tested were longer than 10 μm and the study was therefore inconclusive.]

Groups of 24 male and 24 female Wistar IOPS AF/Han rats, 8–9 weeks of age, were exposed by whole-body inhalation to dust at concentrations of 5 mg/m^3 (respirable particles) from French (Saint Gobain) glass fibre [type and composition not stated] (42% of fibres < 10 μm in length, 69% < 1 μm in diameter; 48 WHO fibres/cm^3) or a Canadian chrysotile fibre (6% respirable fibres > 5 μm in length; 5901 WHO fibres/cm^3) for 5 h per day on five days per week for 12 or 24 months. An unspecified number of rats were

Table 60. Chronic inhalation studies of insulation glass wools and special-purpose glass fibres in rodents (rats and hamsters)

Test substance	Aerosol fibres (numbers and dimensions)	Positive control	Test system (no. at risk); observation time	Exposure	Lung dose	No. of thoracic tumours/no. of animals	Comments (positive control tumour incidence)	Reference
Insulation glass wools								
Rat								
Glass fibre	~168 WHO f/cm^3	Amosite, ~1178 WHO f/cm^3	Sprague-Dawley rats, male; 24 mo	Whole-body, 5 h/d, 5 d/wk, for 3 mo		2/11 (adenomas)	Short exposure period, small number (11) of animals at risk (amosite: 3/12 tumours)	Lee *et al.* (1981)
Glass fibre + resin	240 WHO f/cm^3 (10 f L > 20 μm, 10 mg/m^3 respirable dust; D, 52% < 1 μm; L, 72% 5–20 μm	Chrysotile, 10 mg/m^3, 3800 WHO f/cm^3	56 (48) SPF Fischer rats, male and female; 24 mo; lifetime	Whole-body, 7 h/d, 5 d/wk, for 12 mo	mg f/lung: 1.9 at 12 mo 0.5 at 21 mo	1/48 (adeno-carcinoma); fibrosis, 0	Type of glass fibre not specified (chrysotile: 12/48 tumours; fibrosis)	Wagner *et al.* (1984)
Glass fibre – resin	323 WHO f/cm^3 (19 f L > 20 μm, 10 mg/m^3 respirable dust; D, 47% < 1 μm; L, 58% 5–20 μm	Chrysotile, 10 mg/m^3, 3800 WHO f/cm^3	56 (47) SPF Fischer rats, male and female; 24 mo; lifetime	Whole-body, 7 h/d, 5 d/wk, for 12 mo	mg f/lung: 0.9 at 12 mo 0.2 at 21 mo	1/47 (adenoma); fibrosis, 0	Type of glass fibre not specified (chrysotile: 12/48 tumours; fibrosis)	Wagner *et al.* (1984)
Glass fibre; French (Saint Gobain)	48 WHO f/cm^3, 5 mg/m^3 respirable dust; D, 69% < 1 μm; L, 42% > 10 μm	Chrysotile, 5 mg/m^3; L, 6% > 5 μm	45 Wistar rats, male/female; 28 mo	Whole-body for 24 mo	ND	1/45	Type of glass fibre not specified (chrysotile: 9/47 tumours; fibrosis)	Le Bouffant *et al.* (1984)
Insulsafe II building insulation	30 f/cm^3; L, > 10 μm; D, < 1 μm; 100 total f/cm^3; 10 mg/m^3; D, 1.4 μm mean; GMD, 1.2 μm; L, 37 μm mean; GML, 24 μm	Crocidolite, 3000 total f/cm^3, 90 f/cm^3 (L, > 10 μm)	52 Osborne-Mendel rats, female; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	3×10^4 f/mg dry lung at 3 mo	Tumours: 0/52; fibrosis, 0	Aerosolized fibres were short. Asbestos: low tumour incidence (3/57; ~5%) + fibrosis (some)	Smith *et al.* (1987)
Manville 901 building insulation	232 WHO f/cm^3 (73 f L > 20 μm), 30 mg/m^3; D, 1.4 μm mean; GMD, 1.3 μm; L, 16.8 μm mean; GML, 13.1 μm	Chrysotile, 10 mg/m^3, 10 600 WHO f/cm^3	140 (119) Fischer rats, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	f L > 5 μm/ lung: 42×10^6 at 12 mo; 82×10^6 at 24 mo f L > 20 μm/ lung: 3×10^6 at 12 mo; 5×10^6 at 24 mo	Lung tumours: 7/119 (1 carci-noma); fibrosis, 0	Chrysotile: 13/69 lung tumours; 1/69 mesothelioma	Hesterberg *et al.* (1993); Hesterberg & Hart (2001)

Table 60 (contd)

Test substance	Aerosol fibres (numbers and dimensions)	Positive control	Test system (no. at risk); observation time	Exposure	Lung dose	No. of thoracic tumours/no. of animals	Comments (positive control tumour incidence)	Reference
Insulsafe II building insulation	246 WHO f/cm^3 (90 f L > 20 μm), 30 mg/m^3; D, 0.9 μm mean; GMD, 0.7 μm; L, 18.3 μm mean; GML, 13.7 μm	Chrysotile, 10 mg/m^3, 10 600 WHO f/cm^3	140 (112) Fischer rats, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	f L > 5 μm/lung: 69×10^6 at 12 mo; 182×10^6 at 24 mo f L > 20 μm/lung: 7×10^6 at 12 mo; 6×10^6 at 24 mo	Lung tumours: 3/112; fibrosis, 0	Chrysotile: 13/69 lung tumours; 1/69 mesothelioma	Hesterberg *et al.* (1993); Hesterberg & Hart (2001)
Manville building insulation	25 f/cm^3; L, > 10 μm; D, < 1 μm; 100 total f/cm^3; 12 mg/m^3; D, 1.4 μm mean; GMD, 1.1 μm; L, 31 μm mean; GML, 20 μm	Crocidolite, 3000 f/cm^3, total f/cm^3, 90 f/cm^3; L, > 10 μm	57 Osborne-Mendel rats, female; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	2000 f/mg dry lung at 3 mo	Tumours: 0/57; fibrosis, 0	Aerosolized fibre concentration was very low. Asbestos: few tumours (5%) + fibrosis (some)	Smith *et al.* (1987)
Owens Corning building insulation	5 f/cm^3; L, > 10 μm; D, < 1 μm; 25 total f/cm^3; 9 mg/m^3; D, 3 μm mean; GMD, 3 μm; L, 114 μm mean; GML, 83 μm	Crocidolite, 3000 f/cm^3, total f/cm^3, 90 f/cm^3; L, > 10 μm	58 Osborne-Mendel rats, female; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	600 f/mg dry lung at 3 mo	Tumours: 0/58; fibrosis, 0	Aerosolized fibre concentration was very low and most fibres were very coarse and thick. Asbestos: few tumours (5%) + fibrosis (some)	Smith *et al.* (1987)
Owens Corning building insulation	5 and 15 mg/m^3, no fibre dimensions; no aerosol concentrations specified	None	500 Fischer 344 rats; lifetime	Whole-body, 7 h/d, 5 d/wk, for 86 wks	ND	Tumour, 0/500; fibrosis, 0	No data on concentrations of fibres in aerosol or in the lung. No positive asbestos control	Moorman *et al.* (1988)
Hamster								
Insulsafe II building insulation	30 f/cm^3; L, > 10 μm; D, < 1 μm; 100 total f/cm^3; 10 mg/m^3; D, 1.4 μm mean; GMD, 1.2 μm; L, 37 μm mean; GML, 24 μm	Crocidolite, 3000 total f/cm^3, 90 f/cm^3 (L, > 10 μm)	60 Syrian golden hamsters, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	1×10^4 f/mg dry lung at 3 mo	Tumours: 0/60; fibrosis, 0	Aerosolized fibres were short. Asbestos-exposed animals had no tumours (some did have fibrosis)	Smith *et al.* (1987)
Glass fibre	~168 WHO f/cm^3	Amosite ~1178 WHO f/cm^3	Hamsters; 24 months	Whole-body, 5 h/d, 5 d/wk, for 3 mo	ND	0/9	Short exposure period, small number (9) of animals at risk (amosite: 0/5 tumours)	Lee *et al.* (1981)

Table 60 (contd)

Test substance	Aerosol fibres (numbers and dimensions)	Positive control	Test system (no. at risk); observation time	Exposure	Lung dose	No. of thoracic tumours/no. of animals	Comments (positive control tumour incidence)	Reference
Manville 901 building insulation	339 WHO f/cm^3 (134 f L > 20 μm), 30 mg/m^3; GMD, 0.84 μm; GML, 12.4 μm	Amosite (mid), 165 WHO f/cm^3 (38 f L > 20 μm); amosite (high), 263 WHO f/cm^3 (69 f L > 20 μm)	125 (81) Syrian golden hamsters, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 18 mo	f L > 5 μm/lung: 32×10^6 at 12 mo; 77×10^6 at 18 mo f L > 20 μm/lung: 1×10^6 at 12 mo; 5×10^6 at 18 mo	Lung tumours or meso-theliomas, 0/81; fibrosis, 0	Amosite (mid): 22/85 mesotheliomas, no lung tumours, fibrosis Amosite (high) 17/87 mesotheliomas, no lung tumours, fibrosis	Hesterberg *et al.* (1999); McConnell *et al.* (1999)
Manville building insulation	25 f/cm^3; L, > 10 μm; D, < 1 μm; 100 total f/cm^3; 12 mg/m^3; D, 1.4 μm mean; GMD, 1.1 μm; L, 31 μm mean; GML, 20 μm	Crocidolite, 3000 f/cm^3, total f/cm^3, 90 f/cm^3; L, > 10 μm	66 Syrian golden hamsters, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	1000 f/mg dry lung at 3 mo	Tumours: 0/66; fibrosis, 0	Aerosolized fibre concentration was very low. Asbestos-exposed animals had no tumours (some did have fibrosis)	Smith *et al.* (1987)
Owens Corning building insulation	5 f/cm^3; L, > 10 μm; D, < 1 μm; 25 total f/cm^3; 9 mg/m^3; D, 3 μm mean; GMD, 3 μm; L, 114 μm mean; GML, 83 μm	Crocidolite, 3000 f/cm^3, total f/cm^3, 90 f/cm^3; L, > 10 μm	61 Syrian golden hamsters, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	500 f/mg dry lung at 3 mo	Tumours: 0/61; fibrosis, 0	Aerosolized fibre concentration was very low and most fibres were very coarse and thick. Asbestos-exposed animals had no tumours (some did have fibrosis)	Smith *et al.* (1987)
Special-purpose glass fibres								
Rat								
JM 100	10 mg/m^3 respirable dust; D, 0.3 μm; L, 71% < 10 μm	Chrysotile, 10 mg/m^3	100 Fischer rats, 50 male, 50 female; lifetime	Whole-body, 7 h/d, 5 d/wk, for 12 mo	ND	0/55; fibrosis, 0	JM 100 fibres were short (chrysotile: 11/56 tumours; fibrosis)	McConnell *et al.* (1984)
JM 100	1436 WHO f/cm^3 (108 f L > 20 μm), 10 mg/m^3 respirable dust; D, 97% < 1 μm; L, 93% 5–20 μm	Chrysotile, 10 mg/m^3, 3800 WHO f/cm^3	56 (48) SPF Fischer rats, male and female; 24 mo; lifetime	Whole-body, 7 h/d, 5 d/wk, for 12 mo	mg f/lung: 4.5 at 12 mo 2.1 at 21 mo	1/48 (adeno-carcinoma); fibrosis, 0	JM 100 fibres were short (chrysotile: 12/48 tumours; fibrosis)	Wagner *et al.* (1984)

Table 60 (contd)

Test substance	Aerosol fibres (numbers and dimensions)	Positive control	Test system (no. at risk); observation time	Exposure	Lung dose	No. of thoracic tumours/no. of animals	Comments (positive control tumour incidence)	Reference
JM 100	332 WHO f/cm^3, 5 mg/m^3 respirable dust; D, 95% < 1 μm; L, 60% > 10 μm, 25% > 20 μm	Chrysotile, 5 mg/m^3, 6000 WHO f/cm^3	48 Wistar rats, male and female; 28 mo	Whole-body, 5 h/d, 5 d/wk, for 24 mo	ND	0/48	JM 100 fibres were short (chrysotile: 9/47 tumours; fibrosis)	Le Bouffant *et al*. (1984; 1987)
JM 104/475	252 WHO f/cm^3, 3 mg/m^3; D, 0.42 μm median; L, 4.8 μm median	Crocidolite, 162 WHO f/cm^3; chrysotile, 131 WHO f/cm^3	108 Wistar rats, female; lifetime	Nose-only, for 12 mo	WHO f × 10^6: 70 at 12 mo 25 at 24 mo	1/107	JM 104/475 fibres were short (crocidolite: 1/50 tumours; chrysotile: 0/50 tumours)	Muhle *et al*. (1987)
Manville Code 100	530 f/cm^3; L, > 10 μm; D, ≤ 1 μm; 3000 total f/cm^3; 3 mg/m^3; D, 0.4 μm mean; GMD, 0.4 μm; L, 7.5 μm mean; GML, 4.7 μm	Crocidolite, 3000 f/cm^3, total f/cm^3, 90 f/cm^3; L, > 10 μm	57 Osborne-Mendel rats, female; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	2 × 10^6 f/mg dry lung at 2 mo	Tumours: 0/57; fibrosis, 0	Low survival rates: < 50% of rats survived to 24 mo (including controls). Asbestos: few tumours (3/57; 5%) + fibrosis	Smith *et al*. (1987)
JM 475	5 and 15 mg/m^3; no fibre dimensions; no aerosol concentrations specified	None	500 Fischer 344 rats; 18 mo; lifetime	Whole-body, 7 h/d, 5 d/wk, for 86 wks	ND	Tumours, 0; fibrosis, 0	No data on fibre concentrations in aerosol or in the lung. No positive asbestos control	Moorman *et al*. (1988)
JM 475	1066 WHO f/cm^3 (38 f > 20 μm/cm^3); 0.2 μm < D (more than 60% of fibres) < 0.4 μm	Amosite, 981 WHO f/cm^3 (89 f > 20 μm/cm^3)	24 mo, 83 (38) Wistar rats	Whole body, 7 h/d, 5 d/wk, for 12 mo	2241 × 10^6 WHO f/lung; 11 × 10^6 f > 20 μm/lung	4/38 (adenomas); fibrosis: negligible	Authors concluded tumours/fibrosis similar to that of controls (5% tumours in air control).	Davis *et al*. (1996a); Cullen *et al*. (2000)
104 E	975 WHO f/cm^3 (72 f > 20 μm/cm^3); 0.2 μm < D (more than 60% of fibres) < 0.4 μm	Amosite, 981 WHO f/cm^3 (89 f > 20 μm/cm^3)	24 mo, 83 (43) Wistar rats	Whole body, 7 h/d, 5 d/wk, for 12 mo	2356 × 10^6 WHO f/lung; 83 × 10^6 f > 20 μm/lung	12/43 (7 carcinomas, 3 adenomas and 2 mesothe-liomas); fibrosis: +	Authors concluded that amosite and 104 E were fibrogenic and tumorigenic.	Davis *et al*. (1996a); Cullen *et al*. (2000)

Table 60 (contd)

Test substance	Aerosol fibres (numbers and dimensions)	Positive control	Test system (no. at risk); observation time	Exposure	Lung dose	No. of thoracic tumours/no. of animals	Comments (positive control tumour incidence)	Reference
Hamster								
Manville Code 100	530 f/cm^3; L, > 10 μm; D, ≤ 1 μm; 3000 total f/cm^3; 3 mg/m^3; D, 0.4 μm mean; GMD, 0.4 μm; L, 7.5 μm mean; GML, 4.7 μm	Crocidolite, 3000 f/cm^3, total f/cm^3, 90 f/cm^3; L, > 10 μm	69 Syrian golden hamsters, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 24 mo	1 × 10^6 f/mg dry lung at 2 mo	Tumours: 0/69; fibrosis, 0	Low survival rates: < 25% of hamsters survived to 24 mo (including controls). Asbestos-exposed animals had no tumours (some did have fibrosis).	Smith *et al.* (1987)
JM 475	310 WHO f/cm^3 (109 f L > 20 μm), 37 mg/m^3; GMD, 0.70 μm; GML, 11.8 μm	Amosite (mid), 165 WHO f/cm^3 (38 f L > 20 μm), 3.5 mg/m^3; amosite (high) 263 WHO f/cm^3 (69 f L > 20 μm), 7 mg/m^3	125 (83) Syrian golden hamsters, male; lifetime	Nose-only, 6 h/d, 5 d/wk, for 18 mo	f L > 5 μm/lung: 49 × 10^6 at 12 mo 234 × 10^6 at 18 mo f L > 20 μm/lung: 6 × 10^6 at 12 mo 30 × 10^6 at 18 mo	Lung tumours: 0/83; mesothelioma, 1/83; fibrosis, 0	Amosite (mid): 22/85 mesotheliomas, no lung tumours, fibrosis Amosite (high) 17/87 mesotheliomas, no lung tumours, fibrosis	Hesterberg *et al.* (1999); McConnell *et al.* (1999)

f, fibre; L, length; D, diameter; total f, any particle having L:D ≥ 3; f > 20, fibres with length > 20 μm; f/cm^3, no. of fibres per cm^3 of air. Some concentrations were expressed as fibres > 5 μm length, others as total fibres; authors did not always specify. GMD, geometric mean diameter; GML, geometric mean length; lifetime: until survival rate of air controls is ≤ 20%; typically ~30 months. Thoracic tumours: lung tumours, including adenomas and carcinomas; WHO, respirable fibres as defined by World Health Organization, L > 5 μm, D < 3 μm, L:D, ≥ 3; h, hour; d, day; wk, week; mo, month; ND, not determined

killed either immediately after treatment or after various periods of observation (4, 7, 12 and 16 months after exposure for rats exposed for 12 months and 4 months after exposure for rats exposed for 24 months). The incidences of pulmonary carcinoma in the various treatment groups were 1/45 (French glass fibre), 9/47 (chrysotile) and 0/47 (control rats) (Le Bouffant *et al*., 1984). [The Working Group noted that, because data on survival were not reported, the exact incidence of tumours could not be ascertained.]

Groups of 56 SPF Fischer rats (equal numbers of males and females) [age unspecified] were exposed by inhalation to dust at concentrations of approximately 10 mg/m^3 glass fibre or chrysotile for 7 h per day on five days per week for 12 months (cumulative exposure, 17 500 mg × h/m^3 for each group). The samples of fibrous dust used (and the size distributions of airborne fibres > 5 μm in length) were: glass fibre with resin coating [chemical composition not given] (72% fibres < 20 μm in length; 52% < 1 μm in diameter; 240 WHO fibres/cm^3), glass fibre without resin coating [source unspecified] (58% < 20 μm in length; 47% < 1 μm in diameter; 323 WHO fibres/cm^3) and UICC Canadian chrysotile (39% > 10 μm in length; 29% > 0.5 μm in diameter). To study dust retention, groups of six rats per exposure group were killed at the end of the exposure period or one year after the end of exposure. The remainder were kept until natural death [survival times not reported]. During the period 500–1000 days after the start of exposure, the incidences of pulmonary adenomas and carcinomas were: 1/48 (adenocarcinoma; glass fibre with resin), 1/47 (adenoma; glass fibre without resin), 12/48 (1 adenoma and 11 adenocarcinomas; chrysotile) and 0/48 (untreated controls) (Wagner *et al*., 1984). [The Working Group noted that because data on survival were inadequate, the exact incidence of tumours could not be ascertained.]

Groups of 52–61 female Osborne-Mendel rats, 100 days old, were exposed by inhalation (nose-only) to dusts from various types of glass wool and special purpose fibres, UICC crocidolite asbestos or clean air (negative controls) for 6 h per day on five days per week for two years and then observed for life. A group of 125 untreated rats served as additional negative controls. The three glass wools tested were:

— CT loose 'blowing wool' building insulation Insulsafe II [chemical composition similar to MMVF11] (mean dimensions of aerosol fibres, 37 μm × 1.4 μm; 4.4 mg/m^3; 100 total fibres/cm^3; 30 fibres/cm^3 with length > 10 μm and diameter < 1 μm);
— Manville building insulation with binder [chemical composition similar to MMVF10] (mean dimensions of aerosol fibres, 31 μm × 1.4 μm; 9.9 mg/m^3; 100 total fibres/cm^3; 25 fibres/cm^3 with length > 10 μm and diameter < 1 μm); and
— Owens Corning binder-coated appliance insulation [chemical composition not given] (mean dimensions of aerosol fibres, 3.0 μm × 114 μm; mass concentration, 7.0 mg/cm^3; 19% respirable; 25 total fibres/cm^3; 5 fibres/cm^3 > 10 μm in length and ≤ 1.0 μm in diameter).

The mass of human respirable fibre was estimated for each aerosol from data obtained from nylon cyclone air samplers. [The Working Group noted that no infor-

mation was available on respirability in the rat and that for the Manville building insulation and the Owens Corning appliance insulation fibres, the retained lung burden was relatively low.] No pulmonary tumours were observed in any of the groups exposed to any of the types of glass fibre or in the controls. There was no effect of glass fibre on survival and little pulmonary cellular change. The tumour incidences in rats exposed to UICC crocidolite asbestos were: 3/57 rats (one mesothelioma and two carcinomas) (Smith *et al*., 1987). [The Working Group noted that the counting criteria for the retained fibres were not stated.]

A total of 500 Fischer 344 rats, five weeks of age, were exposed in whole-body chambers to Owens Corning insulation glass wool [chemical composition not given] or air filtration fibre glass fibres for 7 h per day on five days per week for 86 weeks. The target exposure concentration was 15 mg/m^3 for both treatments. [The Working Group noted that the physical dimensions (length and diameter) of the fibres and the numbers of fibres in the exposure aerosol and the lungs were not characterized and the lung burden was not given.] No lung fibrosis, lung cancer or mesotheliomas were observed in the rats exposed to fibres. The incidence of mononuclear-cell leukaemia was statistically elevated in the groups exposed to fibres when compared with the air control group. However, this tumour type is observed at relatively high incidences (22% in the current study) in ageing Fischer 344 rats (Moorman *et al*., 1988).

Groups of 140 male Fischer 344/N rats, eight weeks of age, were exposed by nose-only inhalation to glass wool building insulation fibres (Manville 901 (MMVF10) or CertainTeed® Insulsafe II (MMVF11)) for 6 h per day on five days per week for up to two years and observed for life (until 20% survival). The target aerosol concentrations were 3, 16 or 30 mg/m^3. At a concentration of 30 mg/m^3, MMVF10 contained an average of 232 WHO fibres/cm^3 and MMVF11, 246 WHO fibres/cm^3 (including 73 and 90 fibres/cm^3 longer than 20 μm, respectively). The average fibre dimensions were 1.4 μm × 16.8 μm (MMVF10) and 0.9 μm × 18.3 μm (MMVF11). The negative control rats were exposed under similar conditions to filtered air. Another group of rats was exposed to size-selected NIEHS medium-chrysotile asbestos at 10 mg/m^3 (10.6 × 10^3 WHO fibres/cm^3, no detectable fibre > 20 μm). Lung fibre burdens and lung pathology and histopathology were evaluated after 3, 6, 12, 18 and 24 months of exposure and after various combinations of exposure and recovery periods (3 + 21, 6 + 18, 12 + 12, 18 + 6, 24 + 6 months; no exposure to fibres occurred during the recovery period). Lung fibre burdens were dose- and time-dependent, increasing with both level of exposure and duration. After 24 months of exposure to ~250 WHO fibres/cm^3, the lung fibre burdens, expressed as fibres > 20 μm/lung × 10^6 were 5 and 6 for MMVF10 and MMVF11, respectively. During exposure of rats to either of the two glass wools, dose-related and time-dependent lung inflammation was observed; however, after a post-exposure recovery period of several months, the lungs showed no evidence of inflammation. In the rats exposed to glass wool, no lung fibrosis or mesothelial tumours were seen and the incidences of lung tumours were not significantly elevated over background levels for this species (range, 0.8–7.5% in 112–120 rats at risk; 3.3% in concurrent air

controls). The incidences of tumours in the rats exposed to chrysotile were 13/69 (18.9%) lung tumours and 1/69 (1.4%) pleural mesotheliomas (Hesterberg *et al*., 1993; Hesterberg & Hart, 2001).

(*b*) *Hamster*

Groups of 60–70 male Syrian golden hamsters, 100 days old, were examined after exposure by nose-only inhalation to dusts of various types of glass wool and special-purpose fibres, UICC crocidolite asbestos or clean air (negative controls) for 6 h per day on five days per week for two years, and were then observed for life. A group of 125 untreated hamsters served as additional negative controls. The three glass wools tested were:

— CT loose 'blowing wool' building insulation Insulsafe II [chemical composition similar to MMVF11] (37 μm × 1.4 μm; 4.4 mg/m^3; 100 total fibres/cm^3);
— Manville building insulation with binder [chemical composition similar to MMVF10] (31 μm × 1.4 μm; 9.9 mg/m^3; 100 total fibres/cm^3); and
— Owens Corning binder-coated appliance insulation [chemical composition not given] (3.0 μm diameter; mass concentration, 7.0 mg/cm^3; 19% respirable, 25 fibres/cm^3 with 5 fibres/cm^3 > 10 μm in length and ≤ 1.0 μm in diameter).

A second group of 38 animals was also exposed to Owens Corning binder-coated appliance insulation because of a high death rate in the first group that was unrelated to exposure to fibres. No pulmonary tumours were observed in the group exposed to glass fibre or the room-control group. The incidences of pulmonary tumours in the other groups were: 1/58 (chamber controls; carcinoma) and 0/58 (crocidolite). None of the types of glass fibre affected survival or caused pulmonary lesion (Smith *et al*., 1987). [The Working Group noted that the counting criteria for the retained fibres were not stated and that the lifetime of the hamsters was surprisingly long.]

Groups of 30–35 hamsters [sex, strain and age unspecified] were exposed by whole-body inhalation to glass fibre (700 fibres/cm^3 > 5 μm in length; 24% with length < 3 μm) or amosite asbestos (3100 fibres/cm^3 > 5 μm in length; 38% with length < 3 μm) [no information on diameters was given] for 5 h per day on five days per week for three months and were then observed for 21 months. [Aerosol concentrations estimated as WHO fibre/cm^3 (length > 5 μm, diameter < 3 μm, length/diameter ≥ 3) were approximately 168 for glass fibre and 1178 for amosite.] One group of 30 unexposed animals served as controls. Groups of 1–8 animals per exposure group were killed at 50 days and 3, 6, 12 and 18 months, and the remainder at 24 months. No pulmonary tumour was observed in any group (0/9 in the exposed group killed at 24 months) (Lee *et al*., 1981). [The Working Group noted the short exposure period and the small number of animals evaluated.]

A total of 125 male Syrian golden hamsters, 13–15 weeks of age (~145 g bw), were exposed by nose-only inhalation to building insulation glass fibre (Manville 901, coded MMVF10a) at a concentration of 30 mg/m^3 (339 WHO fibres/cm^3, including 134 fibres > 20 μm/cm^3) for 6 h per day on five days per week for up to 78 weeks (18 months) and

observed for life (i.e. until 20% survival; ~88 weeks). The MMVF10a sample was a thinner version of the original MMVF10 used in the chronic inhalation studies in rats summarized above. In order to compare results obtained using MMVF10a with those of a known human carcinogen, three additional groups of 125 hamsters were concurrently exposed to amosite asbestos at the following aerosol concentrations: 36 (10 fibres > 20 μm), 165 (38 fibres > 20 μm) and 263 (69 fibres > 20 μm) WHO fibres/cm^3. The geometric mean dimensions of the aerosol fibres were 0.84 μm × 12.4 μm for MMVF10a and 0.55 μm × 8.4 μm for amosite. Another group of 125 hamsters was exposed under similar conditions to filtered air only (negative controls). After 13, 26, 52 and 78 weeks of exposure and after various combinations of exposure and recovery periods, 9–10 hamsters were randomly selected from each exposure group and killed; five of the hamsters were evaluated for lung burden and for histopathology and the remaining four or five were evaluated for lung and pleural cell proliferation. Lung depositions after 6 h of exposure were fairly similar in animals exposed to MMVF10a and high dose amosite: 8.4×10^5 WHO fibres/lung (1.6 fibres > 20 μm) for MMVF10a and 20.8×10^5 WHO fibres/lung (2 fibres > 20 μm) for high-dose amosite. However, after 78 weeks of exposure, lung burdens for the two fibres were no longer similar: numbers of fibres > 20 μm/lung were 4.6×10^6 for MMVF10a but 144×10^6 for high-dose amosite; numbers of WHO fibres per lung were ninefold greater for amosite than for MMVF10a; and the number of fibres > 20 μm/lung were fivefold greater for amosite. After 78 weeks of exposure followed by six weeks of recovery, the lung burdens of MMVF10a were reduced by 66% (WHO fibres/lung) and 95% (fibres > 20 μm/lung); in contrast, the lung burdens of amosite were reduced by only 21% and 38%, respectively. Exposure to MMVF10a induced lung inflammation, which was no longer evident after a recovery period of six weeks; no pulmonary fibrosis or tumours were observed in these hamsters (81 hamsters at risk). In contrast, all three doses of amosite induced widespread lung fibrosis and 3%, 22% and 17% pleural mesotheliomas in the 36, 165 and 263 WHO fibres/cm^3 dose groups, respectively (87 hamsters at risk) (Hesterberg *et al*., 1997, 1999; McConnell *et al*., 1999).

3.2.2 *Intraperitoneal injection*

(*a*) *Rat*

Groups of female Wistar rats, 8–12 weeks of age, received either single intraperitoneal injections of 2 or 10 mg, or four weekly injections of 25 mg, German glass wool [chemical composition not given] (59% fibres < 3 μm in length), different doses of UICC chrysotile A or 100 mg of one of seven suspensions of granular dust in 2 mL saline. The German glass wool was administered at doses of 24, 120 and 1200×10^6 fibres > 5 μm in length. The animals were kept until natural death. In the groups treated with glass fibre, lesions reported by the authors as mesotheliomas and spindle-cell sarcomas were observed at incidences of 1/34, 4/36 and 23/32 for the doses of 24, 120 and 1200×10^6 fibres > 5 μm in length, respectively, with corresponding

average survival times of 518, 514 and 301 days. Tumour incidences ranged from 6/37 (2 mg dose) to 25/31 (25 mg dose) in the groups treated with chrysotile, with average survival times of 468–407 days. No abdominal tumours were observed in the control animals treated with saline (Pott *et al*., 1976).

Groups of female Wistar rats, 8–10 weeks old, were given intraperitoneal injections containing a total dose of either 70 mg (two weekly injections) or 180 mg (six weekly injections) of MMVF11 (manufactured by CertainTeed®). The number of fibres was 0.4×10^9 or 1.0×10^9 WHO fibres/rat for the 70 mg and 180 mg doses, respectively, and the approximate median fibre dimensions were 14.6 μm × 0.77 μm. Rats were kept for life and then examined for tumours. Forty rats served as concurrent negative controls and received intraperitoneal injections of saline. The incidences of peritoneal mesotheliomas for the 70 mg and 180 mg dose groups were 12/40 (30%) and 16/23 (70%), respectively. Negative control rats did not develop any mesotheliomas (Pott, 1995; Roller *et al*., 1996; Roller & Pott, 1998).

Five groups of 40–53 male or female Wistar rats, 8–10 weeks old, were given 5–40 weekly intraperitoneal injections of 25 mg B-01-09 glass wool to a total dose of 125–1000 mg glass wool. The doses expressed as fibres > 5 μm/rat for each of the five groups were 2.5, 5, 10 (females), 10 (males) and 20×10^9, respectively, the highest dose being 20 times more than the highest dose recommended by the European Union guidelines (see section 1.5 for details). Approximate median fibre dimensions were 9 μm × 0.7 μm. Rats were kept for life (~30 months) and then examined for tumours. Two hundred and eight male and female rats were kept as concurrent negative controls and treated with 1–20 weekly intraperitoneal injections of saline. The incidences of peritoneal mesotheliomas for the five groups treated with glass wool were 3/40 (8%), 4/40 (11%), 3/40 (8%), 10/40 (21%) and 33/40 (66%), respectively. The incidence of abdominal tumours in negative control rats was 1/208 (Pott, 1995; Roller *et al*., 1996).

Four groups of 40 female Wistar rats, 8–10 weeks old, were given a series of weekly intraperitoneal injections containing either a thin or thicker version of an experimental glass wool (B-09-0.6 or B-09-2.0). The median dimensions of the two fibres were 3.3 μm × 0.49 μm and 10.5 μm × 1.2 μm, respectively. The total dose per rat (in saline) was: 100 or 300 mg (B-09-0.6) and 150 or 450 mg (B-09-2.0) (injections ranging from 2 × 50 mg to 9 × 50 mg/animal). The numbers of fibres for the four doses were: 2 and 6×10^9 WHO fibres per rat for B-09-0.6 and 1 and 3.2×10^9 WHO fibres per rat for B-09-2.0. Forty rats were kept as concurrent negative controls and were given three weekly intraperitoneal injections of 2 mL physiological saline solution. Rats were kept for life (30 months) and then examined for tumours. The incidences of peritoneal mesotheliomas for the four dose groups were 3% (100 mg B-09-0.6), 10% (300 mg B-09-0.6), 23% (150 mg B-09-2.0) and 53% (450 mg B-09-2.0). No tumours were observed in the negative control rats. Exposure to longer and thicker fibres resulted in more tumours (Roller *et al*., 1996, 1997).

Groups of 22–24 male Wistar rats [age unspecified] received an intraperitoneal injection of 144 mg of a glass fibre standard building insulation wool (MMVF10) or

6.1 mg of amosite asbestos and were observed for life to monitor the development of peritoneal mesotheliomas. The doses of the two fibres were 973×10^6 WHO fibres/rat (MMVF10) and 410×10^6 WHO fibres/rat (amosite). The numbers of fibres > 20 μm for each of the two fibres were 555×10^6 (including 119 with a diameter < 0.95 μm) and 71×10^6 (including 63 with a diameter < 0.95 μm), respectively. Tumours were diagnosed by the macroscopic presence of peritoneal mesotheliomas; when the diagnosis was in doubt, microscopy was performed. The incidence of tumours for MMVF10 was 13/22 (59%) and for amosite 21/24 (88%). [Estimated survival fractions for deaths from mesothelioma were reported.] In their consideration of these fibres and others tested, the authors suggested a link between the incidence of intraperitoneal tumours and two other factors:

— the number of fibres > 20 μm injected; and
— the biopersistence of fibres > 5 μm in rat lungs following intratracheal instillation (biopersistence is described more fully in section 4 of this monograph) (Miller *et al*., 1999).

(*b*) *Hamster*

Groups of 40 female Syrian golden hamsters, 8–12 weeks old, received single intraperitoneal injections of 2 or 10 mg glass wool (59% of fibres shorter than 3 μm) [chemical composition not given] or UICC chrysotile A in 1 mL saline. Animals were observed for life. No tumour of the abdominal cavity was found (Pott *et al*., 1976).

3.3 Special-purpose glass fibres

3.3.1 *Inhalation exposure*

(*a*) *Rat*

Groups of 24 male and 24 female Wistar IOPS AF/Han rats, 8–9 weeks of age, were exposed by whole-body inhalation to dust of US JM 100 glass fibre [chemical composition not given] at concentrations of 5 mg/m^3 (respirable particles) (97% respirable fibres < 5 μm in length; 43% total fibres < 0.1 μm in diameter; 332 respirable fibres > 5 μm/cm^3) or a Canadian chrysotile fibre (6% respirable fibres > 5 μm in length; 5901 WHO fibre/cm^3) for 5 h per day on five days per week for 12 or 24 months. An unspecified number of rats were killed either immediately after treatment or after a period of observation (4, 7, 12 and 16 months after exposure for rats exposed for 12 months; 4 months after exposure for rats exposed for 24 months). The incidences of pulmonary carcinoma were 9/47 (chrysotile), 0/48 (JM 100 glass fibre) and 0/47 (control rats) (Le Bouffant *et al*., 1984). [The Working Group noted that, because of the lack of data on survival, the exact incidence of tumours could not be ascertained.]

Two studies of similar design, A and B, using animals from the same source were conducted concurrently in different laboratories (study B was part of the study by Wagner *et al.* (1984), reviewed in detail below). Groups of 50 male and 50 female SPF

Fischer 344 rats, 7–8 weeks of age, were exposed by whole-body inhalation to approximately 10 mg/m^3 respirable dust [size unspecified] of JM 100 [chemical composition not given] or UICC Canadian chrysotile for 7 h per day on five days per week for 12 months and were observed for life. Fifty rats of each sex served as chamber controls. Groups of 3–5 rats per group were killed at 3, 12 and 24 months. No pulmonary tumours were observed after 3 or 12 months of exposure. The incidences of pulmonary tumours in the chrysotile-exposed male rats killed after 24 months of exposure were 2/4 (one adenoma, one adenocarcinoma; study A) and 0/4 (study B). The incidences of pulmonary tumours (adenomas and adenocarcinomas combined) in rats from study A observed for life were: chrysotile, 9/29 males and 2/27 females; JM 100 glass fibre, 0/28 males and 0/27 females; control, 3/27 males and 0/26 females. The incidences in study B were: chrysotile, 7/24 males and 5/24 females; JM 100 glass fibre, 1/24 males and 0/24 females; control, 0/24 males and 0/24 females. Thus, chrysotile asbestos was positive for carcinogenicity, while JM 100 glass was negative (McConnell *et al*., 1984). [The Working Group noted that the dimensions of the fibres used in study A were not reported.]

Groups of 56 SPF Fischer rats (equal numbers of males and females) [age unspecified] were exposed by inhalation to dust at concentrations of approximately 10 mg/m^3 glass fibre [chemical composition not given] or chrysotile for 7 h per day on five days per week for 12 months (cumulative exposure, 17 500 mg × h/m^3 for each group). The fibrous dust samples used (and the size distributions of those airborne fibres > 5 μm in length) were: JM 100 glass fibre (93% < 20 μm in length; 97% < 1 μm in diameter; 1436 WHO fibres/cm^3) and UICC Canadian chrysotile (39% > 10 μm in length; 29% > 0.5 μm in diameter). To study dust retention, groups of six rats per exposure group were killed at the end of the exposure period and one year after the end of exposure. The remainder were kept until natural death [survival times not reported]. During the period 500–1000 days after the start of exposure, the incidence of pulmonary adenomas and carcinomas was: 1/48 (adenocarcinoma; JM 100), 12/48 (one adenoma and 11 adenocarcinomas; chrysotile) and 0/48 (untreated controls) (Wagner *et al*., 1984). [The Working Group noted that, because of inadequate data on survival, the exact tumour incidence could not be ascertained.]

In the study by Smith *et al.* (1987), described in detail in section 3.2.1, exposure of female Osborne-Mendel rats by nose-only inhalation to Manville code 100 fibres without binder (mean dimensions of aerosol fibres, 7.5 μm × 0.4 μm; highest concentration, 2.4 mg/m^3; 3000 total fibres/cm^3; 530 fibres/cm^3, length > 10 μm, and diameter < 1 μm) for 6 h per day on five days per week for two years did not produce pulmonary tumours.

Female Wistar rats, 12 weeks old, were exposed by nose-only tubes to fibre aerosols for 5 h per day on four days per week for up to one year (total exposure time, 1000 h). One group of 108 rats was exposed to JM 104/475 glass microfibre (median fibre dimensions, 4.8 μm × 0.42 μm; 90% < 12.4 μm long) and two groups of 50 rats were exposed either to South African crocidolite (median dimensions, 1.5 μm × 0.27 μm) or

to Calidria chrysotile (from California, USA; median fibre dimensions, 6.0 μm × 0.67 μm). Aerosol concentrations (expressed as mg/m^3 and as WHO fibres/cm^3) for the three fibres were as follows: JM 104/475, 3.0 mg/m^3 (252 fibres/cm^3), crocidolite, 2.2 mg/m^3 (162 fibres/cm^3) and chrysotile, 6.0 mg/m^3 (131 fibres/cm^3). Two groups of negative controls were also kept. One group comprised 55 rats exposed to clean air and the other 50 untreated rats. The lung burdens were: JM 104/475, 0.4 mg fibre/lung; crocidolite, 0.6 mg fibre/lung and chrysotile, 0.3 mg fibre/lung after six months of exposure; 0.6, 0.7 and 0.3 after 12 months of exposure; and 0.2, 0.4 and 0.03 after 12 months of exposure and a 12-month post-exposure recovery period. The proportions for pulmonary tumours were 1/107 (glass fibre; carcinoma), 1/50 rats (crocidolite; adenocarcinoma), 0/50 (chrysotile) and 0/105 (negative controls). The authors suggested that the low incidence of tumours seen after exposure to crocidolite might have been because the lung burden of < 1 mg dust was relatively low, and the absence of tumours after exposure to Calidria chrysotile might be because these fibres were less persistent than those of UICC chrysotile (Muhle *et al*., 1987).

In a study by Moorman *et al*. (1988), described in detail in section 3.2.1, whole-body exposure of 500 Fischer 344 rats to JM 475 special-purpose fibres for 7 h per day on five days per week for 86 weeks, at target exposure concentrations of 5 and 15 mg/m^3, did not produce lung fibrosis, lung cancer or mesotheliomas. The incidence of mononuclear-cell leukaemia was statistically elevated in the groups exposed to fibres when compared to the air control group, but this tumour type is typically observed at relatively high incidences (22%) in ageing Fischer 344 rats. [The Working Group noted that the physical dimensions and the number of fibres in the exposure aerosol and the lungs were not characterized and that the lung burden was not stated.]

In a series of inhalation studies (Davis *et al*., 1996b; Searl *et al*., 1999; Cullen *et al*., 2000), 475 and 104E glass fibres were compared with amosite asbestos. Groups of male Wistar rats were exposed to aerosols containing approximately 1000 fibres longer than 5 μm/cm^3, more than 60% of which had diameters between 0.2 and 0.4 μm. Lung burdens and some clearance data for each fibre type (by length category) were presented. The changes in chemistry of fibres during their residence in the lungs were monitored by SEM-EDXA. The numbers of thoracic tumours observed in animals at risk were: 2/38 for controls, 12/43 for 104E, 18/42 for amosite and only 4/38 for 475 glass. Mesotheliomas occurred only in the groups treated with 104E and amosite. All the tumours observed in animals treated with 475 glass were adenomas. There was little correlation between final lung burdens and severity of effect and the authors suggested a role for fibre leaching that was seen only with the relatively inert 475 glass.

(*b*) *Hamster*

In the study by Smith *et al.* (1987), described in detail in section 3.2.1, exposure of male Syrian golden hamsters by nose-only inhalation to Manville code 100 fibres without binder (mean dimensions of aerosol fibres, 7.5 μm × 0.4 μm; highest concen-

tration, 2.4 mg/m^3; 3000 total fibres/cm^3; 530 fibres/cm^3 with length > 10 μm and diameter < 1 μm) for 6 h per day on five days per week for two years did not affect survival and caused no pulmonary lesions or tumours.

In a study described in section 3.2.1, 125 Syrian golden hamsters were exposed by nose-only inhalation to JM 475 glass fibre (coded MMVF33) at a concentration of 37 mg/m^3 (310 WHO fibres/cm^3, including 109 fibres > 20 μm/cm^3). The geometric mean dimensions of fibres in the aerosol were 0.7 μm × 11.8 μm. Time-points, evaluations and numbers of hamsters tested were similar to those described above. The lung deposition of MMVF33 (11.5×10^5 WHO fibres, including 2.2×10^5 fibres > 20 μm) after 6 h of exposure, and of MMVF10a and high-dose amosite was similar. However, after 78 weeks of exposure, the lung burden for MMVF33 was between those of MMVF10a and amosite asbestos, i.e. 5, 30 and 144×10^6 fibres > 20 μm/lung for MMVF10a, MMVF33 and high-dose amosite, respectively; or, expressed as WHO fibres × 10^6/lung, 77, 234 and 612, respectively. After 78 weeks of exposure plus six weeks of recovery, the lung burdens in animals treated with MMVF33 were reduced by 40–62% (compared with reductions of 66–95% in animals treated with MMVF10a and little or no significant clearance after treatment with the high dose of amosite). All hamsters exposed to MMVF33 had lung fibrosis after six months of exposure and one pleural mesothelioma was seen in 83 animals at risk (1.2%) (Hesterberg *et al*., 1997, 1999; McConnell *et al*., 1999).

(*c*) *Guinea-pig*

Groups of 31 male albino guinea-pigs [age unspecified] were exposed by whole-body inhalation to fibres > 5 μm in length, at concentrations of 700 fibres/cm^3 of ball-milled glass fibre (24.2% fibres with diameter < 3 μm) or 3100 fibres/cm^3 UICC amosite asbestos (38% with diameter < 3 μm), for 6 h per day on five days per week for three months and were then observed for 21 months. One group of 31 unexposed animals served as controls. Groups of 1–10 animals per exposure group were killed at 50 days and at 3 months of exposure, and at 6, 12 and 18 months post-exposure; the remainder were killed at 24 months post-exposure. No pulmonary tumour was observed in animals that were killed or died before the end of the study. Bronchoalveolar adenomas were observed in 2/7 animals treated with glass fibre, 0/5 animals treated with amosite and 0/5 controls killed at the end of the study (Lee *et al*., 1981). [The Working Group noted the short exposure period and the small number of animals evaluated.]

3.3.2 *Intraperitoneal injection*

Rat: Groups of female Wistar rats, 8-12 weeks old, received single intraperitoneal injections of 2, 10 or 50 mg (the latter in two doses) [JM 104] glass fibre (code104; approximate mean fibre dimensions, 10 μm × 0.2 μm) [source of production and chemical composition not given], 20 mg JM 112 glass fibre (code 112; approximate

mean dimensions, 30 μm × 1 μm) [source of production and chemical composition not given], 2 mg UICC crocidolite or 50 mg corundum. Average survival times were 673, 611 and 361 days for the groups treated with 2, 10 and 50 mg JM 104, respectively, and 610 and 682 days for the groups treated with JM 112 and crocidolite, respectively. Abdominal tumours were diagnosed macroscopically and some were also identified by microscopy. Dose-related increases in the incidences of abdominal tumours (mesotheliomas, sarcomas and, rarely, carcinomas) were observed in the groups treated with the finer JM 104 glass fibre: 20/73 (2 mg), 41/77 (10 mg) and 55/77 (50 mg). The incidences in the groups treated with JM 112 and with crocidolite were 14/37 and 15/39, respectively. Of the rats that received injections of granular corundum, 3/37 had tumours in the abdominal cavity; mean survival time was 746 days (Pott *et al*., 1976).

To test the effect of fibre durability on tumorigenicity, eight groups of 46–48 female Wistar rats (range of body weights, 158–166 g) were injected intraperitoneally with either a low-durability glass wool (Bayer B1 or B2 [chemical composition not given]) developed for filters, or with two durable glass fibres (Bayer B3 and JM 475 glass microfibre) of very similar chemical compositions, but different size distributions. Several sizes of fibres of the low durability glass wool were administered in one or two doses as follows: thick (~1.5 μm median diameter) fibres in short, medium and long fibre ranges, designated B1K, B1M and B1L (median lengths, 7.4, 10.7 and 17.8 μm, respectively), with a maximum dose of 0.24×10^9 fibres per rat; and thin (0.5 μm mean diameter) fibres, in two length ranges, B2S and B2L (median lengths, 4.2 and 6 μm, respectively), to a maximum dose of 10^9 fibres > 5 μm per rat. The two more-durable glass fibre compositions, B3 (two length ranges, B3K and B3L, 3.3 and 5.6 μm, respectively) and JM 475, were administered at maximum doses of 0.45 and 0.33×10^9 fibres > 5 μm per rat, respectively. Negative control rats were given five intraperitoneal injections of 2 mL physiological saline solution at weekly intervals: 2/50 (4%) developed abdominal tumours. Exposure to low-durability glass fibre (B1 or B2) at any fibre number, dose or dimension tested did not significantly elevate the number of abdominal tumours. The incidence of tumours in the rats exposed to B1 or B2 ranged from 0–11% (11% in the group exposed to the thickest and longest fibres). Both of the durable glass fibres induced elevated incidences of tumours: B3K and B3L, 64–66%; JM 475, 17%. When the effects of two doses of the same fibre were compared, some dose–response relationships became apparent, but when two length populations of the same fibre were compared, no consistent relationship between tumorigenicity and fibre number or fibre length was apparent (Pott *et al*., 1991).

In a study using fibres similar to those used by Pott *et al*. (1976), three groups of 44 female Wistar rats, four weeks of age, were given intraperitoneal injections of 2 or 10 mg [JM 104E] glass fibre (code 104; milled for 2 h [size not given]) [source of production and chemical composition not given] or 2 mg of [JM 475] glass fibre (code 100; median fibre dimensions, 2.4 μm × 0.33 μm) [source of production and chemical composition not given]. Abdominal tumours were observed in 14/44 rats that received 2 mg JM 104E, in 29/44 rats that received 10 mg JM 104E and in 2/44 rats that received

2 mg JM 475. The first tumour was observed 350 days (50 weeks), 252 days (36 weeks) or 664 days (95 weeks) after the start of treatment with 2 mg JM 104E, 10 mg JM 104E and 2 mg JM 475, respectively. In three positive-control groups that received intraperitoneal injections of 0.4, 2 or 10 mg UICC chrysotile B, tumours developed in 9/44, 26/44 and 35/44 rats, respectively; the first tumour-bearing rat was found at 522 days (75 weeks), 300 days (43 weeks) and 255 days (36 weeks) after start of treatment in the groups treated with 0.4, 2 and 10 mg UICC chrysotile B, respectively. A negative-control group treated with 2 mg granular corundum dust had a tumour incidence of 1/45; the first tumour was found 297 days (42 weeks) after injection. The tumours observed in both the test and control groups were mesotheliomas or sarcomas. The groups treated with 0.4 mg chrysotile B or with JM 475 contracted an infection during month 21 which might have reduced the tumour incidence (Pott *et al*., 1984a).

Groups of female Sprague Dawley rats, 8 weeks of age, received single intraperitoneal injections of 2 mg or 10 mg [JM 475] glass fibre (JM 100; median fibre dimensions, 2.4 μm × 0.33 μm) [chemical composition not given] in 2 mL saline. The median survival times were 90 weeks and 79 weeks for the groups treated with 2 mg and 20 mg, respectively. Sarcomas, mesotheliomas and (rarely) carcinomas were seen in 21/54 animals treated with the 2 mg dose and 24/53 animals treated with the 10 mg dose; the first tumour was seen after 53 weeks in each group. Three tumours were found in both groups of 54 rats that received two injections each of either 20 mg Mount St Helen's volcanic ash or 20 mL saline alone (median survival time, 93 and 94 weeks, respectively; first tumour after 79 weeks of administration of volcanic ash and 94 weeks of administration of saline) (Pott *et al*., 1987).

Groups of 32 female Wistar rats, five weeks of age, received single intraperitoneal injections of 0.5 mg or 2.0 mg [JM 475] glass fibre (code 104; median fibre dimensions, 3.2 μm × 0.18 μm), 2.0 mg of [JM 475] glass fibre treated with 1.4 M hydrochloric acid for 24 h; or 0.5 mg or 2.0 mg South African crocidolite (median fibre dimensions, 2.1 μm × 0.20 μm) in 1 mL saline or saline alone. A group of 32 animals that received three intraperitoneal injections of titanium dioxide (total dose, 10 mg) served as another control. The animals were observed for life. Median survival times were 116, 110, 107, 109, 71, 130 and 120 weeks for rats that received 0.5 mg and 2.0 mg glass fibre, acid-treated glass fibre, 0.5 and 2.0 mg crocidolite, titanium dioxide and saline only, respectively. After exclusion of tumours of the uterus, the observed incidences of sarcomas, mesotheliomas and (rarely) carcinomas of the abdominal cavity were 5/30 (first tumour after 88 weeks) in animals treated with 0.5 mg glass fibre, 8/31 (first tumour after 84 weeks) with 2.0 mg glass fibre and 16/32 (first tumour after 56 weeks) with acid-treated glass fibre. The incidences of tumours in the groups treated with crocidolite were 18/32 (low-dose crocidolite; first tumour after 79 weeks) and 28/32 (high-dose crocidolite; first tumour after 52 weeks); the incidence of tumours in the saline-treated control group was 2/32 (first tumour after 113 weeks). None of the above-mentioned tumours was found in the group treated with titanium dioxide (Muhle *et al*., 1987; Pott *et al*., 1987).

Groups of female Sprague-Dawley rats [initial numbers not specified], eight weeks of age, received one injection of 5 mg [JM 104E] glass fibre (code 104; cut and ground for 1 h in an agate mill; median fibre dimensions, 4.8 μm × 0.29 μm). A negative-control group received 5 mg granular titanium dioxide. In the group treated with JM 104E glass fibre, 44/54 rats developed abdominal tumours; the median survival time was 64 weeks, and the average survival time of the tumour-bearing animals was 67 weeks. In the group treated with titanium dioxide, 2/52 rats had abdominal tumours; the median survival time was 99 weeks, and the average survival time of the tumour-bearing animals was 97 weeks (Pott *et al*., 1987).

Groups of Wistar rats [initial number and sex unspecified], four weeks of age, received one injection of 5 mg [JM 104E] glass microfibre (code 104, cut and ground for 1 h in an agate mill: median fibre dimensions, 4.8 μm × 0.29 μm). A negative control group received 5 mg granular titanium dioxide. Treatment with the JM 104E glass fibre induced abdominal tumours in 20/45 rats; the median survival time of the group was 34 weeks, and average survival time of the tumour-bearing rats was 49 weeks. None of the 47 rats treated with titanium dioxide developed abdominal tumours; median survival time of the group was 102 weeks (Pott *et al*., 1987).

A group of 25 female Osborne-Mendel rats, 100 days of age, received a single intraperitoneal injection of 25 mg Manville code 100 fibres (geometric mean fibre dimensions, 4.7 μm × 0.4 μm; 19% of fibres > 10 μm in length and 0.2–0.6 μm in diameter) in 0.5 mL saline. A group of 25 rats was injected with saline only and another group of 125 rats was untreated. All animals were observed for life. The median average life span was significantly shorter in rats treated with fibres (593 days) than in animals treated with saline (744 days) or untreated controls (724 days). Mesotheliomas were found in 8/25 of the rats treated with glass fibres and in 20/25 rats injected with 25 mg UICC crocidolite (5% ≥ 5 μm in length; mean, 3.1 μm), but in neither of the control groups (Smith *et al*., 1987).

Groups of 54 female Sprague-Dawley rats, eight weeks of age, were injected intraperitoneally with 5 mg of an untreated or treated fibre derived from JM code 104 glass microfibre that had been chopped and ground; 50% were < 4.8 μm in length. Treated fibres were soaked in either 1.4 mol/L hydrochloric acid (HCl) or 1.4 mol/L sodium hydroxide (NaOH) at room temperature for either 2 or 24 h. The weight loss of the fibres treated with HCl was 25% (2 h) or 33% (24 h) [weight loss with NaOH not reported]. Imaging by SEM revealed no modifications of the fibres. [The Working Group noted that presumably this applied to all of the treatments, although this was not stated. The incidence of abdominal tumours was reported in the form of a graph showing tumour incidence over time, but the actual numbers and types of tumour were not reported.] The incidences of tumours (estimated from the graph) in animals that received untreated JM 104 were the same as those that received the NaOH-treated JM 104 (approximately 80%). Administration of JM 104 treated with HCl, however, markedly reduced the tumour incidence: ~60% tumour incidence for 2-h HCl treated fibres and < 10% tumour incidence for 24-h HCl treated fibres. To test their biodurability 2 mg of the treated

(24 h-HCl) and untreated JM 104 fibres were injected intratracheally into rats, and their lungs were examined nine months later for fibres. The number of fibres × 10^6/lung for HCl-treated, NaOH-treated and untreated JM 104 were 31, 76 and 295, respectively. These data suggest greater biopersistence of untreated JM 104 than of NaOH- or HCl-treated JM 104, which does not completely parallel tumour incidences for the three test fibres (Pott *et al*., 1988).

Female Wistar rats [initial number not specified], eight weeks of age, were given intraperitoneal injections of saline solution containing suspensions of JM 475 glass microfibre (5 mg; 680 × 10^6 WHO fibres) or UICC Canadian chrysotile asbestos. The rats treated with chrysotile were given one of three gravimetric doses (0.05, 0.25 or 1.00 mg containing 40 × 10^6, 202 × 10^6 or 808 × 10^6 WHO fibres, respectively). The median dimensions for JM 475 test fibres were: length, 2.6 μm; diameter, 0.15 μm; those for chrysotile fibres were: length, 0.67 μm; diameter, 0.05 μm. The lengths of the JM 475 and chrysotile fibres were 90% < 9.6 μm and 90% < 2.1 μm, respectively. [A number of other fibres were also included in this study.] A group of 102 control rats was injected with saline. Rats were kept until ~130 weeks post-injection, at which time all surviving rats were killed, necropsied and examined for abdominal tumours. When tumours were present, they were examined histologically, and rats with uterine tumours were eliminated from the study. [These tumours metastasize quickly to other organs and therefore could be mistaken for sarcomas or mesotheliomas (the incidence of uterine tumours in controls treated with saline was ~15%).] The numbers of rats at risk for abdominal sarcoma/mesothelioma examined were 53 for the JM 475 and 34 for the chrysotile experiments, respectively. The incidences of tumours (excluding rats with uterine tumours) for JM 475 and the three increasing doses of chrysotile were 34/53, 12/36, 23/34 and 30/36, respectively; 2/102 control rats developed mesotheliomas (Pott, 1989; Pott *et al*., 1989).

Groups of 40 female Wistar rats, 8–10 weeks of age, were given intraperitoneal injections containing a total dose of either 17 mg or 50 mg JM 753 glass fibre. The numbers of fibres for the two doses were 1 × 10^9 and 3 × 10^9 WHO fibres per rat, respectively. The approximate median dimensions of fibres were 3.3 μm × 0.22 μm. Rats were maintained for life (until 30 months) and examined for tumours. A group of 40 concurrent negative control rats were given intraperitoneal injections of saline. The incidences of peritoneal mesotheliomas for the two doses of JM 753 were 30/36 (83%) and 36/39 (92%), respectively. Negative control rats did not develop mesotheliomas (Roller *et al*., 1996).

In two series of intraperitoneal injection studies, groups of 24 male Wistar rats, 12 weeks of age, were each injected with approximately 10^9 fibres longer than 5 μm. The fibres were counted by phase contrast optical microscopy (PCOM). The fibres tested were: JM 475 glass, amosite (Davis *et al*., 1996a; Miller *et al*., 1999) and JM 104E glass (Cullen *et al*., 2000). After injection of JM 475 glass, 8/24 of the rats developed abdominal tumours compared with 21/24 after treatment with amosite or 104E glass.

No control groups were included in these studies, but sizes of fibres and data on animal survival were fully reported.

3.3.3 *Intratracheal instillation*

(*a*) *Rat*

Groups of female Wistar rats aged 11 weeks at the start of the study received 20 weekly intratracheal instillations of a high dose of 0.5 mg/dose (total dose, 10 mg) JM 104/475 [composition not specified] glass fibre (median dimensions of fibres, 3.2 μm × 0.18 μm) or South African crocidolite (median dimensions of fibres, 2.1 μm × 0.2 μm) in 0.3 mL saline or saline alone. The median lifespans were 107, 126 and 115 weeks for the groups receiving glass fibres, crocidolite and saline, respectively. In the group treated with glass fibres, 5/34 rats developed pulmonary tumours (one adenoma, four carcinomas); the mean lifespan of rats with tumours was 113 weeks. In the group treated with crocidolite, 15/35 rats developed lung tumours (11 carcinomas and four mixed tumours); the mean lifespan of tumour-bearing animals was 121 weeks and the first tumour was observed after 89 weeks). Pulmonary tumours did not occur in the 40 control animals or in historical controls of this strain (Pott *et al*., 1987).

A group of 22 female Osborne-Mendel rats, 100 days old, received five weekly intratracheal instillations of a high dose of 2 mg [JM 475] glass fibres (10 mg total dose; geometric mean dimensions of fibres, 4.7 μm × 0.4 μm; 19% of fibres > 10 μm in length and 0.2–0.6 μm in diameter) in 0.2 mL saline. A group of 25 rats was injected with saline only and another group of 125 animals was untreated. All animals were observed for life. The median lifespan was longer in rats treated with glass fibres (783 days) than in the rats treated with saline (688 days) or untreated controls (724 days). No tumour of the respiratory tract was observed in any group. Of 25 rats treated under similar conditions with UICC crocidolite (5% fibres > 5 μm in length; mean, 3.1 ± 10.2 μm), two developed bronchoalveolar tumours (Smith *et al*., 1987). [The Working Group noted the relatively small number of animals in the study and the low tumour response in positive controls, which made interpretation of the results difficult.]

(*b*) *Hamster*

Two groups of 136 or 138 male Syrian golden hamsters [age unspecified] were given eight weekly intratracheal instillations in 0.15 mL saline of 1 mg of two different samples prepared from JM 104 glass fibre [chemical composition not given]. The two samples were wet-milled in a ball mill for 2 or 4 h, respectively, resulting in different length distributions: fibres milled for 2 h; length, 50% < 7.0 μm; diameter, 50% < 0.3 μm; fibres milled for 4 h: length, 50% < 4.2 μm; diameter, 50% < 0.3 μm). Two control groups received eight intratracheal instillations of 1 mg of either UICC crocidolite (length, 50% > 2.1 μm; diameter, 50% > 0.2 μm) as a positive control, or granular titanium dioxide as a negative control (total doses, 8 mg). The incidences of thoracic tumours in the group treated with the longer glass fibres were: 48/136 (5 lung carci-

nomas, 37 mesotheliomas, 6 sarcomas); in the group treated with shorter glass fibres, 38/138 (6 lung carcinomas, 26 mesotheliomas, 6 sarcomas); in the group treated with crocidolite, 18/142 (9 lung carcinomas, 8 mesotheliomas, 1 sarcoma); and in the group treated with titanium dioxide, 2/135 (sarcomas). The total duration of the experiment was 113 weeks. Nearly all of the tumour-bearing animals survived for up to 18 months after the first instillation (Pott *et al*., 1984b).

Six groups of 35 male and 35 female Syrian golden hamsters, 16 weeks of age, received intratracheal instillations in 0.2 mL 0.005% gelatine in saline of 1 mg JM 104 glass fibres (58% < 5 μm in length; 88% < 1.0 μm in diameter) [chemical composition not given], 1 mg glass fibre plus 1 mg benzo[*a*]pyrene, 1 mg crocidolite (UICC standard reference sample; 58% > 5 μm in length; 63% > 0.25 μm in diameter), 1 mg crocidolite plus 1 mg benzo[*a*]pyrene, 1 mg benzo[*a*]pyrene in gelatine solution in saline or vehicle alone, once every two weeks for 52 weeks. The experiment was terminated at 85 weeks, at which time 53, 43, 43, 50, 48 and 46 animals were still alive in the six groups, respectively. Tumours of the respiratory tract were found only in hamsters treated with benzo[*a*]pyrene alone. The incidences of respiratory tract tumours were as follows: in the group given benzo[*a*]pyrene alone, 3/63 (2 carcinomas and 1 sarcoma, plus 4 papillomas); in the group given crocidolite plus benzo[*a*]pyrene, 3/52 (2 carcinomas and 1 sarcoma, plus 1 papilloma); and in the group given glass fibre plus benzo[*a*]pyrene, 2/66 (2 sarcomas, plus 2 papillomas) (Feron *et al*., 1985). [The Working Group noted the relatively short observation time and the absence of tumours in the positive, crocidolite-treated groups.]

3.3.4 *Intrapleural injection*

(*a*) *Mouse*

Four groups of 25 BALB/c mice [sex and age unspecified] received single intrapleural injections of a high dose of 10 mg of one of four different samples of borosilicate glass fibres [chemical composition not given] in 0.5 mL distilled water. The material for injection was obtained by separating each of two original samples with average diameters of 0.05 μm and 3.5 μm into two samples, one with lengths of several hundred microns and the other with lengths of < 20 μm. Animals were killed at intervals of two weeks until 18 months (a total of 37 mice survived at this time). No pleural tumour was found in any of the treated animals, whereas mesotheliomas were observed in 2/150 mice given intrapleural injections of chrysotile or crocidolite [dose not stated] in a parallel experiment. The author concluded that the pleural cavity of mice might be very resistant to tumour induction by any type of mineral fibre (Davis, 1976). [The Working Group noted the small number of animals used, the relatively short observation time and the low response in the positive controls.]

(*b*) *Rat*

Groups of 32–36 SPF Wistar rats (twice as many males as females), 13 weeks of age, received single intrapleural injections in 0.4 mL saline of 20 mg glass fibre (a borosilicate; 30% of fibres 1.5–2.5 μm in diameter; maximum diameter, 7 μm; 60% > 20 μm in length) [chemical composition not given], 20 mg glass powder (a borosilicate; diameter < 8 μm) or 20 mg of one of two different samples of Canadian SFA chrysotile. Rats were kept until natural death; the average survival times were 774, 751, 568 and 639 days for the groups treated with glass fibre, glass powder and the two chrysotile samples, respectively. No injection-site tumour was observed in the group treated with glass fibre; a single mesothelioma occurred in the group treated with glass powder (after 516 days). The incidences of tumours in the two groups treated with chrysotile were 23/36 and 21/32; the first deaths of animals with tumours occurred after 325 and 382 days (Wagner *et al*., 1973).

Three groups of 16 male and 16 female Wistar rats, 10 weeks of age, received single intrapleural injections of 20 mg of a fine US JM 100 glass fibre (99% of fibres < 0.5 μm in diameter; median diameter, 0.12 μm; 2% > 20 μm in length; median length, 1.7 μm) [chemical composition not given] or a coarser US JM 110 glass fibre (17% of fibres < 1 μm in diameter; median diameter, 1.8 μm; 10% > 50 μm in length; median length, 22 μm) [chemical composition not given] in 0.4 mL saline or saline alone. Animals were kept until natural death; mean survival times were 716, 718 and 697 days, for the mice treated with fine fibres, coarse fibres and saline, respectively. Between 663 and 744 days after inoculation, 4/32 animals given the finer glass fibre had mesotheliomas. No pleural tumour occurred in animals treated with the coarser glass fibre or in controls that received saline (Wagner *et al*., 1976).

Groups of 30–130 female Osborne-Mendel rats, 12–20 weeks old, received a single intrathoracic implantation of one of 72 different types of natural and man-made mineral fibres [chemical compositions not given], 19 of which were uncoated or resin-coated glass fibres. The test substances were mixed in 10% gelatine, and 40 mg of each type of glass in 1.5 mL gelatine was smeared on a coarse fibrous glass pledget which was implanted into the left thoracic cavity. The rats were observed for 24 months after treatment and were compared with untreated controls and controls implanted with the pledget alone. The incidence of pleural mesothelioma in animals that survived for more than 52 weeks varied from 0/28 to 20/29 depending on fibre size. The most carcinogenic fibres were those < 1.5 μm in diameter and > 8 μm in length. When two of the glass fibre preparations (diameter, > 0.25 μm) were leached to remove all elements except silicon dioxide, the incidences of pleural mesotheliomas were 2/28 and 4/25 (Stanton *et al*., 1977, 1981).

Groups of 32–45 male SPF Sprague-Dawley rats, three months of age, received single intrapleural injections of 20 mg JM 104 glass fibre (mean length, 5.89 μm; mean diameter, 0.229 μm) [chemical composition not given], 20 mg UICC chrysotile A (mean dimensions, 3.21 μm × 0.063 μm), 20 mg UICC crocidolite (mean dimensions,

3.14 μm × 0.148 μm) in 2 mL saline, or received 2 mL saline alone. Animals were kept until natural death; the mean survival times for whole groups (and for animals with tumours) were 513 (499), 388 (383), 452 (470) and 469 days, respectively. The incidences of thoracic tumours were as follows: group that received glass fibre, 6/45 (mesotheliomas); groups treated with chrysotile and crocidolite, 15/33 (1 carcinoma and 14 mesotheliomas) and 21/39 (mesotheliomas), respectively. No thoracic tumours occurred in the 32 control animals (Monchaux *et al*., 1981).

Groups of 48 SPF Sprague-Dawley rats [sex and age unspecified] received single intrapleural injections of 20 mg fibrous glass dusts in 0.5 mL saline or chrysotile in 0.5 mL saline. The dust samples used (and the size distributions of those fibres longer than 1 μm) were: English glass fibre with resin coating (70% fibres < 5 μm in length; 85% < 1 μm in diameter), English glass fibre after removal of resin (57% < 5 μm in length; 85% < 1 μm in diameter), US JM 100 glass fibre (88% < 5 μm in length; 98.5% ≤ 1 μm in diameter) [chemical composition not given] and UICC African chrysotile [fibre sizes unspecified]. The animals were kept until natural death [survival times unspecified]. One mesothelioma occurred in the group treated with English glass fibre [whether coated or uncoated was not specified], four in the group treated with JM 100 glass fibre and six in the group treated with chrysotile. No mesothelioma was observed in a group of 24 controls treated with saline (Wagner *et al*., 1984).

3.4 Rock (stone) wool

3.4.1 *Inhalation exposure* (see Table 61)

Rat: Groups of 24 male and 24 female Wistar IOPS AF/Han rats, eight to nine weeks old, were exposed by inhalation to dust at concentrations of 5 mg/m^3 (respirable particles) of French (Saint-Gobain) resin-free rock (stone) wool [type of rock unspecified] (40% of fibres < 10 μm in length, 23% < 1 μm in diameter) or a Canadian chrysotile fibre (6% respirable fibres > 5 μm in length) for 5 h per day on five days per week for 12 or 24 months. An unspecified number of rats was killed either immediately after treatment or after a period of observation (of 7, 12 or 16 months after exposure for animals exposed for 12 months; 4 months after exposure for those exposed for 24 months). No pulmonary tumour was observed in 47 rats treated with rock (stone) wool or in 47 untreated controls; nine pulmonary tumours were seen in the 47 rats treated with chrysotile (Le Bouffant *et al.*, 1984). [The Working Group noted that, because of the lack of survival data, the exact incidences of tumours could not be ascertained.]

Groups of 48 SPF Fischer rats [sex and age unspecified] were exposed by inhalation to dust concentrations of approximately 10 mg/m^3 resin-free rock (stone) wool [type of rock unspecified] or UICC Canadian chrysotile for 7 h per day on five days per week for 12 months. The size distribution of those airborne fibres longer than 5 μm was: 71% of rock (stone) wool fibres ≤ 20 μm in length, 58% ≤ 1 μm in diameter; 16% of chrysotile fibres ≥ 20 μm in length, 29% ≥ 0.5 μm in diameter. Six rats were

Table 61. Inhalation studies on the carcinogenicity of rock (stone) wool and slag wool in rats

Test substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule/ cumulative exposure ($mg/m^3 \times h$)	Duration of exposure	Period of observation	No. of animals examined	No. of animals with tumours[a]	Histo-logical type[b]	Median or average survival time (weeks)
Exposure by inhalation to respirable dust at a concentration of 5 mg/m^3 rock (stone) wool (male and female Wistar rats, 8–9 weeks old) (Le Bouffant et al., 1984)								
French resin-free rock (stone) wool	40% fibres < 10 µm length, 23% < 1 µm diameter	ND	5 h/day, 5 days/week, for 52 or 104 weeks	12–28 months	47	0	–	NG
Canadian chrysotile	6% fibres > 5 µm length	ND	5 h/day, 5 days/week, for 52 or 104 weeks	12–28 months	47	9	9A	NG
Control	–	–	5 h/day, 5 days/week, for 52 or 104 weeks	12–28 months	47	0	–	NG
Exposure by inhalation to respirable dust at a concentration of ~10 mg/m^3 rock (stone) wool (SPF Fischer rats) (Wagner et al., 1984)								
Resin-free rock (stone) wool	71% fibres ≤ 20 µm length, 58% ≤ 1 µm diameter	17495	7 h/day, 5 days/week, for 52 weeks	12 months–lifetime[c]	48	2	2A	NG
UICC Canadian chrysotile	16% fibres ≥ 20 µm length, 29% ≥ 0.5 µm diameter	17499	7 h/day, 5 days/week, for 52 weeks	12 months–lifetime[c]	48	12	11AdCa 1A	NG
Control	–	–	7 h/day, 5 days/week, for 52 weeks	12 months–lifetime[c]	48	0	–	NG

Table 61 (contd)

Test substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule/ cumulative exposure ($mg/m^3 \times h$)	Duration of exposure	Period of observation	No. of animals examined	No. of animals with tumours[a]	Histo-logical type[b]	Median or average survival time (weeks)
Exposure by nose-only inhalation to dust clouds (7.8 mg/m^3) of slag wool (female Osborne-Mendel rats, 100 days old) (Smith et al., 1987)								
Slag wool	GML, 22 µm GMD, 0.9 µm	200 fibres/cm^3	6 h/day, 5 days/week, for 104 weeks	Lifetime[c]	55	0	–	97
Crocidolite	L, 5% > 5 µm	3000 fibres/cm^3	6 h/day, 5 days/week, for 104 weeks	Lifetime[c]	57	3	1M, 2 BT	109
Chamber controls	–	–	6 h/day, 5 days/week, for 104 weeks	Lifetime[c]	59	0	–	108
Room controls	–	–	6 h/day, 5 days/week, for 104 weeks	Lifetime[c]	125	0	–	103
Exposure by nose-only inhalation to respirable dust at concentrations of 3, 16 and 30 mg/m^3 rock (stone) wool (MMVF21) (male Fischer 344 rats, 8 weeks old) (McConnell et al., 1994)								
Rock (stone) wool (MMVF21)	GML, 13.0 µm GMD, 0.94 µm	9 672	6 h/day, 5 days/week, for 104 weeks	28 months	114	5	4A 1Ca	~104
	GML, 15.4 µm GMD, 0.90 µm	50 232	6 h/day, 5 days/week, for 104 weeks	28 months	115	5	4A 1Ca	~104
	GML, 14 µm GMD, 0.98 µm	94 848	6 h/day, 5 days/week, for 104 weeks	28 months	114	5	4A 1Ca	~104
Crocidolite	GML, 4.1 µm GMD, 0.28 µm	13 000	6 h/day, 5 days/week, for 44 weeks	28 months	106	14	10A 5Ca 1M	~100

Table 61 (contd)

Test substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule/ cumulative exposure ($mg/m^3 \times h$)	Duration of exposure	Period of observation	No. of animals examined	No. of animals with tumours[a]	Histo-logical type[b]	Median or average survival time (weeks)
Control	–	–	6 h/day, 5 days/week, for 104 weeks	28 months	126	2	2A	~104
Exposure by nose-only inhalation to respirable dust at concentrations of 3, 16 and 30 mg/m^3 slag wool (MMVF22) (male Fischer 344 rats, 8 weeks old) (McConnell et al., 1994)								
Slag wool (MMVF22)	GML, 12.3 μm GMD, 0.84 μm	9 672	6 h/day, 5 days/week, for 104 weeks	28 months	116	2	1A 1Ca	~104
	GML, 13.2 μm GMD, 0.84 μm	50 232	6 h/day, 5 days/week, for 104 weeks	28 months	115	0		~104
	GML, 15.2 μm GMD, 0.87 μm	93 288	6 h/day, 5 days/week, for 104 weeks	28 months	115	3	2A 1Ca	~104
Crocidolite	GML, 4.1 μm GMD, 0.28 μm	13 000	6 h/day, 5 days/week, for 44 weeks	28 months	106	14	10A 5Ca 1M	~100
Control	–	–	6 h/day, 5 days/week, for 104 weeks	28 months	126	2	2A	~104

NG, not given; GML, geometric mean length; GMD, geometric mean diameter

[a] Tumours of the lung, pleura, thorax or abdominal cavity

[b] A, adenoma; AdCa, adenocarcinoma; BT, bronchoalveolar tumour; Ca, relatively undifferentiated epidermoid carcinoma; M, mesothelioma

[c] Lifetime, until survival rate was ≤ 20%

removed from each group at the end of the exposure period to study dust retention, and a similar number of animals was killed one year later for the same purpose. The remainder were kept until natural death [survival times not reported]. During the period 500–1000 days after the start of exposure, lung adenomas (1 with some malignant features) occurred in 2/48 rats in the group treated with rock (stone) wool; 11 adenocarcinomas and one adenoma (with some malignant features) were observed in 48 rats treated with chrysotile. No lung tumour was observcd in a group of 48 untreated controls (Wagner *et al*., 1984). [The Working Group noted that, because of inadequate data on survival, the exact tumour incidences could not be established.]

Groups of 140 male Fischer 344 rats, eight weeks of age, were exposed in nose-only inhalation chambers to rock (stone) wool (MMVF21) at concentrations of 3, 16 or 30 mg/m^3 for 6 h per day on five days per week for 104 weeks. These concentrations corresponded to average numbers of WHO fibres/cm^3 of 34, 150 and 243 and numbers of fibres > 20 μm long of 13, 74 and 114, respectively. The geometric mean diameter of the test fibres was about 0.95 μm and the geometric mean of the fibre length was about 14 μm. The retained lung burdens/mg of dry lung tissue after 24 months were: 242×10^3 WHO fibres and 39.5×10^3 fibres > 20 μm for the 30 mg/m^3 dose, 217×10^3 and 25.6×10^3, respectively, for the 16 mg/m^3 dose and 55.7×10^3 and 9.0×10^3, respectively, for the 3 mg/m^3 dose. A subsequent post-exposure period lasted until approximately 20% of the animals in the air-control group survived which occurred at approximately 28 months. Mortality of the treated animals was similar to that of unexposed control animals. Four lung adenomas and one lung carcinoma each were observed among 114, 115 and 115 rats in the 3 mg/m^3, 16 mg/m^3 and 30 mg/m^3 dose groups, respectively. In the control group, two adenomas were observed in 126 rats. A positive control group was treated with crocidolite asbestos (10 mg/m^3) with a geometric mean diameter of 0.28 μm, determined by SEM, and a geometric mean length of 4.1 μm, determined by light microscopy. [The Working Group noted that exposure to crocidolite asbestos was terminated after 10 months because of increased morbidity and mortality.] The lung burden/mg dry lung tissue retained after 104 weeks was 759×10^3 WHO fibres and 41.1 fibres $\times 10^3 > 20$ μm. Ten pulmonary adenomas, five pulmonary carcinomas and one mesothelioma were reported among 106 crocidolite-treated rats (McConnell *et al*., 1994).

3.4.2 *Intratracheal instillation* (see Table 62)

(*a*) *Rat*

Groups of 40–59 female Wistar rats, 15 weeks of age, were treated by intratracheal instillation with 5 mg (10 weekly doses of 0.5 mg each) and 10 mg (20 weekly doses of 0.5 mg each) of a rock (stone) wool suspension and were then observed for 131 weeks. The low-dose group was exposed to a total of 4×10^6 fibres and the high-dose group, to 8×10^6 fibres (length, > 5 μm; diameter, < 2 μm; aspect ratio > 5:1) [chemical composition and size distribution not given]. At the end of the experiment, the lungs were

Table 62. Carcinogenicity studies on the intratracheal instillation of rock (stone) wool in rats and hamsters

Substance	Instillation schedule (if > 1 injection/ week)	Total number of fibres	Observation period (weeks)	No. of animals with tumours/ no. of animals examined[a,b]	Histological types[c]	Comments	Reference
Rock (stone) wool	5 mg (10 × 0.5 mg)	[d]4×10^6	131	0/59 rats 5/59 rats	PLT OLT		Pott *et al*. (1994)
Rock (stone) wool	10 mg (20 × 0.5 mg)	[d]8×10^6	131	0/40 rats 4/40 rats	PLT OLT		
Control	Saline solution (20 × 0.4 mL)		131	0/40 rats 2/40 rats	PLT OLT		
Tremolite (positive control)	2.5 mg (5 × 0.5 mg)	[d]70×10^6	131	3/38 rats 6/38 rats	PLT OLT	[$p = 0.023$][e]	
Tremolite (positive control)	7.5 mg (15 × 0.5 mg)	[d]300×10^6	131	8/37 rats 4/37 rats	PLT OLT	[$p = 0.0025$][e]	
Rock (stone) wool	10 mg (5 × 2 mg)		104	0/20 hamsters	–	GML, 296 μm GMD, 6.1 μm	Adachi *et al.* (1991)
Control	Saline solution		104	0/20 hamsters	–		

GML, geometric mean length; GMD, geometric mean diameter

[a] Female Syrian hamsters, ~ 80 g

[b] Female Wistar rats, 15 weeks of age

[c] PLT, primary lung tumours: adenomas, adenocarcinomas, squamous cell tumours and cystic keratinizing squamous-cell tumours; OLT, other lung tumours: fibrosarcomas, lymphosarcomas, mesotheliomas or lung metastases from tumours at other sites

[d] D < 2 μm; L > 5 μm; aspect ratio > 5:1

[e] Versus control group treated with saline solution

perfused with formalin and examined histopathologically. None of the 59 rats treated with 5 mg of the suspension developed a primary lung tumour, but other lung tumours (fibrosarcomas, lymphosarcomas, mesotheliomas and lung metastases from tumours at other sites) were found in 5/59 rats. In the group that received 10 mg of the suspension, no primary lung tumours were observed in 40 rats; 4/40 animals had other lung tumours. A control group (40 rats) was treated with 20 weekly injections of 0.4 mL saline solution. No primary lung tumours were observed; other lung tumours were found in 2/40 rats. In a positive-control group, 38 and 37 female Wistar rats were injected intratracheally with tremolite: 2.5 mg (5 doses of 0.5 mg each) or 7.5 mg (15 doses of 0.5 mg each). [No information was given on the fibre dimensions.] The total number of fibres in the low dose was 70×10^6 and in the high dose, it was 300×10^6 fibres (length, > 5 μm; diameter, < 2 μm; aspect ratio, > 5:1). In the group that received the low dose, three primary tumours of the lung and six other tumours of the lung were observed in the 38 rats examined. In the group that received the high dose, the numbers were eight primary tumours of the lung and four other tumours of the lung in 37 rats (Pott *et al*., 1994).

(*b*) *Hamster*

A group of 20 female Syrian hamsters (weight, 80 g) was injected intratracheally with 2 mg rock (stone) wool suspended in 0.2 ml saline once a week for five weeks. The average diameter of the fibres was 6.1 μm and the average length was 296 μm. Two years after the first administration, all hamsters were killed and routine autopsies were performed. No tumours were reported in the treated group or in a control group of 20 hamsters (Adachi *et al*., 1991). [The Working Group noted that the relevance of this study is limited because of the very long and thick fibres used in this experiment.]

3.4.3 *Intraperitoneal injection* (see Table 63)

Rat: Groups of female Sprague-Dawley rats [initial numbers unspecified], eight weeks of age, received three weekly intraperitoneal injections of 75 mg Swedish rock (stone) wool [type of rock unspecified] (median fibre length, 23 μm; median diameter, 1.9 μm), a single injection of 10 mg of a fine fraction prepared from the rock (stone) wool sample (median fibre length, 4.1 μm; diameter, 0.64 μm) or 40 mg (two injections) of granular volcanic ash from Mount St Helen's in 2 mL saline solution. The median survival times were 77, 97 and 93 weeks for the animals given the 75 mg and 10 mg doses of rock (stone) wool and volcanic ash, respectively; the median lifespan of a control group that received two injections of 2 mL saline was 94 weeks. A high incidence of tumours in the abdominal cavity was observed following treatment with 75 mg of the original rock (stone) wool sample: (45/63; lifespan of first animals with tumour, 39 weeks) and a slight increase in tumour incidence in animals treated with 10 mg of the fine fraction (6/45; first tumour after 88 weeks) compared with a tumour incidence of 3/54 in the groups of controls treated with volcanic ash and saline (Pott *et al*., 1987). [The thicker and longer fibres induced more tumours than the thinner and shorter ones.]

Table 63. Carcinogenicity studies on the intraperitoneal injection of fibres of rock (stone) and slag wool in rats

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
Rock (stone) wool									
Rock (stone) wool (Sweden)	3 × 25 mg	NG	L, 23; D, 1.9	134, SpD, F	77	45/63	meso/sarc	[$p < 0.001$]	Pott *et al*. (1987)
Rock (stone) wool (Sweden), fine	1 × 10 mg	NG	L, 4.1; D, 0.64	134, SpD, F	97	6/45	meso/sarc		
Volcanic ash	2 × 20 mg	(granular dust *control*)[j]		134, SpD, F	93	3/54	meso/sarc		
Saline control	5 × 2 mL	(saline control)		134, SpD, F	94	3/54	meso/sarc		
Rock (stone) wool	1 × 25 mg	NG	NG	104, SpD, M and F	NG	3/40	meso	duration of study 104 weeks [results not conclusive]	Maltoni & Minardi (1989)
Water control	NG	(water control)				0/40			
Basalt wool, G & H	5 × 15 mg	59×10^6 WHO with L/D > 5/1	L, 17; D, 1.1 L/D > 5/1	111 (death of last rat), W, F	79	30/53	meso/sarc	strong adhesions of the abdominal organs [$p < 0.0001$]	Pott *et al*. (1989)
Titanium dioxide	5 × 20 mg	(granular dust *control*)[j]		130, W, F	109	2/53	meso/sarc		
Saline control	5 × 2 mL	(saline control)		130, W, F	111	2/102	meso/sarc		

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
Basalt wool, G & H	1 × 25 mg	5×10^6; L/D > 5/1; L > 5; D < 2 μm	L, 13.8; D, 1.08 L/D > 5/1	130, W, F	110	1/38	meso/sarc	lung infection months 12–13; mortality 10 of 48 at start	Pott *et al*. (1991)
Basalt wool, G & H	5 × 30 mg	30×10^6; L/D > 5/1; L > 5; D < 2 μm	L, 13.8; D, 1.08 L/D > 5/1	130, W, F	84	15/21	meso/sarc	lung infection months 12–13; mortality 15 of 36 at start [$p < 0.001$]	
Carbon, activated	5 × 50 mg	(granular dust *control*)[j]		131, W, F	122	1/25	meso/sarc	lung infection months 12–13; mortality 11 of 36 at start	
Saline control	5 × 2 mL	(saline control)		130, W, F	106	2/50	meso/sarc	lung infection months 12–13; mortality 22 of 72 at start	
Basalt 'superthin'	2 × 25 mg	NG	17% with L > 5 and D < 3	Lifetime		5/40	meso	proportion of fibres 12% of nonfibres particles 88% [$p < 0.006$]	Nikitina *et al.* (1989)

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
Basalt 'ultrathin'	2 × 25 MG	NG	41% with L > 5 and D < 3	Lifetime		7/50	meso	[$P < 0.01$]	
Chrysotile	2 × 25 MG	NG	76% with L > 5 and D < 3	Lifetime		27/60	meso	[$P < 0.001$]	
Saline control			(saline *control*)[j]			0/110			
M-stone[f]	1 × 8.5 mg	100×10^6	L, 10.1; D, 0.84; L/D > 5/1	130, W, F	104	2/32	meso	[$p = 0.030$]	Davis *et al*. (1996b); Roller *et al*. (1996)
M-stone[f]	1 × 8.5 mg	100×10^6	L, 10.1; D, 0.84; L/D > 5/1	130, W, M	90	2/36	meso	[$p = 0.037$]	
M-stone[f]	1 × 25.5 mg	300×10^6	L, 10.1; D, 0.84; L/D > 5/1	130, W, F	103	9/32	meso	[$p = 0$]	
M-stone[f]	1 × 25.5 mg	300×10^6	L, 10.1; D, 0.84; L/D > 5/1	130, W, M	93	8/36	meso	[$p = 0$]	
M-stone[f]	2 × 42.5 mg	1000×10^6	L, 10.1; D, 0.84; L/D > 5/1	130, W, M	90	22/35	meso	[$p = 0$]	
B-20-2.0[f, g]	1 × 6 mg	80×10^6	L, 7.8; D, 0.77; L/D > 5/1	130, W, F	105	2/32	meso	[$p = 0.030$]	

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
B-20-2.0 [f, g]	1 × 6 mg	80×10^6	L, 7.8; D, 0.77; L/D > 5/1	130, W, M	119	15/36	meso	[$p = 0$]	
B-20-2.0 [f, g]	1 × 18 mg	240×10^6	L, 7.8; D, 0.77; L/D > 5/1	130, W, F	96	7/32	meso	[$p = 0$]	
B-20-2.0 [f, g]	1 × 18 mg	240×10^6	L, 7.8; D, 0.77; L/D > 5/1	130, W, M	107	12/34	meso	[$p = 0$]	
B-20-2.0 [f, g]	2 × 30 mg	800×10^6	L, 7.8; D, 0.77; L/D > 5/1	130, W, M	88	21/35	meso	[$p = 0$]	
Crocidolite	5 × 0.1 mg	42×10^6	L, 1.8; D, 0.19; L/D > 5/1	130, W, F	85	25/32	meso		
Crocidolite	5 × 0.1 mg	42×10^6	L, 1.8; D, 0.19; L/D > 5/1	130, W, M	89	32/48	meso		
Silicon carbide	5 × 50 mg	(granular dust *control*)[j]		130, W, F	105	1/47	meso		
Silicon carbide	5 × 50 mg	(granular dust *control*)[j]		130, W, M	109	0/71			
Silicon carbide	20 × 50 mg	(granular dust *control*)[j]		130, W, F	107	0/45			
Silicon carbide	20 × 50 mg	(granular dust *control*)[j]		130, W, M	104	0/70			
Saline control	20 × 2 mL	(saline *control*)[j]		130, W, F	110	0/93			
Saline control	20 × 2 mL	(saline *control*)[j]		130, W, M	103	1/69	meso		

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
B-20-06 [f, l]	1 × 3.5 mg	400×10^6	L, 3.6; D, 0.30; L/D > 5/1	130, W, F	103	12/40 (6 macr.[h)]	meso	final histol.: 11 rats with meso[k] [$p = 0$]	Davis *et al.* (1996b); Roller *et al.* (1996)
B-20-06 [f, l]	1 × 8.5 mg	1000×10^6	L, 3.6; D, 0.30; L/D > 5/1	130, W, F	96	17/40 (5 macr.[h])	meso	final histol.: 19 rats with meso[k] [$p = 0$]	
B-20-06 [f, l]	1 × 25 mg	3000×10^6	L, 3.6; D, 0.30; L/D > 5/1	130, W, F	79	30/40	meso	final histol.: 31 rats with meso[k] [$p = 0$]	
B-20-06 [f, l]	3 × 25 mg	9000×10^6	L, 3.6; D, 0.30; L/D > 5/1	130, W, F	38	27/31	meso	final histol.: 27 rats with meso[k] [$p = 0$]	
Saline control	3 × 2 mL	(saline control)		130, W, F	121	0/38		final histol.: 0 rat with meso[k]	
Tremolite	1 × 3.3 mg	57×10^6	L, 3.4; D, 0.29	130,W, F	98	10/39	meso		
Tremolite	1 × 15 mg	260×10^6	L, 3.4; D, 0.29	130, W, F	74	30/40	meso		
MMVF21 [f, g]	2 × 30 mg	400×10^6	L, 16.9; D, 1.02; L/D > 5/1	130, W, F	54	37/38	meso	final histol.: 37 rats with meso[k] [$p = 0$]	Davis *et al.* (1996b); Roller *et al.* (1996)

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated [b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats evaluated	Histo-logical tumour types [e]	Comments	Reference
MMVF21 [f, g]	5 × 30 mg	1000×10^6	L, 16.9; D, 1.02; L/D > 5/1	130, W, F	51	33/38	meso	final histol.: 33 rats with meso[k] [$p = 0$]	
R-stone-E3 [f, i]	4 × 28.5 mg	400×10^6	L, 16.9; D, 1.03; L/D > 5/1	130, W, F	120	0/30		final histol.: 0 rat with meso[k] [$p = 1$]	
R-stone-E3 [f, i]	9 × 28.5 mg	900×10^6	L, 16.9; D, 1.03; L/D > 5/1	130, W, F	120	4/35 (4 macr.[h])	meso	final histol.: 1 rat with meso[k] [$p = 0.0005$]	
Crocidolite	5 × 0.1 mg	42×10^6	L, 1.8; D, 0.19; L/D > 5.1	130, W, F	100	20/39	meso		
Untreated control	(untreated control)		130, W, F	115	0/37		final histol.: 0 rat with meso.[k]		
MMVF21	183.1 mg	10^9		Lifetime or until signs of debilitation, W, M	40	19/20	meso		Miller *et al.* (1999)
Amosite	6.1 mg	10^9		Lifetime or until signs of debilitation, W, M	73	21/24	meso		

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
Slag wool									
Slag wool Rheinstahl	2 × 20 mg	NG	L, 26; D, 2.6; L/D > 5/1	158, W, F	111	6/99	meso/sarc		Pott *et al*. (1987)
Slag wool Zimmermann	2 × 20 mg	NG	L, 14; D, 1.5; L/D > 5/1	155, W, F	107	2/96	meso/sarc		
Saline control	2 × 2 mL	(saline control)		150, W, F	101	0/48			
Slag wool	5 × 30 mg	250 × 10^6; L/D > 5/1; L > 5 μm; D < 2 μm	L, 9.0; D, 1.21; L/D > 5/1	131, W, F	106	2/28	meso/sarc	lung infection months 12–13; mortality 8 of 36 at start	Pott *et al.* (1991)
Saline control	5 × 2 mL	(saline control)		130, W, F	106	2/50	meso/sarc	lung infection months 12–13; mortality 22 of 72 at start	
MMVF22 [f, m]	1 × 20 mg	400 × 10^6	L, 8.7; D, 0.77; L/D > 5/1	130, W, F	99	4/40 (3 macr.[h])	meso	final histol.: 5 rats with meso[k]	Davis *et al.* (1996b); Roller *et al.* (1996)
MMVF22 [f, m]	1 × 50 mg	1000 × 10^6	L, 8.7; D, 0.77; L/D > 5/1	130, W, F		8/40	meso	final histol.: 8 rats with meso[k]	

Table 63 (contd)

Test substance	Injection schedule (weekly intervals)	No. of fibres L > 5 μm and differences from WHO definition[a]	L (μm); D (μm); L/D > 3/1 (median of all lengths, all diameters); different fibre definitions stated[b]	Observation period (weeks), strain, gender[c]	Median survival time (weeks)	No. of rats with tumours[d]/ no. of rats examined	Histo-logical tumour types[e]	Comments	Reference
MMVF22 [f, m]	3 × 50 mg	2900×10^6	L, 8.7; D, 0.77; L/D > 5/1	130, W, F	101	18/38	meso	final histol.: 18 rats with meso[k]	
Saline control	3 × 2 mL	(saline control)		130, W, F	121	0/38		Final histol.: 0 rat with meso[k]	
Tremolite	1 × 3.3 mg	57×10^6	L, 3.4; D, 0.29	130, W, F	98	10/39	meso		
Tremolite	1 × 15 mg	260×10^6	L, 3.4; D, 0.29	130, W, F	74	30/40	meso		
MMVF22	129.6 mg	10^9		Lifetime or until signs of debilitation, W, M	94	13/24	meso		Miller *et al.* (1999)
Amosite	6.1 mg	10^9		Lifetime or until signs of debilitation, W, M	73	21/24	meso		

Table 63 (contd)

G & H, Grünszweig & Hartmann; NG, not given; lifetime, until survival rate is ≤ 20%.

[a] Aspect ratio > 5/1 in Pott *et al*. (1989, 1991) and Roller *et al*. (1996); diameter < 2 μm in Pott *et al*. (1991) and Roller *et al*. (1996)

[b] Aspect ratio > 5/1 in Pott *et al*. (1989, 1991) and Roller *et al*. (1996)

[c] SpD, Sprague Dawley; W, Wistar; M, male; F, female; period of observation after first injection

[d] Calculated from the stated percentage for some groups

[e] Correct histopathological diagnosis of tumours of the abdominal cavity requires considerable experience. Differential diagnoses of sarcomatous mesothelioma, sarcoma (sarc) and metastases of uterus carcinoma have to be considered in addition to other rare tumour types. Such experience was available for the large study by Roller *et al*. (1996) at which time it was clear that only mesotheliomas (meso) could be attributed to fibrous dusts. In this study, when mesotheliomas were excluded, 49 tumours of the uterus and 18 other tumours were found in the abdominal cavity of 406 female rats (4.4%) and 32 tumours in 661 male rats (4.8%). Three histopathologists with experience in the pathology of rodent neoplasia reviewed a large series of slides from this study of which a few presented some diagnostic difficulty. This most commonly involved uterine adenocarcinoma with widespread peritoneal metastases. There was good agreement among the histopathologists on the percentage or absence of malignant mesothelioma. In most cases, these neoplasms appeared similar to other malignant mesotheliomas that have been induced in this animal model. The initial diagnoses were unanimous in 85% of the cases. Following discussion, final agreement was reached in 99% of the cases. A comparison of the final diagnosis made by the panel with the original diagnosis made at the Fraunhofer Institute revealed agreement in 98% of the cases (Davis *et al*. 1996b). A summary of former results of Pott *et al*. showed tumours in the abdominal cavity in 23 of 886 female Wistar rats (tumours of the uterus were excluded as far as possible) after intraperitoneal administration of non-fibrous dusts or fibres thicker than 3 μm (2.6 %). After injection of saline, tumours were reported in the abdominal cavity in 13 of 491 rats (2.6%) (Pott *et al.*, 1993).

[f] The chemical composition was published in Roller *et al*. (1996) and a description of the experimental method in Pott *et al*. (1993).

[g] This experimental vitreous fibre was produced with a chemical composition analogous to early commercial rock (stone) wool fibres.

[h] Rats with abdominal tumours were diagnosed only macroscopically at the time of publication.

[i] This experimental vitreous fibre had a chemical composition expected to have a low biodurability and, consequently, a low carcinogenic potential.

[j] Statistical test performed versus the control group(s) in italics or their sum

[k] The final data on the histopathological findings were used for calculation of the dose–response relationships and published subsequently (Roller *et al*., 1997; Roller & Pott, 1998).

[l] Same chemical composition as B-20-2.0 (similar to rock (stone) fibres, see footnote [g]), but with shorter lengths and smaller diameters

[m] This experimental vitreous fibre was produced with a chemical composition analogous to early commercial slag wool fibres.

Groups of 20 male and 20 female Sprague-Dawley rats, 6–8 weeks of age, received an intraperitoneal injection of 25 mg rock (stone) wool fibres [type not specified; no information on size was given for the test material] and were killed after 104 weeks. A complete autopsy was performed on all animals together with a histopathological examination of the peritoneum. Mesotheliomas were reported in 3/40 rats. The average latency time was 80 weeks. The control animals were injected with water and no tumours were reported (Maltoni & Minardi, 1989). [The Working Group noted that the results are not conclusive as the number of fibres administered and other important details were not presented.]

Female Wistar rats [initial numbers not specified], eight weeks of age, received five weekly intraperitoneal injections of 15 mg of a basalt wool (total dose, 75 mg) [produced by Grünzweig and Hartmann, Germany, chemical composition not given]. The median length of the fibres was 17 μm and the median diameter was 1.1 μm. The total number of WHO fibres (with L/D > 5/1) injected was 59×10^6. The median life-span was 79 weeks after first treatment. A post-mortem examination of the abdominal cavity was made. Parts of tumours or organs in which macroscopic tumour tissue was found were investigated by histopathological examination. Mesotheliomas were diagnosed in 30/53 treated animals (lifespan of first animal with tumour, 54 weeks). In a control group that received five weekly injections of saline, mesothelioma was observed in 2/102 animals and the median lifespan was 111 weeks after first treatment, showing a clear increase in the mortality of the fibre-treated group. In a further group of rats, treated with 100 mg titania, the median survival time was 109 weeks and 2/53 rats had mesotheliomas or sarcomas (Pott *et al*., 1989)

In a chronic study, three groups of rats [age, sex and strain not specified] received two monthly intraperitoneal injections of 25 mg of basalt or chrysotile asbestos dusts suspended in saline [chemical composition of dust was not given]. Forty animals in the first group received basalt dust (17% of the dust fibres had length > 5 μm and diameter < 3 μm). Fifty animals of the second group received basalt dust (41% of the dust fibres had length > 5 μm and diameter < 3 μm). Sixty rats in the third group were injected with chrysotile asbestos dust. Fibres with length > 5 μm and diameter < 3 μm comprised 76% of the fibre fraction of the asbestos dust. The granular fractions of all dusts varied from 87.9%–88.9%. Control animals (110 rats) were injected intraperitoneally with the saline solution alone. Animals were observed for life. All the materials tested induced peritoneal mesotheliomas. The numbers were 5/40, 7/50 and 27/60 rats in the first basalt dust-, second basalt dust- and chrysotile asbestos-treated groups, respectively. No mesotheliomas were found in control animals (Nikitina *et al.*, 1989).

Female Wistar rats [initial numbers not specified], weighing approximately 190 g, received a single intraperitoneal injection of 25 mg or five weekly injections of 30 mg (total dose, 150 mg) basalt wool (produced by Grünzweig & Hartmann, Germany) [chemical composition not given]. The median length of the fibres was 13.8 μm and the median diameter was 1.08 μm. The numbers of fibres injected (length, > 5 μm; diameter, < 2 μm; aspect ratio, > 5:1) were 5×10^6 and 30×10^6, respectively. The survival time

was much reduced by an infectious disease of the lungs during months 12 and 13 of the study, the cause of which could not be unequivocally determined. In the low-dose group, 10/48 rats died from the infection and 15/36 in the high-dose group. The mean lifespan was 110 weeks in the low-dose group and 84 weeks in the high-dose group. For those rats that developed tumours, the mean lifespan was 128 weeks in the low-dose group and 89 weeks in the high-dose group. Macroscopic tumours were observed in 1/38 rats in the low-dose group and in 15/21 rats in the high-dose group. A control group of 72 rats was treated with five weekly injections of 2 mL saline solution. Of these animals, 22 died from the infection and the median lifespan was 106 weeks. Two of the remaining 50 rats developed an abdominal tumour (Pott *et al*., 1991). [The Working Group found it difficult to interpret the study since the impact of the infection was unknown.]

Four groups of 40 female Wistar rats, 8–10 weeks of age, received intraperitoneal injections of one of two rock (stone) wools (MMVF21 and R-stone E3) suspended in 2 mL saline administered as two or five weekly injections of 30 mg MMVF21, or four or nine weekly injections of 28.5 mg of R-stone E3. A negative-control group of 40 female Wistar rats received no treatment and a positive-control group of 40 female rats received five intraperitoneal injections of 0.1 mg of a UICC crocidolite sample. The animals were observed up to 130 weeks after first injection. The doses, dose schedules and characteristics of all the fibres tested are given in Table 63, together with data on median survival and incidence of mesotheliomas. The incidences of mesotheliomas were: 37/38 and 33/38 in the rats treated with MMVF21; 0/30 and 4/35 in the rats treated with R-stonewool E3; 20/39 in the rats treated with crocidolite; and 0/37 in untreated control rats (Pott *et al.*, 1993; Davis *et al.*, 1996b; Roller *et al.*, 1996).

A further experiment was reported as part of this study. Five groups of 32–36 male or 32–36 female Wistar rats, 8–10 weeks of age, were administered M-stone (a typical rock (stone) wool; Manville Technical Center, Denver, CO, USA; median length of the fibres was 10.1 μm and the median diameter was 0.84 μm) in 2 mL saline. The rats received either single intraperitoneal injections of 8.5 or 25.5 mg (males and females) or two injections (at a two-week interval) of 42.5 mg (males only). Five other groups of 32–36 male or 32–36 female Wistar rats, 8–10 weeks of age, received intraperitoneal injections of experimental rock (stone) wool B-20-2.0 (Bayer, Germany; median length of the fibres was 6.6 μm and the median diameter was 0.83 μm) suspended in 2 mL saline and administered as a single injection of 6 or 18 mg (males and females) or as two weekly injections of 30 mg (males only). Animals were observed for up to 130 weeks. A control group of 96 female and 72 male Wistar rats received 20 weekly intraperitoneal injections of 2 mL saline alone. Macroscopic tumours were investigated histopathologically. The incidence of peritoneal mesotheliomas in the five groups treated with M-stone was 2/32, 2/36, 9/32, 8/36 and 22/35. In the groups treated with B-20-2.0, mesotheliomas were observed in 2/32 females and 15/36 males in the low-dose group, 7/32 females and 12/34 males in the mid-dose group and in 21/35 animals that received the high dose. In the control group treated with saline, no tumour was observed in any of the 93 females and a mesothelioma was observed in one of the 69 males. Two groups

of positive control animals (one female and one male) received five weekly intraperitoneal injections of 0.1 mg UICC crocidolite. The median length of the fibres was 1.8 μm and the median diameter 0.19 μm. Mesotheliomas were observed in 25/32 females and 32/48 males examined. Two groups of negative-control animals (48 female and 72 male Wistar rats) received 20 weekly intraperitoneal injections of 50 mg granular silicon carbide. No mesotheliomas were observed in any of the 45 female or 70 male rats examined (Pott *et al*., 1993; Davis *et al*., 1996b; Roller *et al*., 1996).

An additional experiment performed as part of the same study was also reported. Four groups of 40 female Wistar rats, 8–10 weeks of age, received intraperitoneal injections of experimental rock (stone) wool B-20-0.6 (Bayer, Germany) suspended in 2 mL saline either as a single injection of 3.5, 8.5 or 25 mg or as three weekly injections of 25 mg. A negative-control group consisted of 40 female Wistar rats that received three intraperitoneal injections of 2 mL saline alone and two groups of positive controls received injections of either 3.3 or 15 mg tremolite (Libby, Montana). The incidences of mesotheliomas were: 12/40, 17/40, 30/40 and 27/31 in the rats treated with B-20-0.6; 10/39 and 30/40 in the rats treated with tremolite compared to 0/38 in the control rats treated with saline (Pott *et al.*, 1993; Davis *et al.*, 1996b; Roller *et al.*, 1996).

Groups of about 24 male Wistar rats, approximately 12 weeks of age, received two injections of MMVF21 of a mass dose of 183.1 mg suspended in buffered saline. The target dose was 10^9 WHO fibres; the number of fibres with length > 5 μm was 0.8×10^9 per rat and the diameters were < 0.95 μm and > 0.95 μm. Animals were kept for life or until they showed signs of debilitation. At autopsy, the peritoneal contents were examined macroscopically for the presence of peritoneal mesotheliomas. In addition, specimens from the first six animals to develop mesotheliomas were taken for histopathological examination. The median survival time was 281 days. The median survival time of rats that developed mesotheliomas was 284 days. An abdominal mesothelioma was diagnosed in 19/20 animals examined. Tumours were diagnosed by the macroscopic presence of peritoneal mesotheliomas and by microscopy when the diagnosis was in doubt. In a group of positive controls, male Wistar rats were treated with 6.1 mg amosite fibres with the same target concentration of 10^9 WHO fibres. About 99% of the fibres had diameters below 0.95 μm. Mesotheliomas were observed in 21/24 of the rats in this group (Miller *et al*., 1999). [The Working Group noted that no vehicle control group was included in this study.]

3.4.4 *Intrapleural injection*

Rat: Groups of 48 SPF Sprague-Dawley rats [sex and age unspecified] received single intrapleural injections of 20 mg Swedish rock (stone) wool [type of rock unspecified] or chrysotile in 0.5 mL saline. The dust samples used (and the size distributions of fibres) were: Swedish rock (stone) wool with resin coating (70% fibres < 5 μm in length; 52% < 0.6 μm in diameter), Swedish rock (stone) wool after removal of resin (70% < 5 μm in length; 58% < 0.6 μm in diameter) and UICC African

chrysotile [fibre sizes unspecified]. The animals were kept until natural death [survival times unspecified]. Three mesotheliomas occurred in the group treated with rock (stone) wool with resin and two in the group treated with rock (stone) wool without resin; six mesotheliomas occurred in the group treated with chrysotile. No tumour was observed in a group of 24 controls treated with saline (Wagner *et al.*, 1984).

3.5 Slag wool

3.5.1 *Inhalation exposure* (see Table 61)

Rat: A group of 55 female Osborne-Mendel rats, 100 days of age, was exposed by inhalation (nose only) to slag wool dust [type of slag unspecified] (mass concentration, 7.8 mg/m^3; 15.2% respirable fibres — geometric mean diameter, 0.9 μm; geometric mean length, 22 μm; chamber concentration, 200 fibres/cm^3 with 76 fibres > 10 μm in length and ≤ 1.0 μm in diameter) for 6 h per day on five days per week for two years and then observed for life. Groups of 59 chamber and 125 room controls were also kept. No tumour of the respiratory tract was observed in any group. Average survival time in the group treated with slag wool was shorter (677 days) than that of chamber (754 days) and room (724 days) controls. Of 57 rats exposed to UICC crocidolite (3000 fibres/cm^3; 5% fibres ≥ 5 μm in length; mean, 3.1 ± 10.2 μm), two developed bronchoalveolar tumours and one, a mesothelioma (Smith *et al*., 1987). [No information was given on chemical composition of the slag wool or on animal respirability. The number of slag wool fibres retained was relatively low.]

Groups of 140 male Fischer 344 rats, eight weeks of age, were exposed in nose-only inhalation chambers to three concentrations (3, 16 and 30 mg/m^3) of slag wool (MMVF22) for 6 h per day on five days per week for 104 weeks. These exposure conditions correspond to average numbers of WHO fibres/cm^3 of 30, 131 and 213. The geometric mean diameter of the test fibres was approximately 0.85 μm and the geometric mean length approximately 13 μm. The retained lung burden/mg dry lung tissue after 24 months was: 44.4 × 10^3 WHO fibres and 1.8 × 10^3 fibres > 20 μm for the 3 mg/m^3 dose, 96.7 × 10^3 and 4.5 × 10^3, respectively, for the 16 mg/m^3 dose and 177 × 10^3 and 11.0 × 10^3, respectively, for the 30 mg/m^3 dose. The post-exposure period was continued until approximately 20% of the animals in the air-control group survived; this occurred four months after the end of exposure. Mortality was similar to that observed in the unexposed controls. In the group that received the 3 mg/m^3 dose, one adenoma and one carcinoma were observed in 116 rats. No tumours were observed in the group that received the 16 mg/m^3 dose. In the group treated with 30 mg/m^3 slag wool dust, two pulmonary adenomas and one lung carcinoma were observed in 115 rats. In the control group, two lung adenomas were found in 126 rats. A positive control group was exposed to crocidolite asbestos (10 mg/m^3), with a geometric mean diameter of 0.28 μm determined by SEM and a geometric mean length of 4.1 μm determined by light microscopy. Exposure to crocidolite asbestos was terminated after 10 months

because of increased morbidity and mortality. The retained lung burden/mg dry lung tissue after 104 weeks was 759×10^3 WHO fibres and 41.1×10^3 fibres > 20 μm. Ten pulmonary adenomas, five pulmonary carcinomas and one mesothelioma were reported in 106 rats (McConnell *et al*., 1994).

3.5.2 *Intraperitoneal injection* (see Table 63)

(*a*) *Rat*

Groups of female Wistar rats, 15 weeks old, received two weekly intraperitoneal injections of 20 mg of one of two samples of Rheinstahl and Zimmermann (Germany) slag wool [chemical composition not given] in 2 mL saline. The Rheinstahl sample had a median fibre length of 26 μm and a median fibre diameter of 2.6 μm; the Zimmermann sample had a median fibre length of 14 μm and a median fibre diameter of 1.5 μm. The animals were observed for life; median survival times were 111, 107 and 101 weeks for the groups given coarser (Rheinstahl) and finer (Zimmermann) slag wool and for a control group treated with saline alone, respectively. Slight increases in the incidence of sarcomas, mesotheliomas and (rarely) carcinomas of the abdominal cavity were observed with the slag wool samples: 6/99 with the coarser sample (first tumour after 88 weeks) and 2/96 with the finer sample (first tumour after 67 weeks). No tumour occurred in any of the 48 control animals (Pott *et al*., 1987). [The Working Group noted that, in other studies in this laboratory, the historical incidence of abdominal tumours in animals treated with saline ranged from 0%–6.3%.]

Female Wistar rats [initial numbers not specified], weighing approximately 190 g, received either five weekly intraperitoneal injections of 30 mg slag wool [chemical composition not given] suspended in 2 mL saline or 2 mL saline alone (control group of 72 females). The median length of the fibres was 9.0 μm, the median diameter was 1.21 μm and the number of fibres injected (length > 5 μm, diameter < 2 μm, aspect ratio > 5/1) was 250×10^6. The lifespan of the animals was much reduced by an infectious disease of the lung in months 12 and 13 (the cause could not be diagnosed) which killed 8/36 rats in the treated group. The mean lifespan in both groups was 106 weeks. The mean lifespan for rats that developed tumours was 77 weeks. Macroscopic tumours were observed in 2/28 rats. In the control group, 22/72 animals died from the infection and two of the remaining 50 rats developed an abdominal tumour (Pott *et al*., 1991). [The Working Group found it difficult to interpret this study since the impact of the infection was unknown.]

Three groups of 40 female Wistar rats, 8–10 weeks old, received a single intraperitoneal injection of 20 or 50 mg or three weekly injections of 50 mg MMVF22 suspended in 2 mL saline. The median length of the fibres was 8.7 μm and the median diameter was 0.77 μm. A control group of 40 female Wistar rats received three intraperitoneal injections of 2 mL saline only. The length of the observation period was 130 weeks. The median survival time was 99 weeks in the group treated with the 20-mg dose, 102 weeks in the group treated with the single 50-mg dose and 95 weeks in the group that received

the three 50-mg doses. Necropsy was followed by a macroscopic investigation of the thoracic and abdominal areas for tumours and the organs were transferred into buffered formalin. Macroscopic tumours were investigated histopathologically. The incidence of mesotheliomas was 4/40 in the group given the 20-mg dose, 8/40 after treatment with the single 50-mg dose and 18/38 in the group that received the three 50-mg doses. In the control group treated with saline, no tumour was observed in 38 rats. Two groups of positive controls received an intraperitoneal injection of either 3.3 mg or 15 mg tremolite (Libby, Montana). The median fibre length was 3.4 μm and the median diameter was 0.29 μm; the numbers of fibres injected were 57×10^6 and 260×10^6, respectively. In the group that received 3.3 mg tremolite, 10/39 rats examined had developed mesotheliomas, and mesotheliomas were seen in 30/40 rats in the group treated with 15 mg tremolite (Pott *et al*., 1993; Davis *et al*., 1996b; Roller *et al*., 1996)

Groups of approximately 24 male Wistar rats, approximately 12 weeks of age, received two injections of a slag wool fibre (MMVF22) of a mass dose of 129.6 mg suspended in buffered saline. The target dose was 10^9 WHO fibres. Animals were kept for life or until they showed signs of debilitation. At autopsy, the peritoneal contents were examined macroscopically for the presence of peritoneal mesotheliomas. Tumours were diagnosed by the macroscopic presence of peritoneal mesotheliomas and by microscopy when the diagnosis was in doubt. In addition, specimens from the first six animals to develop mesotheliomas were taken for a histopathological examination. The median survival time was 658 days, and the median survival time of animals with mesothelioma was 695 days. An abdominal mesothelioma was diagnosed in 13/24 rats examined. In a group of positive controls, male Wistar rats were treated with 6.1 mg amosite fibres with the same target concentration of 10^9 WHO fibres. About 99% of the fibres had diameters below 0.95 μm. Mesotheliomas were observed in 22/24 of the rats in this group [no vehicle control group was included in this study] (Miller *et al*., 1999).

(*b*) *Hamster*

A group of 69 male Syrian golden hamsters, 100 days of age, was exposed by inhalation (nose-only) to slag wool dust [type of slag unspecified] (mass concentration, 7.8 mg/m^3; 15.2% respirable fibres; geometric mean diameter, 0.9 μm; geometric mean length, 22 μm; chamber concentration, 200 fibres/cm^3 with 76 fibres/cm^3 > 10 μm in length and ≤ 1.0 μm in diameter) for 6 h per day on five days per week for two years and then observed for life. Groups of 58 chamber and 112 room controls were included in the study. No tumour of the respiratory tract was observed in the treated animals or in room controls; one of 58 chamber controls had a bronchoalveolar tumour. There was no decrease in lifespan (about 660 days). In a group of 58 hamsters exposed to UICC crocidolite (3000 fibres/cm^3; 5% fibres ≥ 5 μm in length; mean, 3.1 ± 10.2 μm), no pulmonary tumour was seen (Smith *et al*., 1987). [The Working Group noted that the counting criteria for retained fibres were not stated and that the number of slag wool fibres retained was relatively low.]

3.5.3 *Intrapleural injection*

Rat: Groups of 48 SPF Sprague-Dawley rats [sex and age unspecified] received either a single intrapleural injection of 20 mg German slag wool [type of slag unspecified] or chrysotile in 0.5 mL saline. The dust samples used (and the size distributions of the fibres) were: German slag wool (67% < 5 μm in length; 42% < 0.6 μm in diameter), German slag wool after removal of resin (80% < 5 μm in length; 62% < 0.6 μm in diameter and UICC African chrysotile [sizes of fibres unspecified]. The animals were kept until natural death [survival times unspecified]. No tumour was observed in the group treated with slag wool or in a group of 24 controls treated with saline. Six mesotheliomas occurred in the group treated with chrysotile (Wagner *et al.*, 1984).

3.6 Refractory ceramic fibres

3.6.1 *Inhalation exposure* (see Table 64)

(*a*) *Rat*

A group of 48 SPF Wistar AF/HAN rats [sex and source unspecified], 12 weeks old, was exposed by whole-body inhalation [exposure chamber parameters (size, flow rate, temperature, humidity) and animal husbandry (single or group caging, environmental controls, light/dark cycle, basal diet and water supply) unspecified] to a target concentration of 10 mg/m^3 respirable dust from bulk fibrous ceramic aluminium silicate glass [chemical composition not given] for 7 h per day on five days per week for 12 months (cumulative exposure, 224 days). A group of 40 unexposed rats housed within the same laboratory unit served as controls [age, sex, source and treatment of controls (whether exposed to room or chamber air) unspecified]. Following cessation of exposure to the dust, all the remaining animals were kept until either their natural death or the termination of the experiment at 32 months. The mean concentration of respirable dust was 10.0 ± 4.8 mg/m^3 and that of total dust, 9.6 ± 8.4 mg/m^3. The analysis of respirable dust, by phase contrast optical microscopy (PCOM), showed that the animals were exposed to 95 WHO fibres/cm^3 (> 5 μm in length and < 3 μm in diameter; aspect ratio, > 3:1). Approximately 90% of the fibres were < 3 μm in length and < 0.3 μm in diameter and the ratio of particles (> 1 μm in diameter) to fibres (> 5 μm in length) was approximately 4:1. Survival times did not differ significantly between control and treated animals. Eight animals treated with dust (8/48, 17%) developed pulmonary neoplasms (one adenoma, three carcinomas and four malignant histiocytomas). Pulmonary tumours were not observed in any of the control animals. In addition to the tumours associated with the lung, eight benign and eight malignant tumours [unspecified], including one peritoneal mesothelioma were also found in the group treated with dust. The dust burden of ceramic aluminium silicate in the left lung was converted to whole lung values and ranged from 2.8–6.8 mg (Davis *et al.*, 1984).

Table 64. Studies of the carcinogenicity of refractory ceramic fibres in rats and hamsters

Fibre type	Route of administration	Strain	Sex	Exposure conc. (mg/m^3)	Exposure conc. (f/cm^3)	Exposure duration (h/d, d/wk), total exposure (weeks), observation (weeks)	No. of animals/ group	Lung tumours (adenoma and carcinoma)	Tumours (pleural or peritoneal)	Lung burden	Reference
Rat											
RCF (NS)	Whole-body inhalation	SPF Wistar AF/HAN	NS	10	95 WHO f/cm^3, 90% with D, < 3 μm; prt/fib, 4:1	(7, 5), 52, lifetime or 128	48	8/48 (17%); 0 in controls	0	–	Davis *et al*. (1984)
Fibrefrax®	Nose-only inhalation	Osborne-Mendel	F	10.8	88 f/cm^3 with L, > 10 μm; GMD, 0.9 μm; GML, 25 μm; prt/fib, 33:1	(6, 5), 104, lifetime	55	0/55	–	2.18×10^4 f/mg dry lung	Smith *et al*. (1987)
Kaolin-based RCF1	Nose-only inhalation	Fischer 344	M	30	187 WHO f/cm^3; GMD, ~0.8 μm; GML, 12.8–17.4 μm	(6, 5), 104, lifetime	140	16/123 (13%); controls, 2/130 (1.5%)	2/123 (1.6%); controls, 0	$2–7 \times 10^5$ WHO f/mg dry lung	Mast *et al*. (1995a)
Alumina-zirconia silica RCF2	Nose-only inhalation	Fischer 344	M	30	220 WHO f/cm^3; GMD, ~0.8 μm; GML, 12.8–17.4 μm	(6, 5), 104, lifetime	140	9/121 (7.4%); controls, 2/130 (1.5%)	3/121 (2.5%); controls, 0	$2–7 \times 10^5$ WHO f/mg dry lung	

Table 64 (contd)

Fibre type	Route of administration	Strain	Sex	Exposure conc. (mg/m^3)	Exposure conc. (f/cm^3)	Exposure duration (h/d, d/wk), total exposure (weeks), observation (weeks)	No. of animals/ group	Lung tumours (adenoma and carcinoma)	Tumours (pleural or peritoneal)	Lung burden	Reference
High-purity RCF3	Nose-only inhalation	Fischer 344	M	30	182 WHO f/cm^3; GMD, ~0.8 μm; GML, 12.8–17.4 μm	(6, 5), 104, lifetime	140	19/121 (10.7%); controls, 2/130 (1.5%)	2/121 (1.7%); controls, 0	$2–7 \times 10^5$ WHO f/mg dry lung	
After-service RCF4	Nose-only inhalation	Fischer 344	M	30	206 WHO f/cm^3; GMD, 1.22 μm; GML, 9.8 μm	(6, 5), 104, lifetime	140	4/118 (3.4%); controls, 2/130 (1.5%)	1/118 (0.8%); controls, 0	$2–7 \times 10^5$ WHO f/mg dry lung	
Kaolin-based RCF1	Nose-only inhalation	Fischer 344	M	3 9 16	26 75 120 WHO f/cm^3; GMD, 0.8 μm; GML, 14 μm	(6, 5), 104, lifetime	140	2 (1.6%) 5 (3.9%) 2 (1.6%); controls, 1 (0.8%)	0 1 (0.8%) 0; controls, 0	4.3×10^4 NS 22.1×10^4 WHO f/mg dry lung	Mast *et al.* (1995b)
Fibrefrax®	Intra-tracheal instillation	Osborne-Mendel	F	5 × 2 mg (weekly)	(Elutriated from inhalation chamber)	lifetime	22	0	–		Smith *et al.* (1987)

Table 64 (contd)

Fibre type	Route of adminis-tration	Strain	Sex	Exposure conc. (mg/m^3)	Exposure conc. (f/cm^3)	Exposure duration (h/d, d/wk), total exposure (weeks), observation (weeks)	No. of animals/ group	Lung tumours (adenoma and carcinoma)	Tumours (pleural or peritoneal)	Lung burden	Reference
Refractory ceramic fibre (NS)	Intrapleural injection	SPF Wistar	M/F	20 mg in 0.4 mL	(Ball mill grinding)	lifetime	31	–	3/31 (9.7%), pleural meso	–	Wagner *et al*. (1973)
Kaolin (fibre A)/ alumina and silica (fibre B)	Intrapleural injection	Alpk:AP (Wistar-derived)	M/F	20 mg in 0.2 mL	(Ball mill grinding and sieving)	lifetime	24 M/ 24 F	–	Fibre A, 0 Fibre B: 1/48, (2.1%, pleural meso); 2/48 (4.1%, peritoneal meso)	–	Pigott & Ishmael (1992)
High Duty™ grade alumino-silicate, vitreous or devitrified	Intrapleural injection	Wistar-Porton	M	20 mg in 0.4 mL	NS	lifetime	19	–	0	–	Carthew *et al*. (1995)
Refractory ceramic fibre (NS)	Intraperi-toneal injection	Wistar AF/HAN	NS	25 mg in 2 mL	NS; L, 90% < 3 μm; D, < 0.3 μm	NS	32	–	3/32 (9.4%) peritoneal tumours	–	Davis *et al*. (1984)

Table 64 (contd)

Fibre type	Route of administration	Strain	Sex	Exposure conc. (mg/m^3)	Exposure conc. (f/cm^3)	Exposure duration (h/d, d/wk), total exposure (weeks), observation (weeks)	No. of animals/ group	Lung tumours (adenoma and carcinoma)	Tumours (pleural or peritoneal)	Lung burden	Reference
Fibrefrax®	Intraperitoneal injection	Wistar WU/ Kißlegg	F	45 mg in 2mL	L, 8.3 μm; D, 0.91 μm	28 months after injection	~50	–	32/47 (68%)	–	Pott *et al.* (1987, 1989)
Manville				75 mg in 2 mL	L, 6.9 μm; D, 1.1 μm				12/54 (22%) TiO_2 control, 5/53 (9.4%)		
Fibrefrax®	Intraperitoneal injection	Osborne-Mendel	F	25 mg in 0.5 mL	(Elutriated from inhalation chamber)	lifetime	25	–	19/23 (83%); control, 0/25	–	Smith *et al.* (1987)
RCF1	Intraperitonealinjection	Charles River Wistar	M	110 mg in 2 mL	228×10^6 f > 10 μm	lifetime	18–24	–	21/24 (88%)	–	Miller *et al.* (1999)
RCF2				188 mg in 2 mL	320×10^6 f > 10 μm				13/18 (72%)		
RCF4				90 mg in 2 mL	81×10^6 f > 10 μm or 10^9 f > 5 μm				0/22		

Table 64 (contd)

Fibre type	Route of adminis-tration	Strain	Sex	Exposure conc. (mg/m^3)	Exposure conc. (f/cm^3)	Exposure duration (h/d, d/wk), total exposure (weeks), observation (weeks)	No. of animals/ group	Lung tumours (adenoma and carcinoma)	Tumours (pleural or peritoneal)	Lung burden	Reference
Hamster											
Fibrefrax®	Nose-only inhalation	Syrian golden	M	10.8	200 f/cm^3, GMD, 0.9 μm; GML, 25 μm; prt/fib, 33:1	(6, 5), 104, lifetime	70	0; control, 1/58	1/70; control, 0	0.86×10^4 f/mg dry lung	Smith *et al.* (1987)
RCF1	Nose-only inhalation	Syrian golden	M	30	215 WHO f/cm^3; GMD, 0.78 μm; GML, 15.9 μm	(6, 5), 78, lifetime	140	0	42/102 (41%); control, 0	1.59×10^5 f/mg dry lung	McConnell *et al.* (1995)
Fibrefrax®	Intra-tracheal instillation	Syrian golden	M	5 × 2 mg (weekly)	(Elutriated from inhalation chamber)	lifetime	25	0	–	–	Smith *et al.* (1987)
Fibrefrax®	Intraperi-toneal injection	Syrian golden	F	25 mg in 0.5 mL	(Elutriated from inhalation chamber)	lifetime	56	0	7/36; control, 0		Smith *et al.* (1987)

NS, not specified; prt/fib, ratio of particles to fibres; f., fibre; RCF, refractory ceramic fibres; GMD, geometric mean diameter; GML, geometric mean length; lifetime, until survival rate is ≤ 20%; D, diameter; L, length; M, male; F, female; meso, mesothelioma

A group of 55 female Osborne-Mendel rats, 100 days of age, was exposed by nose-only inhalation to an aerosol of a refractory ceramic fibre (Fibrefrax®) [chemical composition not given] at a mass concentration of 10.8 mg/m^3 for 6 h per day on five days per week for two years and then observed for life. A group of 60 controls was exposed to filtered air by nose-only inhalation and 125 room cage controls were kept. The aerosol fibres had a geometric mean diameter of 0.9 μm and a geometric mean length of 25 μm; it contained approximately 88 fibres/cm^3 > 10 μm in length having diameters ≤ 1.0 μm and a particulate to fibre ratio of 33:1. [No information was available on animal respirability.] The fibre content of the lungs was assessed in only a small sample of animals [number unspecified]. Exposure to refractory ceramic fibres had no effect on overall health or survival of the animals. The lung burden of fibres was $2.18 \pm 0.99 \times 10^4$ fibres/mg dry lung weight [particulates and size distribution of fibres unspecified]. [The lung burden of retained fibres was relatively low.] No pulmonary tumours were observed in any of the treated (0/55) or control (0/60) rats (Smith *et al*., 1987).

Six groups of 140 male weanling Fischer 344 rats were exposed to refractory ceramic fibres by nose-only inhalation. A control group exposed to high-efficiency particulate air (HEPA)-filtered air was included. Four groups of animals were exposed to 30 mg/m^3 of one of three types (kaolin-based RCF1; alumina zirconia silica — AZS RCF2, or high-purity RCF 3) of size-separated refractory ceramic fibres or to an after-service heat-treated (1316 °C for 24 h to simulate after-service material) kaolin-based fibre containing approximately 27% crystalline silica in the form of cristobalite) (RCF4) for 6 h per day on five days per week for 24 months. An additional group of 80 animals was exposed to 10 mg/m^3 chrysotile asbestos. Following the 24-month exposure period, the rats were kept for lifetime observation (until approximately 20% survival at 30 months), at which time they were killed. Aerosol exposure concentrations of 30 mg/m^3 for the refractory ceramic fibres and 10 mg/m^3 for chrysotile were achieved and maintained during the study. The counts of WHO fibres at these concentrations corresponded to 187 ± 53, 220 ± 52, 182 ± 66, 206 ± 48 and 1.06×10^4 fibres/cm^3 for RCF1, RCF2, RCF3, RCF4 and chrysotile, respectively. The GMD of RCF1, RCF2 and RCF3 in the aerosol ranged from 0.82–0.88 μm and the GML ranged from 12.8–17.4 μm. The RCF4 was somewhat thicker (GMD, 1.22 μm) and shorter (GML, 9.8 μm). The corresponding dimensions for chrysotile were 0.08 μm and 1.2 μm, respectively. [In view of the known association between fibre length and carcinogenic potential, the differences in mean length between the refractory ceramic fibres and chrysotile make direct comparison difficult.] The particulate (< 3 μm diameter) to fibre ratio was reported to range between 1.02:1 and 1.88:1 for the refractory ceramic fibres. [This estimate has been revised in Maxim *et al.* (1997), Mast *et al*. (2000a,b) and Bellmann *et al*. (2001).] Lung burdens at 24 months ranged from approximately 2×10^5 to 7×10^5 WHO fibres/mg dry lung tissue. The numbers of exposure-related pulmonary neoplasms (bronchoalveolar adenomas and carcinomas) were significantly increased in all the groups exposed to fibres (except that exposed to RCF4) (RCF1, 16/123 (13%);

RCF2, 9/121 (7.4%); RCF3, 19/121 (15.7%); RCF4, 4/118 (3.4%); the number in the group that received chrysotile was 13/69 (19%) compared with 2/130 (1.5%) in the untreated chamber controls. A few mesotheliomas were observed in each of the groups exposed to fibres (RCF1, 2/123 (1.6%); RCF2, 3/121 (2.5%); RCF3, 2/121 (1.7%); RCF4, 1/118 (0.8%); chrysotile, 1/69 (1.4%)), compared with 0/130 in the untreated controls (Mast *et al*., 1995a).

The study by Mast *et al.* (1995a) was followed up with a multiple-dose study of the kaolin-based refractory ceramic fibre (RCF1) conducted in the same laboratory using the identical lot of RCF1, animal source and experimental design. Four groups of 140 weanling Fischer 344 rats were exposed by nose-only inhalation to HEPA-filtered air (chamber controls) or to 3, 9 or 16 mg/m^3 of RCF1 (corresponding to 26, 75 and 120 WHO fibres/cm^3 and a reported particulate to fibre ratio of 0.9–1.5:1 [as noted above, this has since been recalculated]; GMD, 0.8 μm; GML, 14 μm) for 6 h per day on five days per week for 24 months. Following the 24-month period of exposure, the rats were kept for lifetime observation (until approximately 20% survival), at which time they were killed (30 months). The lung fibre burden (WHO fibres/mg dry lung weight) at 24 months ranged from 4.3×10^4 in the animals treated with 3 mg/m^3 to 22.1×10^4 in the group that received 16 mg/m^3. The numbers of pulmonary neoplasms (adenoma and carcinoma combined) showed no statistically significant increase at any of the concentrations of RCF1 tested when compared with control animals and were within the range reported as typical in the male Fischer 344 rat. At 30 months, when the animals were killed, a single very small mesothelioma was seen in one rat that had been exposed to 9 mg/m^3 RCF1 (Mast *et al*., 1995b).

[The Working Group noted that the greater particulate fraction of RCF1 (than other RCFs) could have influenced the development of inflammation and subsequent carcinogenic response in the chronic inhalation studies of RCF1. The extent of this influence is difficult to assess quantitatively (Yu *et al*., 1994; Hesterberg *et al.*, 1995a,b; Mast *et al*., 1995a; Gelzleichter *et al*., 1996a; Bernstein *et al*., 1997; Creutzenberg *et al*., 1997; Maxim *et al*., 1997; Brown, 2000; Brown *et al.*, 2000a; Mast *et al*., 2000a,b; Bellmann *et al*., 2001).]

(*b*) *Hamster*

A group of 70 male Syrian golden hamsters, 100 days old, was exposed by nose-only inhalation to refractory ceramic fibres (Fibrefrax®) [chemical composition not given] at a mass concentration of 10.8 mg/m^3 for 6 h per day on five days per week for 24 months. The control groups consisted of 58 chamber controls exposed to air and 112 room cage controls. A group of 58 hamsters was exposed to UICC crocidolite (7 mg/m^3; 3000 fibres/cm^3; 95% ≤ 5 μm in length; 90 fibres/cm^3 > 10 μm long). The exposure aerosol fibres had a GMD of 0.9 μm and a GML of 22 μm. Assuming a concentration of 200 fibres/cm^3, the aerosol was calculated to contain approximately 88 fibres/cm^3 > 10 μm in length and ≤ 1.0 μm in diameter. A respirable mass fraction of 35 ± 7% (percentage weight) was estimated. The particle-to-fibre ratio was reported to be 33:1.

The fibre content of the lungs (0.86×10^4 fibres/mg dry lung) was assessed in a small sample of animals [actual number of animals and time of sampling unspecified]. At 10 months of treatment, one hamster exposed to the refractory ceramic fibre (1/70) developed a mesothelioma. A chamber control hamster (1/58) had a secretory bronchoalveolar tumour. No pulmonary tumours were seen in the hamsters exposed to crocidolite although there was a significant incidence of bronchoalveolar hyperaplasia (11/58) and interstitial fibrosis (14/58) (Smith *et al*., 1987).

Two groups of 140 weanling male Syrian golden hamsters were exposed by nose-only inhalation to either HEPA-filtered air (chamber controls) or to 30 mg/m^3 (215 WHO fibres/cm^3; GMD, 0.78 μm; GML, 15.9 μm) size-selected refractory ceramic fibres (RCF1) for 6 h per day on five days per week for 18 months. (This study was conducted with the identical lot of refractory ceramic fibres and in the same laboratory as the studies reported by Mast *et al*., 1995a,b.) Once the period of exposure had ended the hamsters were kept until approximately 20% survival and then killed (20 months). A group of 80 hamsters exposed to 10 mg/m^3 (3.0×10^3 WHO fibres/cm^3; GMD, 0.08 μm; GML, 0.98 μm) NIEHS chrysotile acted as the positive control. Survival was unaffected by exposure to RCF1 but was significantly reduced by chrysotile. Lung burden of fibres (WHO fibres/mg dry lung) at 18 months was 1.59×10^5 and 16.5×10^5 in the groups treated with RCF1 and chrysotile, respectively. No pulmonary neoplasms occurred in any experimental group. Mesotheliomas developed in 42/102 (41%) of the animals exposed to RCF1, but not in any other group. While the first mesothelioma was found at 10 months of treatment, most (24/42) were not seen until after 18 months. Many were readily visible at necropsy (57%) and involved both the visceral and parietal pleura (McConnell *et al*., 1995). [The Working Group noted that all the hamsters had been treated with tetracycline for an intestinal infection that they developed during the study.]

3.6.2 *Intratracheal instillation*

(*a*) *Rat*

A group of 22 female Osborne-Mendel rats, 100 days old, received five weekly intratracheal instillations of 2 mg refractory ceramic fibres (Fibrefrax®) [chemical composition not given], which had been elutriated from the chambers of a nose-only inhalation study. The fibres in the inhalation aerosol had a GMD of 0.9 μm, a GML of 25 μm, contained approximately 88 fibres/cm^3 with lengths > 10 μm and diameters ≤ 1.0 μm, and a particulate-to-fibre ratio of 33:1. [The length and diameter distributions and particulate count of the instilled material were unspecified.] A group of 25 control females was treated with saline. The treated animals and controls were then kept for their natural lifespan. Treatment with the refractory ceramic fibre had no effect on median average lifespan (698 days) compared with controls treated with saline (688 days). No pulmonary tumours developed in any of the rats treated with refractory ceramic fibres or with saline. Of 25 rats treated similarly with UICC crocidolite [size

distribution of elutriated material unspecified], 2/25 (8%) developed bronchoalveolar tumours (Smith *et al*., 1987).

(*b*) *Hamster*

A group of 25 male Syrian golden hamsters, 100 days old, received five weekly intratracheal instillations of 2 mg refractory ceramic fibres (Fibrefrax®) [chemical composition not given], which had been elutriated from the chambers of a nose-only inhalation study. The fibres in the inhalation aerosol had a GMD of 0.9 μm, a GML of 25 μm, contained approximately 88 fibres/cm^3 with lengths > 10 μm and diameters ≤ 1.0 μm, and a particulate-to-fibre ratio of 33:1. [The length and diameter distributions and particulate count of the instilled material were unspecified.] A group of 24 males treated with 0.2 mL saline acted as controls. The treated animals and controls were then kept for their natural lifespan. The median average lifespan of hamsters treated with refractory ceramic fibres (446 days) was significantly shorter than that of the controls treated with saline (567 days). No pulmonary tumours were seen either in any hamster treated with refractory ceramic fibres or in any control animal treated with saline. Of 27 hamsters treated similarly with UICC crocidolite [size distribution of elutriated material unspecified], 20/27 (74%) developed bronchoalveolar tumours. Of these primary tumours, 13 were benign and seven were malignant (Smith *et al*., 1987).

3.6.3 *Intrapleural injection*

Rat: Groups of 31–36 SPF Wistar rats (twice as many males as females) [number of each sex unspecified], 13 weeks old, received a single intrapleural injection, in 0.4 mL sterile saline, containing 20 mg suspended solids of numerous different fibres and dusts including ceramic aluminium silicate fibres [chemical composition not given] prepared by grinding. The mean survival time was 736 days, which was similar to that in controls (728 days) being used at the same time in other experiments. Thirty-one of the animals injected with refractory ceramic fibres were examined by microscopy and three were found to have pleural mesotheliomas (9.7%). The first appeared 743 days after treatment. In this experiment, pleural mesotheliomas were observed in 23/36 (64%) of the rats injected with SFA chrysotile (20 mg); the first tumour appeared 325 days after injection [non-neoplastic disease unspecified] (Wagner *et al*., 1973).

Groups of 24 male and 24 female Alpk:AP (Wistar-derived) rats, eight weeks old, received a single intrapleural injection of 20 mg suspended solids of aluminosilicate fibres in 0.2 mL saline (fibre A made from kaolin and fibre B from alumina and silica, prepared by grinding and sieving) [source and purity unspecified] (treatment was arranged in replicates and took place over a period of five weeks) and animals were observed for life or until 85% mortality when the survivors were killed [months of total exposure at termination unspecified]. The fibre dimensions were as follows: fibre A:

diameter ≤ 3 μm, 66%; length ≥ 10 μm, 80%; fibre B: diameter ≤ 3 μm, 92%; length ≥ 10 μm, 46%). Control animals received an equivalent injection of 0.2 mL sterile saline. A similar group of animals was injected with UICC chrysotile A [length and diameter distribution unspecified] at the same concentration of suspended solids. At 24 months, survival was not affected by the treatment (control, 79%; fibre A, 96%; fibre B, 83%; chrysotile, 100%) and was not significantly different between groups at termination (control, 29%; fibre A, 41%; fibre B, 29%; chrysotile, 29%). No mesotheliomas were observed in animals treated with fibre A. Treatment-related neoplastic disease was limited to the development of pleural (1/48, 2.1%) or peritoneal mesotheliomas in (2/48, 4.1%) of the rats treated with fibre B (the peritoneal mesotheliomas were the result of a partial deposition of the dose in the peritoneum) and 7/48 (14.5%) pleural mesotheliomas in rats treated with chrysotile A (Pigott & Ishmael, 1992).

Three groups of 19, 21 and 24 male Wistar-Porton rats, weighing 200 g, received a single intrapleural injection of 0.4 mL saline containing 20 mg suspended solids of High Duty™ grade aluminosilicate fibres prepared from commercially available fibre blanket [length, diameter, chemical composition and quantity of non-fibrous particulates unspecified] or one of two samples of fibres that had previously been devitrified at 1400 °C or 1200 °C for 14 days. A control group of 30 rats received intrapleural saline only. Following treatment, the rats were observed for life. There was no statistically significant difference in mean survival between control and treated animals and cumulative mortality curves for control and treated groups were very similar. The study was apparently [read from a graph] terminated 900 days after treatment [actual time of termination unspecified]. No pleural mesotheliomas were observed in any of the three groups exposed to fibres or in the control group (Carthew *et al*., 1995).

3.6.4 *Intraperitoneal injection*

(*a*) *Rat*

A group of 32 Wistar AF/HAN rats [sex and age unspecified] received a single 2 mL intraperitoneal injection of 25 mg refractory ceramic fibres [chemical composition not given] suspended in buffered saline [treatment of the control group unspecified]. The refractory ceramic fibres were collected from inhalation exposure chambers in use at that time for an ongoing animal study and the material collected was as similar as possible [length, diameter, particle mass and size of refractory ceramic fibres used for injection unspecified] to that entering the inhalation chamber (approximately 90% of the fibres were < 3 μm in length and < 0.3 μm in diameter and the ratio of particles (> 1 μm in diameter and > 5 μm in length) to fibres was approximately 4:1). At the end of the study [duration unspecified], 3/32 (9.4%) of the treated and 2/29 of the control rats had developed peritoneal tumours [unspecified] (Davis *et al*., 1984).

In two preliminary studies, groups of approximately 50 female Wistar WU/Kiβlegg rats, 8 weeks old, received intraperitoneal injections (once a week for five weeks) of one of the following: Fibrefrax® refractory ceramic fibre wool (total dose, 45 mg; fibre

length, 8.3 μm; fibre diameter, 0.91 μm) [chemical composition not given], Manville refractory ceramic fibre wool (total dose, 75 mg; fibre length, 6.9 μm; fibre diameter, 1.1 μm) [chemical composition not given] or titanium dioxide (total dose, 100 mg) [P25 from Degussa, Germany] suspended in 2 mL saline. A group of 102 animals served as controls and received 2 mL of saline once a week for five weeks. Necropsy at 28 months after injection showed the incidence of macroscopic abdominal tumours was 32/47 (68%) in the first group treated with Fibrefrax® (first tumour death at 30 weeks), 12/54 (22%) in the second group treated with Manville (first tumour death at 60 weeks), 5/53 (9.4%) in the group treated with titanium dioxide (first tumour death at 38 weeks) and 2/102 (2%) in the control group treated with saline (first tumour death at 93 weeks). The median survival time in both the groups of animals treated with refractory ceramic fibres was reduced (51 and 91 weeks, respectively) compared with that in the controls treated with saline (111 weeks) (Pott *et al*., 1987, 1989). [The Working Group noted that these two publications presented essentially the same data, with minor discrepancies. It is unclear whether any histopathological examination was performed.]

A group of 25 female Osborne-Mendel rats, 100 days old, received a single intraperitoneal injection in 0.5 mL saline of 25 mg refractory ceramic fibre (Fibrefrax®) [chemical composition not given], which had been elutriated from the chambers of a nose-only inhalation study. The fibres in the inhalation aerosol had a GMD of 0.9 μm and a GML of 25 μm. [The length and diameter distributions and particulate counts for the elutriated material injected were unspecified.] A group of 25 females treated with 0.5 mL saline alone served as controls. Treated and control animals were then kept for their natural lifespan. The mean lifespan in rats treated with refractory ceramic fibres was significantly shorter (480 ± 32 days) that that in controls (744 ± 28 days). Abdominal mesotheliomas [histological description and type unspecified] developed in 19/23 (included a single fibrosarcoma) of the animals injected with refractory ceramic fibres and in none of the controls (0/25) (Smith *et al*., 1987).

Groups of 18–24 male Charles River Wistar rats, approximately 12 weeks old, were injected intraperitoneally on two consecutive days with either RCF1 (total dose, 110 mg; 228×10^6 fibres > 10 μm length and < 0.95 μm diameter), RCF2 (total dose, 188 mg; 320×10^6 fibres > 10 μm length and < 0.95 μm diameter) or RCF4 (total dose, 90 mg; 81×10^6 fibres > 10 μm length and < 0.95 μm diameter) suspended in 2 mL sterile saline. The refractory ceramic fibres were obtained from the same original stock as those used in the earlier inhalation studies by Mast *et al*. (1995a,b) and McConnell *et al*. (1995). The mass of refractory ceramic fibres required to deliver a target dose of 10^9 fibres > 5 μm in length was calculated from optical fibre-sizing data. No saline-injected controls were used. Following injection, animals were kept for life or until they became moribund and were killed. By macroscopic examination, the presence of mesotheliomas (responsible for the death of the animal) was found in 21/24 of the rats treated with RCF1, 13/18 of those treated with RCF2 and 0/22 rats injected with RCF4. The median survival time was lower after treatment with the RCF1 (337 days) and RCF2 (376 days) compared with RCF 4 (725 days) (Miller *et al*., 1999).

(*b*) *Hamster*

Two groups of female Syrian golden hamsters (56 in total), 100 days of age, received a single intraperitoneal injection, in 0.5 mL saline of 25 mg refractory ceramic fibres (Fibrefrax®) [chemical composition not given], which had been elutriated from the chambers of a nose-only inhalation study. The fibres in the inhalation aerosol had a GMD of 0.9 μm and a GML of 25 μm. The length and diameter distributions and particulate count of the elutriated material injected were unspecified.] A group of 25 females treated with 0.5 mL saline served as controls. Treated animals and controls were kept for life. After intraperitoneal injection with refractory ceramic fibres, 21/36 hamsters in the first group and 15/36 in the second group died within 30 days from acute haemorrhagic peritonitis and vascular collapse. The mean lifespans of the surviving hamsters were significantly reduced (462 and 489 days, in the first and second group, respectively) compared with that of control animals injected with saline (560 days). Abdominal mesotheliomas were found in 2/15 hamsters in the first group and in 5/21 hamsters in the second group [histological description and type unspecified]. No tumours were found in the controls (Smith *et al*., 1987).

3.7 Newly developed fibres

3.7.1 *Inhalation exposure*

X-607 (alkaline earth silicate) wool

Rat: In a chronic inhalation study, 140 male Fischer 344 rats, 9–11 weeks of age, were exposed to Johns Manville X-607 and 80 male Fischer 344 rats were exposed to NIEHS medium-length chrysotile asbestos. The X-607 wool contains a relatively high proportion of calcium oxide (38%). Rats were exposed by nose-only inhalation for 6 h per day on five days per week for periods of up to two years. The concentration of X-607 fibres in the aerosol was 30 mg/m^3; there were 174 ± 72 × 10^6 WHO fibres/m^3 and 47 ± 23 × 10^6 fibres > 20 μm/m^3, with geometric mean fibre dimensions of 11 μm × 0.9 μm. For chrysotile the concentration was 10 mg/m^3, with 10 600 × 10^6 WHO fibres/m^3 and no detectable fibres > 20 μm; the geometric mean fibre dimensions were 1.2 μm × 0.3 μm. After 104 weeks of exposure to X 607, the lung burden was 58 × 10^6 WHO fibres/lung and 1 × 10^6 fibres > 20 μm/lung. After 104 weeks of exposure to chrysotile, there were 1600 × 10^6 WHO fibres/lung and no fibres > 20 μm were detected. Animals exposed to X-607 did not develop any lung fibrosis. The incidence of lung tumours in rats treated with X-607 was one adenoma and one carcinoma in 121 animals at risk; this was not significantly different from the incidence in controls kept in air which was two adenomas in 130 rats. The tumour incidence after exposure to chrysotile asbestos was six lung adenomas, six lung carcinomas and one mesothelioma in 69 animals at risk (Hesterberg *et al.*, 1998a).

High-alumina, low-silica wool (HT) fibre (MMVF34)

A group of 140 male Fischer 344 rats, 9–10 weeks of age, was exposed by nose-only inhalation for 6 h per day on five days per week for 104 weeks to 30 mg/m^3 high-alumina, low-silica wool (HT) fibre (MMVF34). This fibre, commercialized in 1995, is characterized by a relatively high content of aluminium and a relatively low content of silica compared with the older MMVF21. The gravimetric concentration was selected to obtain a fibre concentration of at least 250 × 10^6 WHO fibres/m^3 throughout the exposure period to enable comparison with previous studies carried out on MMVFs in which 30 mg/m^3 was the highest dose. The actual values of the fibre concentration were 291 × 10^6 WHO fibres/m^3 and 85 × 10^6 fibres > 20 μm/m^3. The geometric mean of the fibre diameter was 0.87 μm and the geometric mean length was 10.8 μm. The retained lung burden after 24 months was 60.2 × 10^6 WHO fibres and 3.1 × 10^6 fibres > 20 μm. A subsequent period of no exposure continued until approximately 20% survival was reached in the control group kept in air at approximately 28 months. Mortality after treatment with HT fibres was comparable with mortality of unexposed controls. The results of the comparative study showed a marked difference in the pathogenicity of the MMVF21 and MMVF34 in terms of their fibrogenic potential: MMVF21 caused pulmonary fibrosis, but MMVF34 did not. In 107 rats exposed to MMVF34, no carcinoma and five adenomas were observed. In the 107 rats in the control group, one carcinoma and three adenomas were found (Kamstrup *et al*., 1998, 2001).

3.7.2 *Intraperitoneal injection*

High-alumina, low-silica wool (HT) fibre

Rat: A group of 50 female Wistar rats (body weight approximately 200–230 g), aged 10–12 weeks, received a dose of 2.1 × 10^9 WHO HT fibres (0.6 × 10^9 fibres > 20 μm) suspended in 2 mL saline, by intraperitoneal injection. The geometric mean diameter of fibres was 0.65 μm and the geometric mean length was 10.7 μm. Fifty female Wistar rats treated with 2 mL saline served as negative controls. After treatment, animals were kept until survival in one group fell below 20%. At this time, all animals were killed. All animals were necropsied; any gross abnormalities observed during necropsy were examined histopathologically. No induction of mesothelioma was observed in either the group treated with HT fibres or in the control group. These results were compared with those of a study in which the fibre D6 (MMVF21; median diameter, 0.8 μm; median length, 8.5 μm; 1 × 10^9 WHO fibres; 0.2 × 10^9 fibres > 20 μm) had been tested by the same laboratory. In that study, mesotheliomas were observed in 32 of 57 treated rats and no mesothelioma in the corresponding control group of 91 rats (Kamstrup *et al*., 2002). [The Working Group noted that these two injection studies were not contemporaneous.]

A and C wools

Rat: Groups of 51 female Wistar rats, 8–9 weeks of age, were given intraperitoneal injections containing 0.7–35 mg of either glass wool A or glass wool C. The doses, expressed as WHO fibres/rat × 10^6, ranged from 9.2–630. Other groups of 51 rats were injected with either crocidolite asbestos (maximum dose, 0.5 mg or 110 × 10^6 WHO fibres) or saline. Rats were kept for 130 weeks, then killed and examined for abdominal tumours. None of the groups exposed to either fibre A or fibre C had a tumour incidence greater than 10%. The tumour incidences were (from lowest dose to highest dose): groups treated with fibre A, 3/51, 1/51, 1/51 and 3/51; groups treated with fibre C, 5/51, 4/51, 1/51 and 1/51; controls treated with saline, 0/51. The tumour incidence in the group exposed to 0.5 mg crocidolite was 25/51 (Lambré *et al*., 1998).

F, G and H wools

[F, G and H wools have not been commercialized, but they are included here because data on biopersistence and chronic effects are available for these fibre types. For many of the newly developed fibres which are commercially available in the European Union, only biopersistence data are available.]

Rat: Groups of 51 female Wistar rats, 8–9 weeks old, received intraperitoneal injections containing 1.1–55 mg of three types of rock (stone) wool fibres (F, G and H) [the Working Group noted that, according to the definition given in section 1, these fibres are not a rock (stone) wool] (all calcium-modified silicates) suspended in saline. The doses administered, the corresponding numbers of fibres, and geometric mean lengths and diameters were reported, together with tumour incidence (all fibres were defined as having length > 5 μm, diameter < 2 μm, length:diameter > 5 μm). Doses expressed as WHO fibres × 10^6/rat ranged 5.2–550. Other groups of 51 rats were injected with either crocidolite asbestos (maximum dose, 0.5 mg or 110 × 10^6 WHO fibres) or saline. Rats were kept for 130 weeks then killed and examined for abdominal tumours. The incidences of peritoneal tumours (from lowest dose to highest dose) were: 3/51, 1/51 and 3/51 in rats treated with F wool; 2/51, 1/51 and 2/51 in rats treated with G wool; 3/51, 1/51 and 9/51 (dose of 55 mg or 260 × 10^6 WHO fibres for the latter incidence) in rats treated with H wool; and 4/51, 10/51 and 25/51 in rats treated with crocidolite. In a control group of 102 females treated with saline, no mesothelioma was reported (Lambré *et al*., 1998).