3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Data used in the evaluation are summarized in Table 41, at the end of this section (p. 106).

Glasswool

(*a*) *Inhalation*

Rat: Groups of 46 young adult male Sprague-Dawley rats were exposed by inhalation to fibres >5 μm in length, at concentrations of 0.7×10^6/l [700 fibres/cm³] ball-milled fibreglass (24.2% fibres with diameter <3 μm) or 3.1×10^6/l [3100 fibres/cm³] UICC amosite asbestos (61.9% fibres 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 46 unexposed animals served as controls. Groups of four to ten animals per exposure group were killed at 20 days, 50 days, 90 days, six months, 12 months and 18 months, and the remainder at 24 months. No pulmonary tumour was observed in animals that were killed or died prior to the end of the study. Nonsignificant [$p > 0.05$] increases in the number of bronchoalveolar tumours were observed in 2/11 (adenomas) fibreglass-treated animals and 3/11 (two adenomas, one carcinoma) amosite-treated animals compared with 0/13 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 available for evaluation.]

Groups of 24 male and 24 female Wistar IOPS AF/Han rats, eight to nine weeks old, were exposed by inhalation to dust concentrations of 5 mg/m³ (respirable particles) French glasswool (42% of fibres <10 μm in length, 69% <1 μm in diameter), US glasswool (JM 100; 97% respirable fibres <5 μm in length, 43% total fibres <0.1 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 different periods of observation (for seven, 12 and 16 months after exposure for animals exposed for 12 months; four months after exposure for those exposed for 24 months). One relatively undifferentiated epidermoid carcinoma of the lung was observed in 1/45 rats treated with French glasswool, and nine pulmonary tumours were seen

among 47 rats treated with chrysotile. No tumour was found among 48 rats treated with US glasswool or among 47 control rats not exposed to dusts (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 50 male and 50 female SPF Fischer 344 rats, seven to eight weeks old, were exposed by inhalation to approximately 10 mg/m^3 respirable dust [size unspecified] of US 'microfibre' glasswool (JM 100) 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 three to five animals per group were killed at three, 12 and 24 months. Two studies of similar design, A and B, using animals from the same source were conducted at the same time in different laboratories; study B was a part of the study by Wagner *et al.* (1984) which is reviewed in detail below. The authors reported that cumulative exposure to chrysotile was approximately the same in both studies, but cumulative exposure to glasswool was significantly less in study A. No pulmonary neoplasm was observed at three or 12 months. Two of four chrysotile-exposed male rats in study A killed at 24 months had bronchoalveolar tumours (one adenoma, one adenocarcinoma); no tumour was found in animals at 24 months in study B. The incidences of pulmonary tumours (adenomas and adenocarcinomas combined) in the rats in study A observed for life were: chrysotile —males, 9/29; females, 2/27; glasswool — males, 0/28; females, 0/27; control — males, 3/27; females, 0/26. The rates in study B were: chrysotile — males, 7/24; females, 5/24; glasswool — males, 1/24; females, 0/24; control — males, 0/24; females, 0/24 (McConnell *et al.*, 1984). [The Working Group noted that the fibre dimensions used in study A were not reported.]

Groups of 48 SPF Fischer rats [equal numbers of males and females (McConnell *et al.*, 1984); age unspecified] were exposed by inhalation to dust concentrations of approximately 10 mg/m^3 glasswool 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 longer than 5 μm) were: glasswool with resin coating ([source unspecified] 72% fibres <20 μm in length, 52% ≤1 μm in diameter), glasswool without resin coating (58% ≤20 μm in length, 47% ≤1 μm in diameter), US glasswool (JM 100; 93% ≤20 μm in length, 97% ≤1 μm in diameter) and UICC Canadian chrysotile (39% >10 μm in length, 29% >0.5 μm in diameter). Six rats were removed from each group at the end of exposure to study dust retention, and a similar number of animals was sacrificed one year later for the same purpose. The remainder were held until natural death [survival times not reported]. During the period 500–1000 days after the start of exposure, one pulmonary adenocarcinoma occurred in 48 rats exposed to glasswool with resin and one in the 48 rats treated with US glasswool. One benign adenoma was observed in 47 rats exposed to glasswool without resin, and 11 adenocarcinomas and one benign adenoma with some malignant features occurred in 48 rats treated with chrysotile. No lung tumour was observed 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 52–61 female, 100-day-old Osborne-Mendel rats were examined after exposure by inhalation (nose only) to various types of glasswool dusts for 6 h per day on five days per week for two years and were then observed for life. Groups of 59 chamber and 125 room controls were available. The types of glass fibres were classified according to geometric mean diameter, as follows: (1) glasswool with no binder — diameter, 0.4 μm; mass concentration, 2.4 mg/m^3; 81% respirable — 3000 fibres/cm^3 with 530 fibres/cm^3 >10 μm in length and $\leqslant$1.0 μm in diameter; or 0.24 mg/m^3 (300 fibres/cm^3); (2) loose 'blowing wool' used for building insulation — diameter, 1.2 μm; mass concentration, 4.4 mg/m^3, 30% respirable — 100 fibres/cm^3 with 30 fibres/cm^3 >10 μm in length and $\leqslant$1.0 μm in diameter; (3) fibrous glass building insulation with binder — diameter, 1.1 μm; mass concentration, 9.9 mg/m^3; 13% respirable — 100 fibres/cm^3 with 25 fibres/cm^3 >10 μm in length and $\leqslant$1.0 μm in diameter; or 1 mg/m^3 (10 fibres/cm^3); (4) binder-coated building insulation — diameter, 3.0 μm; mass concentration, 7.0 mg/m^3; 19% respirable — 25 fibres/cm^3 with 5 fibres/cm^3 >10 μm in length and $\leqslant$1.0 μm in diameter. No respiratory tract tumour was observed in any group. The various forms of fibrous glass did not affect survival and caused little pulmonary cellular change. Of 57 rats exposed to UICC crocidolite asbestos (3000 fibres/cm^3; 5% fibres $\geqslant$5 μm in length: mean, 3.1 $\pm$ 10.2 μm), three developed one mesothelioma and two, bronchoalveolar tumours (Smith *et al.*, 1987).

Female Wistar rats, 12 weeks old, were exposed in nose-only tubes to fibre aerosols for 5 h, four times a week, for a total exposure period of one year (total exposure, 1000 h). The test group was exposed to US glasswool (JM 104) shortened for 50 min in a knife mill (fibre lengths: 10% <2.0 μm, 50% <4.8 μm, 90% <12.4 μm; fibre diameters: 10% <0.23 μm, 50% <0.42 μm, 90% <0.80 μm; aerosol concentration, 3.0 $\pm$ 1.8 mg/m^3, 576 $\pm$ 473 fibres/cm^3; cumulative dose, 3000 mg/m^3 $\times$ h). Two positive-control groups of 50 rats were exposed either to South African crocidolite, containing slightly longer fibres than UICC crocidolite (fibre lengths: 90% >0.72 μm, 50% >1.5 μm, 10% >4.5 μm; fibre diameters: 90% >0.17 μm, 50% >0.27 μm, 10% >0.46 μm; aerosol concentration, 2.2 $\pm$ 1.3 mg/m^3, 2011 $\pm$ 835 fibres/cm^3; cumulative dose, 2200 mg/m^3 $\times$ h), or to Calidria chrysotile (from California, USA; fibre lengths: 90% >2.0 μm, 50% >6.0 μm, 10% >14.0 μm; fibre diameters: 90% >0.28 μm, 50% >0.67 μm, 10% >1.6 μm; aerosol concentration, 6.0 $\pm$ 5.9 mg/m^3, 241 $\pm$ 165 fibres/cm^3; cumulative dose, 6000 mg/m^3 $\times$ h). Two negative-control groups were available: 55 rats were exposed to clean air and 50 rats had no treatment. Only 1/107 rats treated with glasswool developed a primary squamous-cell carcinoma of the lung; median lifetime of the group was 110 weeks. In the group treated with crocidolite, 1/50 rats developed a lung adenocarcinoma; median lifetime of the group was 111 weeks. No lung tumour was detected in the group treated with chrysotile (median lifetime, 109 weeks), or in either of the two negative-control groups (median lifetimes, 108 weeks). A further group treated with glasswool also inhaled 100 ppm (260 mg/m^3) sulphur dioxide for 5 h, five times a week for one year; 1/108 rats had a lung adenoma; median lifetime of the group was 106 weeks. In the corresponding control group of 50 rats exposed only to 100 ppm sulphur dioxide, no lung tumour was detected; median lifetime was 99 weeks. According to the authors, the low tumour incidence in the crocidolite-treated group might have been due to the relatively low lung burden of about 1 mg dust, and the absence of tumours after exposure

to Calidria chrysotile to the lower persistence of these fibres than of UICC chrysotile samples (Muhle *et al.*, 1987).

Hamster: Groups of 30 or 35 hamsters [sex and age unspecified] were exposed by inhalation to fibres >5 μm in length, at concentrations of 0.7×10^6/l [700 fibres/cm^3] ball-milled fibreglass (24.2% fibres with diameter <3 μm) or 3.1×10^6/l [3100 fibres/cm^3] UICC amosite asbestos, for 6 h per day on five days per week for three months and were then observed for 21 months. One group of 30 unexposed animals served as controls. Groups of one to eight animals per exposure group were killed at 50 days, 90 days, six months, 12 months and 18 months, and the remainder at 24 months. No pulmonary tumour was observed in any group (Lee *et al.*, 1981). [The Working Group noted the short exposure period and the small number of animals available for evaluation.]

Groups of 60–70 male, 100-day-old Syrian golden hamsters were examined after exposure by inhalation (nose only) to various types of glasswool dusts for 6 h per day on five days per week for two years and were then observed for life. Groups of 58 chamber and 112 room controls were available. The types of glass fibres were classified according to geometric mean diameter as follows: (1) glasswool with no binder — diameter, 0.4 μm; mass concentration, 2.4 mg/m^3; 81% respirable — 3000 fibres/cm^3 with 530 fibres/cm^3 >10 μm in length and ≤1.0 μm in diameter; or 0.24 mg/m^3 (300 fibres/cm^3); (2) loose 'blowing wool' used for building insulation — diameter, 1.2 μm; mass concentration, 4.4 mg/m^3; 30% respirable — 100 fibres/cm^3 with 30 fibres/cm^3 >10 μm in length and ≤1.0 μm in diameter; (3) fibrous glass building insulation with binder — diameter, 1.1 μm; mass concentration, 9.9 mg/m^3, 13% respirable — 100 fibres/cm^3 with 25 fibres/cm^3 >10 μm in length and ≤1.0 μm in diameter; or 1 mg/m^3 (10 fibres/cm^3); (4) binder-coated building insulation — diameter, 3.0 μm; mass concentration, 7.0 mg/m^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 the latter fibre because of a high death rate in the first group that was unrelated to fibre exposure. No respiratory-tract tumour was observed in the glass fibre-treated or room-control groups; one of the 58 chamber controls had a bronchoalveolar tumour. The various forms of fibrous glass did not affect survival and caused no pulmonary lesion. Among 58 hamsters exposed to UICC crocidolite asbestos (3000 fibres/cm^3; 5% fibres ≥5 μm in length; mean, 3.1 ± 10.2 μm), no pulmonary tumour occurred (Smith *et al.*, 1987).

Guinea-pig: Groups of 31 male albino guinea-pigs [age unspecified] were exposed by inhalation to fibres >5 μm in length, at concentrations of 0.7×10^6/l [700 fibres/cm^3] ball-milled fibreglass (24.2% fibres with diameter <3 μm) and 3.1×10^6/l [3100 fibres/cm^3] UICC amosite asbestos, 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 one to ten animals per exposure group were killed at 50 days, 90 days, six months, 12 months and 18 months, and the remainder at 24 months. No pulmonary tumour was observed in animals that were killed or died prior to the end of the study. Bronchoalveolar adenomas were observed in 2/7 fibreglass-treated animals, 0/5 asbestos-treated animals 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 available for evaluation.]

Baboon: Two groups of ten male baboons (*Papio ursinus*), weighing approximately 6–8 kg, were exposed by inhalation to dust clouds of US glasswool (blend of JM 102 and JM 104; concentration of respirable dust, 5.80 mg/m^3; >60% of fibres <6.3 μm in length, >70% of fibres <1.0 μm in diameter, 35% were <0.5 μm in diameter) or a UICC crocidolite standard reference sample (concentration of respirable dust, 13.45 mg/m^3; <25% of fibres >3.2 μm in length, <20% of fibres >0.5 μm in diameter) for 7 h per day on five days per week for up to 35 or 40 months, respectively. Lung biopsies were carried out on pairs of animals at eight, 18 and 30 months, respectively, and at six to seven months after termination of exposure. Animals that died spontaneously were also subjected to autopsy. No tumour occurred after exposure to either of the dusts. The authors stated that, in inhalation experiments with asbestos carried out on monkeys and baboons over the preceding 25 years, only one animal exposed to crocidolite for 15 months had developed a mesothelioma five years after start of exposure (Goldstein *et al.*, 1983). [The Working Group noted the very short duration of the study in relation to the life span of these animals and that no untreated control was reported.]

(*b*) *Intratracheal instillation*

Rat: Groups of female Wistar rats, 11 weeks of age, received 20 weekly intratracheal instillations of 0.5 mg/dose US glasswool (JM 104; median fibre length, 3.2 μm; median diameter, 0.18 μm) or South African crocidolite (total dose, 10 mg; median fibre length, 2.1 μm; median diameter, 0.2 μm) in 0.3 ml saline or saline alone. Median lifetimes were 107, 126 and 115 weeks for the groups receiving glass fibres, crocidolite and saline only, respectively. A statistically significant increase (5/34 animals) in the incidence of lung tumours was observed with the glass fibres; one tumour was an adenoma, two were adenocarcinomas and two were squamous-cell carcinomas. The mean life span of animals with tumours was 113 weeks; the life span of the first animal with a tumour was 96 weeks. Of 35 rats given crocidolite, 15 developed lung tumours (nine adenocarcinomas, two squamous-cell carcinomas and four mixed tumours; mean life span of tumour-bearing animals, 121 weeks; first tumour after 89 weeks). No such tumour occurred in 40 control animals, or in historical controls of this strain (Pott *et al.*, 1987).

A group of 22 female, 100-day-old Osborne-Mendel rats received five weekly intratracheal instillations of 2 mg glasswool (geometric mean fibre length, 4.7 μm; geometric mean diameter, 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 average life span was longer in treated rats (783 days) than in the saline (688 days) or untreated (724 days) controls. No respiratory-tract tumour was observed in any group. Of 25 rats treated similarly 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 used and the low tumour response in positive controls, which made interpretation of the study difficult.]

Hamster: Two groups of 136 or 138 male Syrian golden hamsters [age unspecified] were examined after eight weekly intratracheal instillations in 0.15 ml saline of 1 mg of two different

glass fibre samples prepared from US glasswool (JM 104) by wet milling in a ball mill for 2 or 4 h, respectively, resulting in different length distributions (2-h sample: length, 50% <7.0 μm; diameter, 50% <0.3 μm; 4-h sample: 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. The incidences of thoracic tumours were: 48/136 2-h glass fibre-treated animals (five lung carcinomas, 37 mesotheliomas, six sarcomas), 38/138 4-h glass fibre-treated animals (six lung carcinomas, 26 mesotheliomas, six sarcomas), 18/142 crocidolite-treated animals (nine lung carcinomas, eight mesotheliomas, one sarcoma) and 2/135 titanium dioxide-treated controls (two sarcomas); lung carcinomas were described as mucoepidermoid carcinomas. The total duration of the experiment was 113 weeks. Nearly all tumour-bearing animals survived up to 18 months after the first instillation, and about 50% lived for longer than two years (Pott *et al.*, 1984a). [The Working Group noted the unusually long life span of the hamsters in this study.]

Six groups of 35 male and 35 female Syrian golden hamsters, 16 weeks of age, received intratracheal instillations in 0.2 ml 0.005% gelatin in saline of 1 mg US glasswool (JM 104; 58% <5 μm in length; 88% <1.0 μm in diameter), 1 mg glasswool 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 gelatin solution in saline or vehicle alone, respectively, 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: in the 63 animals examined in the group given benzo[*a*]pyrene alone, two carcinomas and one sarcoma were observed plus four papillomas; in 52 hamsters receiving crocidolite plus benzo[*a*]pyrene, two carcinomas and one sarcoma plus one papilloma were observed; and two sarcomas (3%) plus two papillomas were found in 66 animals treated with glasswool plus benzo[*a*]pyrene (Feron *et al.*, 1985). [The Working Group noted the relatively short observation time and the absence of tumours in the positive, crocidolite-treated control group.]

(*c*) *Intrapleural administration*

Mouse: Four groups of 25 BALB/c mice [sex and age unspecified] received single intrapleural injections of 10 mg of one of four different samples of borosilicate glass fibres in 0.5 ml distilled water. The injection material was obtained by separating each of two original samples with average diameters of 0.05 μm and 3.5 μm into two samples with lengths of several hundred micrometers and lengths of <20 μm. Animals were killed at intervals of two weeks to 18 months, at which time there were 37 survivors. No pleural tumour was found in any of the treated animals, whereas two mesotheliomas were observed in a total of 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 production 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 positive controls.]

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 fibreglass (a borosilicate; 30% of fibres 1.5–2.5 μm in diameter; maximum diameter, 7 μm; 60%, >20 μm in length), 20 mg glass powder (a borosilicate; projected area diameter, <8 μm) or 20 mg of one of two different samples of Canadian SFA chrysotile. Animals were held until natural death; average survival times were 774, 751, 568 and 639 days for the groups treated with fibreglass, glass powder and the two chrysotile samples, respectively. No injection-site tumour was observed in the fibreglass-treated group; a single mesothelioma occurred in the glass powder-treated group (after 516 days). Tumour incidences in the two chrysotile groups were 23/36 and 21/32; death of the first animals with tumours occurred after 325 and 382 days (Wagner *et al.*, 1973).

Three groups of 16 male and 16 female Wistar rats, ten weeks of age, received single intrapleural injections of 20 mg of a finer US glasswool (JM 100; 99% of fibres <0.5 μm in diameter; median diameter, 0.12 μm; 2%, >20 μm in length; median length, 1.7 μm) or a coarser US glasswool (JM 110; 17% of fibres <1 μm in diameter; median diameter, 1.8 μm; 10%, >50 μm in length; median length, 22 μm) in 0.4 ml saline or saline alone. Animals were held until natural death; mean survival times were 716, 718 and 697 days, respectively. Between 663 and 744 days after inoculation, 4/32 animals given the finer fibreglass had mesotheliomas. No pleural tumour occurred in animals treated with the coarser fibreglass or in saline controls (Wagner *et al.*, 1976).

Groups of 32–45 male SPF Sprague-Dawley rats, three months old, received single intrapleural injections of 20 mg US glasswool (JM 104; mean length, 5.89 μm; mean diameter, 0.229 μm), 20 mg UICC chrysotile A (mean length, 3.21 μm; mean diameter, 0.063 μm), 20 mg UICC crocidolite (mean length, 3.14 μm; mean diameter, 0.148 μm) in 2 ml saline, or saline alone. Animals were held until natural death; mean survival times for total groups (and for animals with tumours) were 513 (499), 388 (383), 452 (470) and 469 days, respectively. Six thoracic mesotheliomas developed in a total of 45 rats injected with glasswool. The incidences of thoracic tumours in chrysotile- and crocidolite-treated animals were 15/33 (one carcinoma and 14 mesotheliomas) and 21/39 (mesotheliomas), respectively. No such tumour 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 or chrysotile in 0.5 ml saline. The dust samples used (and the size distributions of those fibres longer than 1 μm) were: English glasswool with resin coating (70% fibres ≤5 μm in length; 85% ≤1 μm in diameter), English glasswool after removal of resin (57% ≤5 μm in length; 85% ≤1 μm in diameter), US glasswool (JM 100; 88% ≤5 μm in length; 98.5% ≤1 μm in diameter) 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 glasswool [whether coated or uncoated unspecified], four in the group treated with US glasswool and six in the chrysotile-treated group. No such tumour was observed in a group of 24 saline-treated controls (Wagner *et al.*, 1984).

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, 19 of which were uncoated or resin-coated fibrous glass. The materials were mixed in 10% gelatin, and 40 mg of each type of glass in 1.5 ml gelatin were 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 incidences of pleural mesothelioma in animals surviving 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 (Table 39). When two of the fibrous glass preparations (diameter, >0.25 μm) were leached to remove all elements except silicon dioxide, they induced incidences of 2/28 and 4/25 pleural mesotheliomas (Stanton *et al.*, 1977, 1981).

Table 39. Summary of results of implantation of different forms of fibrous glass in the pleural cavity of rats[a]

Fibre type	Incidence of pleural sarcomas[b]	log fibres/μg, ≤ 0.25 μm × >8 μm
Glass 1	9/17	5.16
Glass 2	12/31[c]	4.29
Glass 3	20/29	3.59
Glass 4	18/29	4.02
Glass 5	16/25	3.00
Glass 6	7/22	4.01
Glass 7	5/28	2.50
Glass 8	3/26	3.01
Glass 9	2/28	1.84
Glass 10	2/27	–
Glass 12 (coated)	1/25	–
Glass 13	1/27	–
Glass 14 (coated)	1/25	–
Glass 15 (coated)	1/24	1.30
Glass 16	1/29	–
Glass 17	0/28	–
Glass 18 (coated)[d]	0/115	–
Glass 19 (leached)	2/28[c]	–
Glass 20 (leached)	4/25[c]	–
Control (pleural implants described as noncarcinogenic)	17/615 (2.8%)	–
Control (untreated)	3/491 (0.6%)	–

[a]From Stanton *et al.* (1977, 1981)

[b]Incidence in animals surviving longer than 52 weeks, except where noted (Stanton *et al.*, 1977)

[c]Survival of animals in which incidence was determined is not specified (Stanton *et al.*, 1981).

[d]Control in first series of experiments (Stanton *et al.*, 1977)

(*d*) *Intraperitoneal administration*

Rat: Groups of female Wistar rats, eight to 12 weeks of age, received single intraperitoneal injections of 2 or 10 mg or four weekly injections of 25 mg German glasswool (106; 59% fibres <3 μm in length), different doses of UICC chrysotile A or 100 mg of one of seven kinds of granular dust in 2 ml saline. The animals were held until natural death. In the groups given glasswool or chrysotile, dose-dependent incidences of mesotheliomas and sarcomas were observed: 1/34, 4/36 and 23/32 in the groups receiving 2, 10 and 100 mg glass fibres, respectively, with corresponding average survival times of 518, 514 and 301 days; incidences ranged from 6/37 (2 mg) to 25/31 (25 mg) in the chrysotile-treated groups, with average survival times of 468–407 days. Of 263 animals treated with granular dusts, three rats developed malignant tumours. No abdominal tumour occurred in 72 saline-treated control animals (Pott *et al.*, 1976).

Groups of female Wistar rats, eight to 12 weeks of age, received single intraperitoneal injections of 2, 10 or 50 mg (the latter given in two doses) of one German glasswool (104; mean fibre length, approximately 10 μm; diameter, approximately 0.2 μm), 20 mg of another German glasswool (112; mean length, approximately 30 μm; diameter, approximately 1 μm), 2 mg UICC crocidolite or 50 mg corundum. Average survival times were 673, 611 and 361 days for the groups treated with the finer glasswool (104) and 610 and 682 days for the groups treated with the coarser glasswool (112) or crocidolite. Dose-related increases in the incidences of abdominal tumours (mesotheliomas, sarcomas and, rarely, carcinomas) were observed in the groups treated with the finer glasswool: 20/73 (2 mg), 41/77 (10 mg) and 55/77 (50 mg). The incidences in the groups treated with the coarser glasswool or with crocidolite were 14/37 and 15/39, respectively. Of the 37 rats that received injections of granular corundum, three had tumours in the abdominal cavity; mean survival was 746 days (Pott *et al.*, 1976).

Three groups of 44 female Wistar rats, four weeks old, were examined after intraperitoneal injections of 2 or 10 mg US glasswool (JM 104; milled for 2 h [size not given]) or 2 mg of another US glasswool (JM 100; 50% fibres <2.4 μm in length; 50% <0.33 μm in diameter). Abdominal tumours were observed in 14/44 rats that received 2 mg JM 104 glasswool, in 29/44 rats that received 10 mg JM 104 glasswool and in 2/44 rats that received 2 mg JM 100 glasswool. The first tumour-bearing rat was found 350 days (50 weeks), 252 days (36 weeks) and 664 days (95 weeks) after the start of treatment in the three groups, respectively. In three positive control groups that received intraperitoneal injections of 0.4, 2 or 10 mg UICC chrysotile B, tumours developed in 9, 26 and 35 of 44 rats, respectively; the first tumour-bearing rat was found 522 days (75 weeks), 300 days (43 weeks) and 255 days (36 weeks) after start of treatment in the three groups, respectively. A negative control group treated with 2 mg granular corundum dust had a tumour incidence of 1/45; the first tumour-bearing animal 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 100 glasswool had an infection during the 21st month which might have reduced the tumour incidence. The high tumour incidence in rats treated with JM 104 glasswool was suggested by the authors to be due to the longer fibre

length, and the low incidence in rats treated with JM 100 glasswool to the large proportion of shorter fibres (Pott *et al.*, 1984b).

Groups of female Sprague Dawley rats, eight weeks old, received single intraperitoneal injections of 2 mg or 10 mg US glasswool (JM 100; median fibre length, 2.4 μm; median fibre diameter, 0.33 μm) in 2 ml saline. Median survival times were 90 and 79 weeks for the groups receiving 2 mg and 10 mg glasswool, respectively. Sarcomas, mesotheliomas and (rarely) carcinomas occurred in 21/54 low-dose and in 24/53 high-dose animals (first tumour after 53 weeks in each group). Three tumours were found in two 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, 93 and 94 weeks; first tumour after 79 and 94 weeks, respectively) (Pott *et al.*, 1987).

Groups of 32 female Wistar rats, five weeks old, received single intraperitoneal injections of 0.5 or 2.0 mg US glasswool (JM 104; median length, 3.2 μm; median diameter, 0.18 μm), 2.0 mg of glasswool treated with 1.4 M hydrochloric acid for 24 h, or 0.5 or 2.0 mg South African crocidolite (median length, 2.1 μm; median diameter, 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 receiving 0.5 mg and 2.0 mg glasswool, acid-treated glasswool, 0.5 and 2.0 mg crocidolite, titanium dioxide and saline only, respectively. The incidences of sarcomas, mesotheliomas and (rarely) carcinomas of the abdominal cavity observed with the glasswool were 5/30 (first tumour after 88 weeks) with 0.5 mg, 8/31 (first tumour after 84 weeks) with 2.0 mg and 16/32 (first tumour after 56 weeks) with acid-treated glasswool. Tumour incidences of 18/32 (first tumour after 79 weeks) and 28/32 (first tumour after 52 weeks) occurred in the crocidolite groups, and two tumours (first tumour after 113 weeks) were seen in the saline-control group. No such tumour was found in the group treated with titanium dioxide (Muhle *et al.*, 1987; Pott *et al.*, 1987).

Groups of eight-week-old female Sprague-Dawley rats were injected once with 5 mg of US glasswool (JM 104) cut and ground for 1 h in an agate mill or treated with 1.4 M hydrochloric acid or sodium hydroxide for 2 or 24 h, and administered in 2 ml saline. The loss in weight 2 and 24 h after treatment with acid amounted to 25 and 33%; that after treatment with alkali, 1.7 and 6.8%; and that after treatment with distilled water, 1.7%. A negative control group received 5 mg granular titanium dioxide. The glasswool treated for 2 h with acid induced abdominal tumours (mesotheliomas, sarcomas and, rarely, carcinomas) in 32/54 rats; median survival time of the group was 88 weeks, and average survival time of the tumour-bearing animals was 93 weeks. Glasswool treated for 24 h with acid (fibre length: 50% <5.3 μm; fibre diameter: 50% <0.5 μm) induced tumours in 4/54 rats; median survival time of the group was 99 weeks, and average survival time of the tumour-bearing animals was 111 weeks. Glasswool treated for 2 h with alkali induced tumours in 42/54 rats; median survival time of the group was 71 weeks, and average survival time of the tumour-bearing rats was 69 weeks. Glasswool treated for 24 h with alkali (fibre length: 50% <5.4 μm; fibre diameter: 50% <0.5 μm) induced abdominal tumours in 46/53 rats; median survival time of the group, and average survival time of the tumour-bearing

rats was 72 weeks. In the group administered untreated fibres (fibre length: 50% <4.8 μm; fibre diameter: 50% <0.29 μm), 44/54 rats developed abdominal tumours; median survival time of the group was 64 weeks, and average survival time of the tumour-bearing animals was 67 weeks. In the group treated with titanium dioxide, 2/52 rats were found to have abdominal tumours; median survival time of the group was 99 weeks, and average survival time of the tumour-bearing animals was 97 weeks (Pott *et al.*, 1987).

In another experiment, groups of four-week-old Wistar rats [sex unspecified] received 5 mg of the same glasswool, either untreated or treated for 24 h with acid or alkali, by intraperitoneal injection in 0.8 ml saline. A negative control group received 5 mg granular titanium dioxide. The acid-treated glasswool (fibre length: 50% <5.3 μm; fibre diameter: 50% <0.5 μm) induced abdominal tumours (mesotheliomas, sarcomas and, rarely, carcinomas) in 2/45 rats; median survival time of the group was 113 weeks, and average survival time of the tumour-bearing rats was 126 weeks. The alkali-treated glasswool (fibre length: 50% <5.4 μm; fibre diameter: 50% <0.5 μm) led to the formation of tumours in 27/46 rats; median survival time of the group was 58 weeks, and average survival time of the tumour-bearing rats was 64 weeks. Untreated glasswool (fibre length: 50% <4.8 μm; fibre diameter: 50% <0.29 μm) induced abdominal tumours in 20/45 rats; median survival time of the group was 34 weeks, and average survival time of the tumour-bearing rats was 49 weeks. None of 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, 100-day-old Osborne-Mendel rats received a single intraperitoneal injection of 25 mg glasswool (geometric mean fibre length, 4.7 μm; geometric mean diameter, 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 was untreated. All animals were observed for life; the median average life span was significantly shorter in treated rats (593 days) than in saline (744 days) or untreated (724 days) controls. Mesotheliomas were found in 8/25 of the glasswool-treated rats and in 20/25 rats injected with 25 mg UICC crocidolite (5% ≥5 μm in length; mean, 3.1 ± 10.2 μm) but in neither control group (Smith *et al.*, 1987).

Hamster: Groups of 40 female Syrian golden hamsters, eight to 12 weeks old, received single intraperitoneal injections of 2 or 10 mg German glasswool (59% of fibres shorter than 3 μm) 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). [The Working Group noted that survival times were not reported and that saline controls were not used.]

Glass filament

Intraperitoneal administration

Rat: Groups of 50 female Wistar rats, 12 weeks of age, received 10 or 40 mg of two German glass filaments — a finer filament (ES 5; median diameter, 5.5 μm; 80% of fibres 4.8–6.3 μm in diameter; median length, 39 μm; 10% of fibres longer than 80 μm) and a coarser one (ES 7; median diameter, 7.4 μm; 80% of fibres, 6.8–8.1 μm in diameter; median

length, 46 μm; 10% of fibres longer than 102 μm) — or a granular glass dust [unspecified] by single or double (weekly) intraperitoneal injection in 2 ml saline. Animals were observed for life; median survival times were 111, 107, 121 and 119 weeks for the groups given 10 mg finer glass filament, 40 mg finer glass filament, 40 mg coarser glass filament and 40 mg granular glass dust, respectively. Corresponding mean survival times of animals with tumours were 106, 119, 126 and 129 weeks, respectively. No statistically significant increase in the incidences of sarcomas, mesotheliomas or (rarely) carcinomas of the abdominal cavity was observed in the groups treated with finer glass filament (low dose: 2/50; death of first animal with tumour after 92 weeks; high dose: 5/46; first tumour after 96 weeks) or with coarser glass filament (1/47; first tumour after 126 weeks), when compared with an incidence of 2/45 (first tumour after 121 weeks) in the group treated with granular glass dust (Pott *et al.*, 1987).

Similar groups of female Wistar rats, 12–15 weeks old, received 50 or 250 mg of a very fine German glass filament (ES 3; median diameter, 3.7 μm; 80% of fibres 3.3–4.2 μm in diameter; median fibre length, 16.5 μm; 10% of fibres longer than 50 μm), the finer glass filament (ES 5) described above or granular glass dust by laparatomy in 4 ml saline. Median survival time of the group given 250 mg finer glass filament was 109 weeks; the life span of the other groups was reduced by an infection in month 15: median survival times were 94, 94, 88, 99 and 87 weeks for the groups receiving 50 mg and 250 mg very fine glass filaments, 50 mg and 250 mg granular glass dust and a control group receiving 4 ml saline alone, respectively. Abdominal tumours occurred in 2/28 animals given the finer glass filament (death of first animal with tumour after 76 weeks), in 3/48 given the low dose of the very fine filament (first tumour after 71 weeks) and in 4/46 given the high dose of the very fine filament (first tumour after 87 weeks). Similar numbers of abdominal tumours occurred in the control groups: 4/48 with both the low and high doses of granular glass and 2/45 with saline alone; the first tumours were detected after 62, 91 and 95 weeks, respectively (Pott *et al.*, 1987).

[The Working Group noted that the number of fibres injected was much smaller in these studies than in those with glasswool (<0.3 μm in diameter) carried out in the same laboratory.]

Rockwool and slagwool

(*a*) *Inhalation*

Rat: Groups of 24 male and 24 female Wistar IOPS AF/Han rats, eight to nine weeks old, were exposed by inhalation to dust concentrations of 5 mg/m^3 (respirable particles) French resin-free rockwool [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 different periods of observation (for seven, 12 and 16 months after exposure for animals exposed for 12 months; four months after exposure for those exposed for 24 months). No pulmonary tumour was observed among 47 rats treated with rockwool or in 47 untreated controls; nine pulmonary tumours occurred

among 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 rockwool [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 rockwool fibres $\leqslant$20 μm in length, 58% $\leqslant$1 μm in diameter; 16% of chrysotile fibres $\geqslant$20 μm in length, 29% $\geqslant$0.5 μm in diameter. Six rats were removed from each group at the end of exposure to study dust retention, and a similar number of animals was sacrificed one year later for the same purpose. The remainder were held until natural death [survival times not reported]. During the period 500–1000 days after the start of exposure, lung adenomas (one with some malignant features) occurred in 2/48 rats in the rockwool-treated group; 11 adenocarcinomas and one adenoma (with some malignant features) occurred in 48 rats treated with chrysotile. No lung tumour was observed 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.]

A group of 55 female, 100-day-old Osborne-Mendel rats was exposed by inhalation (nose only) to slagwool dust [type of slag unspecified] (mass concentration, 7.8 mg/m^3; 15.2% respirable — 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 $\leqslant$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 available. No respiratory-tract tumour was observed in any group. Average survival in the slagwool-treated group 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 $\geqslant$5 μm in length; mean, 3.1 $\pm$ 10.2 μm), two developed bronchoalveolar tumours and one, a mesothelioma (Smith *et al.*, 1987).

Hamster: A group of 69 male, 100-day-old Syrian golden hamsters was exposed by inhalation (nose only) to slagwool dust [type of slag unspecified] (mass concentration, 7.8 mg/m^3; 15.2% respirable — 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 $\leqslant$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 available. No respiratory-tract tumour was observed in the treated animals or in room controls; one of 58 chamber controls had a bronchoalveolar tumour. There was no decrease in life span (about 660 days). Of 58 hamsters exposed to UICC crocidolite asbestos (3000 fibres/cm^3; 5% fibres $\geqslant$5 μm in length: mean, 3.1 $\pm$ 10.2 μm), no pulmonary tumour occurred (Smith *et al.*, 1987).

(*b*) *Intrapleural administration*

Rat: Groups of 48 SPF Sprague-Dawley rats [sex and age unspecified] received single intrapleural injections of 20 mg fibrous dusts of various wools or chrysotile in 0.5 ml saline. The dust samples used (and the size distributions of those fibres longer than 5 μm) were:

Swedish rockwool [type of rock unspecified] with resin coating (70% fibres <5 μm in length; 52% <0.6 μm in diameter), Swedish rockwool after removal of resin (70% <5 μm in length; 58% <0.6 μm in diameter), German slagwool [type of slag unspecified] (67% <5 μm in length; 42% <0.6 μm in diameter), German slagwool after removal of resin (80% <5 μm in length; 62% <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 rockwool with resin and two in the group treated with rockwool without resin; six mesotheliomas occurred in the chrysotile-treated group. No tumour was observed in the group treated with slagwool or in a group of 24 saline-treated controls (Wagner *et al.*, 1984).

In the experiment by Stanton *et al.* (1977, 1981) (see pp. 93–94), one sample of slagwool (a silica-slag-derived mineral) was implanted in the pleura. A pleural sarcoma developed in 1/25 animals that survived longer than 52 weeks.

(*c*) *Intraperitoneal administration*

Rat: Groups of female Wistar rats, 15 weeks old, received 40 mg of two samples of German slagwool [type of slag unspecified] by two weekly intraperitoneal injections in 2 ml saline. The coarser sample (RH) had a median fibre length of 26 μm and a median fibre diameter of 2.6 μm; the finer one (ZI) 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 and finer slagwool and for a control group given saline alone, respectively. Slight increases in the incidences of sarcomas, mesotheliomas and (rarely) carcinomas of the abdominal cavity were observed with the slagwool samples: 6/99 with the coarser sample (first tumour after 88 weeks) and 2/96 with the finer one (first tumour after 67 weeks). No tumour occurred in 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 saline-treated controls ranged from 0 to 6.3%.]

Preliminary results after 28 months of observation were available from another experiment carried out on female Wistar rats, eight weeks of age: groups of about 50 animals received five intraperitoneal injections of a German rockwool (from basalt; total dose, 75 mg; median length, 20 μm; median diameter, 1.8 μm) or 100 mg titanium dioxide in 2 ml saline. Median survival times were 79, 109 and 111 weeks for the rockwool group, the titanium dioxide group and a control group receiving five injections of 2 ml saline alone, respectively. In the group that received the rockwool, tumours of the abdominal cavity developed in 32/53 animals, the first tumour occurring 54 weeks after first injection. Tumour incidences in the control groups were 5/53 with titanium dioxide (life span of first animal with tumour, 38 weeks) and 2/102 with saline (first tumour after 93 weeks). In two positive-control groups, single intraperitoneal injections of 0.25 mg actinolite fibres and of 1 mg chrysotile produced tumours in 20/36 and 31/36 rats, respectively (Pott *et al.*, 1987). [The Working Group noted that most of the diagnoses had not been verified by histopathological examination at the time of reporting.]

Groups of female Sprague-Dawley rats, eight weeks old, received intraperitoneal injections of 75 mg Swedish rockwool [type of rock unspecified] (administered in three

injections; median fibre length, 23 μm; diameter, 1.9 μm), 10 mg of a fine fraction prepared from the rockwool sample (single injection; median fibre length, 4.1 μm; diameter, 0.64 μm) or 40 mg granular volcanic ash from Mount St Helen's (two injections) in 2 ml saline. Median survivals were 77, 97 and 93 weeks for the animals given the two forms of rockwool and volcanic ash, respectively; the median life span of a control group that received two injections of 2 ml saline was 94 weeks. A high incidence of tumours of the abdominal cavity was observed with 75 mg of the original rockwool sample: 45/63 (life span of first animals with tumour, 39 weeks); a slightly increased tumour incidence occurred with 10 mg of the fine fraction: 6/45 (first tumour after 88 weeks). This compared to a tumour incidence of 3/54 in the volcanic ash group and in the control group (Pott *et al.*, 1987).

Ceramic fibres

(*a*) *Inhalation*

Rat: Groups of 45–46 young adult male Sprague-Dawley rats were exposed by inhalation to fibres >5 μm in length, at concentrations of 2.9×10^6/l [2900 fibres/cm^3] potassium octatitanate (Fybex; 19.1% fibres <3 μm diameter), 2.0×10^6/l pigmentary potassium titanate (PKT; 45.8% fibres <3 μm diameter) or 3.1×10^6/l [3100 fibres/cm^3] UICC amosite asbestos for 6 h per day on five days per week for three months, and were then observed for 21 months. One group of 46 unexposed animals served as controls. Groups of four to ten animals per exposure group were killed at 20 days, 50 days, 90 days, six months, 12 months and 18 months, and the remainder at 24 months. No pulmonary tumour was observed in animals that were killed or died prior to the end of the study. Bronchoalveolar tumours were observed in 1/14 animals treated with potassium octatitanate (one adenoma), 0/19 animals treated with pigmentary potassium titanate, 3/11 animals treated with amosite (two adenomas, one carcinoma) and 0/13 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 available for evaluation.]

Three groups of about 40 'young' rats [strain, sex and age unspecified] were exposed by inhalation to dust clouds of fibres consisting chemically of >95% alumina with 3–4% silica (Saffil®; median fibre diameter, 3.3 μm), thermally 'aged' fibres (treated at temperatures >1000°C) or UICC chrysotile A for 18 months. The concentration of total dust from the untreated fibres was 20–120 mg/m^3, resulting in a cumulative exposure of approximately 7000 mg × h/m^3 respirable dust for the untreated and aged forms (respirable fraction, 2.5% on average); cumulative exposure to chrysotile was 13 800 mg × h/m^3 respirable dust. The animals were held to 85% mortality. No pulmonary tumour was found in animals exposed to the ceramic fibres or in 34 undusted controls; 9/39 animals exposed to chrysotile had lung tumours (Pigott & Ishmael, 1982). [The Working Group noted that survival times were not reported and that only a small proportion of the dust cloud was respirable.]

A group of 48 SPF Wistar AF/Han rats [sex unspecified], 12 weeks of age, was exposed by inhalation to concentrations of 10 mg/m^3 respirable dust from fibrous ceramic aluminium silicate glass ([source unspecified] approximately 90% of fibres <3 μm in length and <0.3 μm in diameter; particles with aspect ratio >3:1) for 7 h per day on five days per

week for 12 months (cumulative exposure, 224 days). Four animals were removed from the experiment at 12 months and four at 18 months; seven surviving animals in treated and control groups were sacrificed at the end of the experiment at 32 months; the remainder were allowed to live out their life span. Seven of the 48 treated animals developed malignant pulmonary neoplasms, and one had a benign adenoma. No pulmonary tumour was observed in 39 untreated controls, but two malignant tumours of the peritoneum or digestive system were observed (Davis *et al.*, 1984).

A group of 55 female, 100-day-old Osborne-Mendel rats was exposed by inhalation (nose only) to refractory ceramic fibre dust [source unspecified] at a mass concentration of 10.8 mg/m^3, of which 35% was respirable (geometric mean diameter, 0.9 μm; geometric mean length, 25 μm; chamber concentration, 200 fibres/cm^3 with 88 fibres/cm^3 >10 μm in length and $\leqslant 1.0$ 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 available. No respiratory-tract tumour was observed in any group. Exposure to refractory ceramic fibres did not affect survival. Of 57 rats exposed to UICC crocidolite (3000 fibres/cm^3; 5% >5 μm in length; mean, 3.1 $\pm$ 10.2 μm), three developed one mesothelioma and two bronchoalveolar tumours (Smith *et al.*, 1987).

Hamster: Groups of 34 hamsters [sex and age unspecified] were exposed by inhalation to fibres >5 μm in length, at concentrations of 2.9 $\times$ 10^6/l [2900 fibres/cm^3] potassium octatitanate (Fybex®; 19.1% fibres with diameter <3 μm), 2.0 $\times$ 10^6/l [2000 fibres/cm^3] pigmentary potassium titanate (PKT; 45.8% fibres with diameter <3 μm) or 3.1 $\times$ 10^6/l [3100 fibres/cm^3] UICC amosite asbestos, for 6 h per day on five days per week for three months and were then observed for 21 months. One group of 34 unexposed animals served as controls. Groups of four to 12 animals per exposure group were killed at 50 days, 90 days, six months, 12 months and 18 months, and the remainder at 24 months. One of four animals exposed to potassium octatitanate and killed at 18 months had a pleural mesothelioma. No other pulmonary tumour was observed in any of the groups (Lee *et al.*, 1981). [The Working Group noted the short exposure period and the small number of animals available for evaluation.]

A group of 70 male, 100-day-old Syrian golden hamsters was exposed by inhalation (nose only) to refractory ceramic fibre dust [source unspecified] at a mass concentration of 10.8 mg/m^3, of which 35% was respirable (geometric mean diameter, 0.9 μm; geometric mean length, 25 μm; chamber concentration, 200 fibres/cm^3 with 88 fibres/cm^3 >10 μm in length and $\leqslant 1.0$ 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 available. One treated hamster developed a spindle-cell mesothelioma on the posterior left lung; one of 58 chamber controls had a bronchoalveolar tumour. There was no decrease in life span. Among 58 hamsters exposed to UICC crocidolite asbestos (3000 fibres/cm^3; 5% $\geqslant 5$ μm in length; mean, 3.1 $\pm$ 10.2 μm), no pulmonary tumour occurred (Smith *et al.*, 1987).

Guinea-pig: Groups of 35 male albino guinea-pigs [age unspecified] were exposed by inhalation to fibres >5 μm in length, at concentrations of 2.9 $\times$ 10^6/l [2900 fibres/cm^3] potassium octatitanate (Fybex®; 19.1% fibres with diameter <3 μm), 2.0 $\times$ 10^6/l

[2000 fibres/cm^3] pigmentary potassium titanate (PKT; 45.8% with diameter <3 μm) or 3.1×10^6/l [3100 fibres/cm^3] UICC amosite asbestos, 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 one to ten animals per exposure group were killed at 50 days, 90 days, six months, 12 months and 18 months, and the remainder at 24 months. No pulmonary tumour was observed in any of the groups (Lee *et al.*, 1981). [The Working Group noted the short exposure period and the small number of animals available for evaluation.]

(*b*) *Intratracheal instillation*

Rat: A group of 22 female, 100-day-old Osborne-Mendel rats received five weekly intratracheal instillations of 2 mg refractory ceramic fibres ([source unspecified] geometric mean fibre length, 25 μm; geometric mean diameter, 0.9 μm; 83% of fibres >10 μm in length and 86% <2.0 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 average life span was approximately the same in treated rats (698 days) and in saline (688 days) and untreated (724 days) controls. No respiratory-tract tumour was observed in any group. Of 25 rats treated similarly 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 small number of animals per group and the low tumour response in positive controls, which made interpretation of the study difficult.]

Hamster: A group of 25 male, 100-day-old Syrian golden hamsters received five weekly intratracheal instillations of 2 mg refractory ceramic fibres ([source unspecified] geometric mean fibre length, 25 μm; geometric mean diameter, 0.9 μm; 83% of fibres >10 μm in length and 86% <2.0 μm in diameter) in 0.2 ml saline. A group of 24 hamsters was injected with saline only, and another group of 112 animals was untreated. All animals were observed for life; the median average life span was significantly shorter in the treated hamsters (446 days) than in the saline (567 days) or untreated (563 days) controls. No respiratory-tract tumour was observed in any group. Of 27 hamsters treated similarly with UICC crocidolite (5% fibres ≥5 μm in length; mean, 3.1 ± 10.2 μm), 20 developed bronchoalveolar tumours (13 benign, seven malignant) (Smith *et al.*, 1987).

(*c*) *Intrapleural administration*

Rat: Groups of 31–36 SPF Wistar rats (twice as many males as females), 13 weeks of age, received a single intrapleural injection in 0.4 ml saline of 20 mg ceramic aluminium silicate fibres ([source unspecified] 0.5–1 μm in diameter), nonfibrous aluminium oxide (<10 μm projected area diameter) or one of two different samples of Canadian SFA chrysotile. Animals were held until natural death; average survival times were 736, 710, 568 and 639 days for the groups treated with ceramic fibres, aluminium oxide and the two chrysotile samples, respectively. Of the 31 ceramic fibre-treated animals, mesotheliomas developed in three, the first of which died 743 days after injection. One mesothelioma was observed in the aluminium oxide-treated group (after 646 days). Tumour incidences in the chrysotile groups

were 23/36 and 21/32; death of the first animals with tumours occurred after 325 and 382 days (Wagner *et al.*, 1973).

Groups of 30–50 female Osborne-Mendel rats, 12–20 weeks old, received a single intrathoracic implantation of one of 13 different types of ceramic fibres [source unspecified]. The materials were mixed in 10% gelatin, and 40 mg of each type of ceramic fibre in 1.5 ml gelatin were 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 incidences of pleural sarcomas varied, depending on the number of fibres ≤ 0.25 μm diameter and >8 μm length (Table 40) (Stanton *et al.*, 1981).

Table 40. Summary of results of implantation of different ceramic fibres in the pleural cavity of rats[a]

Fibre	Incidence of pleural sarcomas	log fibres/μg, ≤ 0.25 μm × >8 μm
Potassium titanate 1	21/29	4.94
Potassium titanate 2	20/29	4.70
Silicon carbide	17/26	5.15
Aluminium oxide 1	15/24	3.63
Aluminium oxide 2	8/27	2.95
Aluminium oxide 3	9/27	2.47
Aluminium oxide 4	4/25	2.60
Aluminium oxide 5	4/22	3.73
Aluminium oxide 6	2/28	0.82
Aluminium oxide 7	1/25	–
Aluminium oxide 8	1/28	–
Glass filament >80% aluminium oxide	2/47	–
Glass filament >90% zirconium oxide	1/45	–
Control (pleural implants described as noncarcinogenic)	17/615 (2.8%)	–
Control (untreated)	3/491 (0.6%)	–

[a]From Stanton *et al.* (1981)

Groups of 24 male and 24 female rats [strain and age unspecified] received single intrapleural injections of 20 mg fibres consisting chemically of >95% alumina with 3–4% silica (Saffil®; median fibre diameter, 3.3 μm), thermally 'aged' fibres (treated at temperatures >1000°C) or UICC chrysotile A in saline. The animals were held until natural deaths. No mesothelioma occurred in animals treated with either form of ceramic fibre or in 48 saline controls; 7/48 rats treated with chrysotile had mesotheliomas (Pigott & Ishmael, 1982). [The Working Group noted that survival data were not given.]

(*d*) *Intraperitoneal administration*

Rat: A group of 32 Wistar AF/Han rats [age and sex unspecified] received a single intraperitoneal injection of 25 mg fibrous ceramic aluminium silicate glass ([source unspecified] approximately 90% of fibres <3 μm in length and <0.3 μm in diameter) suspended in 2 ml Dulbecco's phosphate buffered saline. Peritoneal tumours developed in three animals (9%), the first tumour occurring approximately 850 days after injection [total length of observation and survival times not reported]. One of the tumours was a typical mesothelioma, and the histology of the others was similar to that of fibrosarcoma. In a group of 39 untreated controls used for a study by inhalation (see pp. 101–102), two malignant tumours (5%) of the peritoneum or digestive system were observed (Davis *et al.*, 1984).

Groups of about 50 female Wistar rats, eight weeks of age, received five intraperitoneal injections of ceramic wool (Fiberfrax®; total dose, 45 mg; median fibre length, 8.3 μm; diameter, 0.91 μm), a US ceramic wool (MAN; total dose, 75 mg; median fibre length, 6.9 μm; diameter, 1.1 μm) or titanium dioxide (total dose, 100 mg) in 2 ml saline. Preliminary results were reported describing tumour incidences 28 months after first injection. Tumours of the abdominal cavity were found in 32/47 animals (median survival time, 51 weeks; life span of first animal with tumour, 30 weeks) treated with the first ceramic wool and in 12/54 animals (median survival, 91 weeks; first tumour after 60 weeks) treated with the US ceramic wool. Of 53 animals receiving titanium dioxide, five developed tumours (median survival, 109 weeks; first tumour after 38 weeks); and two tumours occurred in a total of 102 rats that received saline alone (median survival, 111 weeks; first tumour after 93 weeks). In two positive-control groups, single intraperitoneal injections of 0.25 mg actinolite fibres and of 1 mg chrysotile produced tumours in 20/36 and 31/36 rats, respectively (Pott *et al.*, 1987). [The Working Group noted that most of the diagnoses had not been verified by histopathological examination at the time of reporting.]

A group of 25 female, 100-day-old Osborne-Mendel rats received a single intraperitoneal injection of 25 mg refractory ceramic fibres ([source unspecified] geometric mean fibre length, 25 μm; geometric mean diameter, 0.9 μm; 83% of fibres >10 μm in length and 86% <2.0 μm in diameter) in 0.5 ml saline. A group of 25 rats was injected with saline only, and another group of 125 was untreated. All animals were observed for life; the median average life span was significantly shorter in treated rats (480 days) than in saline (744 days) or untreated (724 days) controls. Mesotheliomas were found in 19/23 of the refractory ceramic fibre-injected rats; no tumour was observed in either control group (Smith *et al.*, 1987).

Hamster: Groups of 15 and 21 male, 100-day-old Syrian golden hamsters received a single intraperitoneal injection of 25 mg refractory ceramic fibres ([source unspecified] geometric mean fibre length, 25 μm; geometric mean diameter, 0.9 μm; 83% of fibres >10 μm in length and 86% <2.0 μm in diameter) in 0.5 ml saline. A group of 25 hamsters was injected with saline only, and another group of 112 was untreated. All animals were observed until natural death; median average life span was significantly shorter in the two groups of treated hamsters (462 and 489 days) than in saline (560 days) or untreated (503 days) controls. Mesotheliomas were found in 2/15 and 5/21 hamsters treated with ceramic fibre; no tumour was observed in either control group (Smith *et al.*, 1987)

Table 41. Summary table of studies used for evaluation of the carcinogenicity of man-made mineral fibres in experimental animals (the studies of Stanton *et al.* (1977, 1981) are summarized separately in Tables 39 and 40)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Cumulative exposure [mg/m³ × h]	Duration of exposure					
Inhalation exposure to glasswool and glass fibres								
Inhalation exposure to respirable dust concentrations of 5 mg/m³ (Wistar IOPS AF/Han rats, equal numbers of females and males 8-9 weeks old) (Le Bouffant et al., 1984)								
Glasswool	L 42% <10 μm D 69% <1 μm	–	5 h/day, 5 days/week, total length of dusting: half the animals, 12 months, the other half, 24 months	Up to 28 months (several animals killed at 12, 16 and 24 months)	45	1	Ca	–
Glasswool	L 97% <5 μm D 43% <0.1 μm	–			48	0	–	–
Chrysotile (Canadian)	L 6% >5 μm	–			47	9	Pulmonary tumours	–
Controls	–	–			47	0	–	–
Inhalation exposure to respirable dust concentrations of 10 mg/m³ (PSF Fischer 344 rats, equal numbers of females and males, 7-8 weeks old) (McConnell et al., *1984)*								
Glasswool	Not given	9 035	7 h/day, 5 days/week, 12 months	Lifetime (several animals killed at 3, 12 and 24 months)	55	0	–	–
UICC Chrysotile (Canadian)		14 559			56	11	4 A, 7 AdCa	–
Controls	–	–			53	3	1 A, 2 AdCa	–
Inhalation exposure to respirable dust concentrations of 10 mg/m³ (SPF Fischer rats, equal numbers of females and males) (Wagner et al., *1984)*								
Glasswool plus resin	L 72% 5–20 μm D 52% ≤1 μm	17 498	7 h/day, 5 days/week, 12 months	Lifetime (some animals killed at 12 and 24 months)	48	1	AdCa	–
Glasswool without resin	L 58% 5–20 μm D 47% ≤1 μm	17 458			47	1	A	–
US glasswool	L 93% 5–20 μm D 97% ≤1 μm	17 510			48	1	AdCa	–
UICC Chrysotile (Canadian)	L 39% >10 μm D 29% >0.5 μm	17 499			48	12	1 A, 11 AdCa	–
Controls	–	–			48	0	–	–

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Cumulative exposure [mg/m³ × h]	Duration of exposure					
Nose-only inhalation exposure to dust clouds of various glass fibres (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
		Dust conc.						
Glasswool	L g. mean, 4.9 μm D g. mean, 0.4 μm	2.4 mg/m³ (3000 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	57	0	–	110
Glasswool	L g. mean, 4.9 μm D g. mean, 0.4 μm	0.24 mg/m³ (300 f/cm³)			57	0	–	108
Glasswool (blowing wool)	L g. mean, 24 μm D g. mean, 1.2 μm	4.4 mg/m³ (100 f/cm³)			52	0	–	115
Nose-only inhalation (Smith et al., *1987)* (contd)								
Glasswool (building insulation)	L g. mean, 20 μm D g. mean, 1.1 μm	9.9 mg/m³ (100 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	57	0	–	94
Glasswool (building insulation)	L g. mean, 20 μm D g. mean, 1.1 μm	1 mg/m³ (10 f/m³)			61	0	–	104
Glasswool (binder-coated)	L g. mean, 80 μm D g. mean, 3.0 μm	7.0 mg/m³ (25 f/cm³)			58	0	–	100
UICC crocidolite	L 5% >5 μm	3000 f/cm³			57	3	1 M, 2 BT	109
Chamber controls	–	–			59	0	–	108
Room controls	–	–			125	0	–	103
Nose-only inhalation exposure to glass fibres in concentrations of 3 mg/m³ (female Wistar rats, 12 weeks old) (Muhle et al., *1987)*								
Glasswool	L 50% <4.8 μm D 50% <0.42 μm	3 000	5 h/day, 4 days/week, 1 year	140 weeks	107	1	ScCa	110
Glasswool with SO_2	L 50% <4.8 μm D 50% <0.42 μm	3 000			108	1	A	106
Crocidolite (S. Africa)	L 50% >1.5 μm D 50% >0.27 μm	2 200			50	1	AdCa	111
Chrysotile (Calidria)	L 50% >6.0 μm D 50% >0.67 μm	6 000			50	0	–	109
SO_2	–	–			50	0	–	99
Clean air	–	–			55	0	–	108
No treatment	–	–			50	0	–	108

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Cumulative exposure [mg/m³ × h]	Duration of exposure					
Nose-only inhalation exposure to dust clouds of various glass fibres (male Syrian golden hamsters, 100 days old) (Smith et al., *1987)*								
		Dust conc.						
Glasswool	L g. mean, 4.9 μm D g. mean, 0.4 μm	2.4 mg/m³ (3000 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	69	0	–	95
Glasswool	L g. mean, 4.9 μm D g. mean, 0.4 μm	0.24 mg/m³ (300 g/cm³)			70	0	–	95
Glasswool (blowing wool)	L g. mean, 24 μm D g. mean, 1.2 μm	4.4 mg/m³ (100 f/cm³)			60	0	–	85
Glasswool (building insulation)	L g. mean, 20 μm D g. mean, 1.1 μm	9.9 mg/m³ (100 f/cm³)			66	0	–	90
Glasswool (building insulation)	L g. mean, 20 μm D g. mean, 1.1 μm	1 mg/m³ (10 f/cm³)			65	0	–	97
Glasswool (binder-coated	L g. mean, 83 μm D g. mean, 3.0 μm	7.0 mg/m³ (25 g/cm³)			61	0	–	93 (1) 88 (2)
UICC crocidolite	L, 7% >5 μm	3000 f/cm³			58	0	–	78
Chamber controls	–	–			58	1	BT	95
Room controls	–	–			112	0	–	80
Inhalation exposure to respirable dust concentrations of 5.8 mg/m³ glass fibres or 13.45 mg/m³ crocidolite (male baboons, 6–8 kg) (Goldstein et al., *1983)*								
Glass fibres	L >60% <6.3 μm D >70% <1.0 μm		7 h/day, 5 days/week, up to 35-40 months	Up to 6-7 months after the end of dusting	10	0	–	Not given
UICC crocidolite	L <25% >3.0 μm D <20% >0.5 μm				10	0	–	Not given

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intratracheal administration of glasswool and glass fibres								
Intratracheal instillation in 0.3 ml saline (female Wistar rats, 11 weeks old) (Pott et al., *1987)*								
Glasswool	L 50% <3.2 μm D 50% <0.18 μm	10	20	126 weeks	34	5	1 A, 2 AdCa, 2 ScCa, 1 T	107
Crocidolite (S. Africa)	L 50% >2.1 μm D 50% >0.20 μm	10	20	126 weeks	35	15	9 AdCa, 2 ScCa, 4 mixed, 1 T	126
Saline	–	–	20	124 weeks	40	0	–	115
Intratracheal instillation of glass fibres in 0.2 ml saline (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
Glasswool	L g. mean, 4.7 μm D g. mean, 0.4 μm	10	5	Lifetime	22	0	–	112
UICC crocidolite	L 5% >5 μm	10	5		25	2	2 BT	91
Saline	–	–			25	0	–	98
No treatment	–	–			125	0	–	103
Intratracheal instillation in 0.15 ml saline (male Syrian golden hamsters) (Pott et al., *1984a) [age unspecified]*								
Glasswool 2-h milled	L 50% <7.0 μm D 50% <0.3 μm	8	8	113 weeks	136	48	5 Ca, 37 M, 6 S	>104
Glasswool 4-h milled	L 50% <4.2 μm D 50% <0.3 μm	8	8		138	38	6 Ca, 26 M, 6 S	>104
UICC crocidolite	L 50% >2.1 μm D 50% >0.2 μm	8	8		142	18	9 Ca, 8 M, 1 S	>104
Titanium dioxide	Granular	8	8		135	2	1 S	>104

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intratracheal instillation in 0.2 ml 0.005% gelatin solution in saline (female and male Syrian golden hamsters, 16 weeks old) (Feron et al., *1985)*								
Benzo[*a*]pyrene (BaP)		26	26	85 weeks	63	7	4 P, 2 Ca, 1 S	No relevant difference in mortality between any of the treatment groups and the control group
Glasswool	L 58% <5 μm D 88% <1 μm	26	(once every 2 weeks for 52 weeks)		64	0		
Glasswool with BaP	L 58% <5 μm D 88% <1 μm	26 + 26			66	4	2 P, 2 S	
Crocidolite	L 58% >5 μm D 63% >0.25 μm	26			60	0	–	
Crocidolite with BaP	L 58% >5 μm D 63% >0.25 μm	26 + 26			52	4	1 P, 2 Ca, 1 S	
Saline	–	–			59	0	–	

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intrapleural administration of glasswool and glass fibres								
Intrapleural injection in 0.5 ml distilled water (BALB/c mice) (Davis, 1976) [sex, age unspecified]								
Glass fibre (borosilicate)	L several hundred μm D average, 0.05 μm	10	1	Up to 18 months	25	0	–	–
Glass fibre (borosilicate)	L <20 μm D average, 0.05 μm	10	1		25	0	–	–
Glass fibre (borosilicate)	L several hundred μm D average, 3.5 μm	10	1		25	0	–	
Glass fibre	L <20 μm D average, 3.5 μm	10	1		25	0	–	–
Asbestos (chrysotile + crocidolite)	Not given	Not given			150	2	2 M	–
Intrapleural injection in 0.4 ml saline (SPF Wistar rats, twice as many males as females, 13 weeks old) (Wagner et al., *1973)*								
Glass fibre (borosilicate)	L 60% >20 μm D 30% 1.5–2.5 μm	20	1	Lifetime	35	0	–	111
SFA chrysotile		20	1		36	23	23 M	81
SFA chrysotile		20	1		32	21	21 M	91
Glass powder	Granular, D < 8 μm	20	1		35	1	1 M	107
Intrapleural injection in 0.4 ml saline (Wistar rats, equal numbers of males and females, 10 weeks old) (Wagner et al., *1976)*								
Glasswool	L median, 1.7 μm D median, 0.12 μm	20	1	Lifetime	32	4	4 M	102
Glasswool	L median, 22 μm D median, 1.8 μm	20	1		32	0	–	103
Saline	–	–	1		32	0	–	100

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	Duration of exposure					
Intrapleural injection in 2 ml saline (male SPF Sprague-Dawley rats, 12 weeks old) (Monchaux et al., *1981)*								
Glasswool	L mean, 5.89 μm D mean, 0.229 μm	20	1	Lifetime	45	6	6 M	73
UICC chrysotile A	L mean, 3.21 μm D mean, 0.063 μm	20	1		33	15	1 Ca, 14 M	55
UICC crocidolite	L mean, 3.14 μm D mean, 0.148 μm	20	1		39	21	21 M	65
Saline	–	–	1		32	0	–	67
Intrapleural injection in 0.5 ml saline (SPF Sprague-Dawley rats) (Wagner et al., *1984) [sex, age unspecified]*								
Glasswool with resin (English)	L 70% ⩽5 μm D 85% ⩽1 μm	20	1	Lifetime	48	1	1 M	–
Glasswool without resin (English)	L 57% ⩽5 μm D 85% ⩽1 μm	20	1		48			
US glasswool	L 88% ⩽5 μm D 98.5% ⩽1 μm	20	1		48	4	4 M	–
UICC African chrysotile A		20	1		48	6	6 M	–
Saline	–	–	1		24	0	–	–

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal administration of glasswool and glass fibre								
Intraperitoneal injection of glasswool in 2 ml saline (female Wistar rats, 8-12 weeks old) (Pott et al., 1976)								
Glasswool (very fine)	L 59% <3 μm	2	1	Lifetime	34	1	1 M	74
	D 50% <0.4 μm	10	1		36	4	2 M, 2 S	73
		100	4 (weekly)		32	23	20 M, 3 S	43
Glasswool (finer)	L 50% <10 μm	2	1		73	20	17 M, 3 S	96
	D 50% <0.2 μm	10	1		77	41	36 M, 4 S, 1 Ca	87
		50	2 (weekly)		77	55	47 M, 8 S	52
Glasswool (coarser)	L 50% <30 μm	20	1		37	14	12 M, 1 S, 1 Ca	87
	D 50% <1.0 μm							
UICC chrysotile A		2	1		37	6	4 M, 2 S	67
		6.25	1		35	27	24 M, 3 S	70
		25	1		31	25	21 M, 3 S, 1 Ca	58
		100	4 (weekly)		33	18	16 M, 2 S	50
UICC crocidolite		2	1		39	15	12 M, 3 S	97
7 kinds of granular dust	–	100	4 (weekly)		263	3	1 M, 1 S, 1 Ca	85
Corundum	–	50	2 (weekly)		37	3	1 M, 2 Ca	107
Saline	–	–	1		72	0	–	85
Intraperitoneal injection of glass fibres in saline (female Wistar rats, 4 weeks old) (Pott et al., 1984b)								
Glass fibres	L 50% <2.4 μm	2	1	Lifetime (some early deaths from infection in month 21)	44	2	2 (M, S)	–
	D 50% <0.33 μm							
Glass fibres		2	1		44	14	14 (M, S)	–
Glass fibres		10	1		44	29	29 (M, S)	–
Actinolite	L 50% >1.4 μm	2.5	1		45	31	31 (M, S)	–
	D 50% >0.16 μm							
UICC chrysotile B	L 50% >0.9 μm	0.4	1		44	9	9 (M, S)	–
	D 50% >0.11 μm							
Corundum	Granular	2	1		45	1	1 (M, S)	–

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal injection of glass fibres in 2 ml saline (female Sprague-Dawley rats, 8 weeks old) (Pott et al., *1987)*								
Glass fibre	L 50% <2.4 μm D 50% <0.33 μm	2	1	134 weeks	54	21	(M, S, Ca)	90
Glass fibre	L 50% <2.4 μm D 50% <0.33 μm	10	1	126 weeks	53	24	(M, S, Ca)	79
Volcanic ash, Mount St Helen's	Granular	40	2	134 weeks	54	3	(M, S, Ca)	93
Saline	–	–	2	134 weeks	54	3	(M, S, Ca)	94
Intraperitoneal injection of glass fibres in 1 ml saline (female Wistar rats, 5 weeks old) (Pott et al., *1987)*								
Glass fibre	L 50% <3.2 μm D 50% <0.18 μm	0.5	1	142 weeks	30	5	(M, S, Ca)	116
Glass fibre	L 50% <3.2 μm D 50% <0.18 μm	2.0	1	142 weeks	31	8	(M, S, Ca)	110
Glass fibre 24-h HCl treated		2.0	1	141 weeks	32	16	(M, S, Ca)	107
Crocidolite (S. Africa)	L 50% >2.1 μm D 50% >0.20 μm	0.5	1	141 weeks	32	18	(M, S, Ca)	109
Crocidolite (S. Africa)	L 50% >2.1 μm D 50% >0.20 μm	2.0	1	103 weeks	32	28	(M, S, Ca)	71
Titanium dioxide	Granular	10	3	142 weeks	32	0	–	130
Saline	–	–	1	142 weeks	32	2	(M, S, Ca)	120

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal injection of glass fibres in 2 ml saline (female Sprague-Dawley rats, 8 weeks old) (Pott et al., *1987)*								
Glass fibre	L 50% <4.8 μm D 50% <0.29 μm	5	1	108 weeks	54	44	(M, S, Ca)	64
Glass fibre 2-h HCl-treated		5	1	133 weeks	54	32	(M, S, Ca)	88
Glass fibre 24-h HCl-treated	L 50% <5.3 μm D 50% <0.5 μm	5	1	142 weeks	54	4	(M, S, Ca)	99
Glass fibre 2-h NaOH-treated		5	1	115 weeks	54	42	(M, S, Ca)	71
Glass fibre 24-h NaOH-treated	L 50% <5.4 μm D 50% <0.5 μm	5	1	106 weeks	53	46	(M, S, Ca)	72
Titanium dioxide	Granular	5	1	142 weeks	52	2	(M, S, Ca)	99
Intraperitoneal injection of glass fibres in 1 ml saline (female Wistar rats, 4 weeks old) (Pott et al., *1987)*								
Glass fibre	L 50% <4.8 μm D 50% <0.29 μm	5	1	65 weeks	45	20	(M, S, Ca)	34
Glass fibre 24-h HCl-treated	L 50% <5.3 μm D 50% <0.5 μm	5	1	146 weeks	45	2	(M, S, Ca)	113
Glass fibre 24-h NaOH-treated	L 50% <5.4 μm D 50% <0.5 μm	5	1	103 weeks	46	27	(M, S, Ca)	58
Titanium dioxide	Granular	5	1	145 weeks	47	0	–	102
Intraperitoneal injection of glass fibres in 0.5 ml saline (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
Glass fibres	L g. mean, 4.7 μm D g. mean, 0.4 μm	25	1	Lifetime	25	8	8 M	85
UICC crocidolite	L 5% > 5 μm	25	1		25	20	20 M	82
Saline	–	–			25	0	–	106
No treatment	–	–			125	0	–	103
Intraperitoneal injection of glasswool in 1 ml saline (female Syrian golden hamsters, 8-12 weeks old) (Pott et al., *1976)*								
Glasswool	L 59% <3 μm	2	1	Lifetime	40	0	–	–
		10	1		40	0	–	–
UICC chrysotile A		2	1		40	0	–	–
		10	1		40	0	–	–

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal administration of glass filaments								
Intraperitoneal injection of glass filaments in 2 ml saline (female Wistar rats, 12 weeks old) (Pott et al., *1987)*								
Glass filament (finer)	L 50% <39 μm D 50% <5.5 μm	10	1	165 weeks	50	2	(M, S, Ca)	111
Glass filament (finer)	L 50% <39 μm D 50% <5.5 μm	40	2	165 weeks	46	5	(M, S, Ca)	107
Glass filament (coarser)	L 50% <46 μm D 50% <7.4 μm	40	2	156 weeks	47	1	(M, S, Ca)	121
Glass	Granular	40	2	165 weeks	45	2	(M, S, Ca)	119
Intraperitoneal administration of glass filaments in 4 ml saline by laparotomy (female Wistar rats, 12 weeks old) (Pott et al., *1987)*								
Glass filament (finer)	L 50% <39 μm D 50% <5.5 μm	250	1	144 weeks	28	2	(M, S, Ca)	109
Intraperitoneal administration of glass filaments in 4 ml saline by laparatomy (female Wistar rats, 15 weeks old) (Pott et al. , *1987)*								
Glass filament, ES 3	L 50% <16.5 μm D 50% <3.7 μm	50	1	135 weeks	48	3	(M, S, Ca)	94
Glass filament, ES 3	L 50% <16.5 μm D 50% <3.7 μm	250	1	139 weeks	46	4	(M, S, Ca)	94
Glass	Granular	50	1	139 weeks	48	4	(M, S, Ca)	88
		250	1	130 weeks	48	4	(M, S, Ca)	99
Saline	–	–	1	139 weeks	45	2	(M, S, Ca)	87

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Cumulative exposure [mg/m³ × h]	Duration of exposure					
Inhalation exposure to rockwool and slagwool								
Inhalation exposure to respirable dust concentrations of 5 mg/m³ (Wistar rats IOPS Af/Han, equal numbers of males and females, 8-9 weeks old) (Le Bouffant et al., *1984)*								
Rockwool	L 40% <10 μm D 23% <1 μm	Not given	5 h/day, 5 days/week; total length of dusting: half the animals, 12 months, the other half, 24 months	Up to 28 months (several animals killed at 7, 12, 16 and 24 months)	47	0	–	–
Chrysotile (Canadian)	L 6% >5 μm				47	9	Pulmonary tumours	
Controls	–	–			47	0	–	–
Inhalation exposure to respirable dust concentrations of 10 mg/m³ (SPF Fischer rats) (Wagner et al., *1984) [sex, age unspecified]*								
Rockwool without resin	L 71% 5-20 μm D 58% ≤1 μm	17 495	7 h/day, 5 days/week, 12 months	Lifetime (some animals killed at 12 and 24 months)	48	2	2 A	–
UICC chrysotile (Canadian)	L 16% >20 μm D 29% >0.5 μm	17 499			48	12	1 A, 11 AdCa	–
Controls	–	–			48	0	–	–
Nose-only inhalation exposure to dust clouds of slagwool (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
		Dust conc.						
Slagwool	L g. mean, 22 μm D g. mean, 0.9 μm	7.8 mg/m³ (200 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	55	0	–	97
UICC crocidolite	L 5% >5 μm	3000 f/cm³						
Chamber controls	–	–			57	3	1 M, 2 BT	109
Room controls	–	–			59	0	–	108
					125	0	–	103
Nose-only inhalation exposure to dust clouds of slagwool (male Syrian golden hamsters, 100 days old) (Smith et al., *1987)*								
		Dust conc.						
Slagwool	L g. mean, 22 μm D g. mean, 0.9 μm	7.8 mg/m³ (200 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	69	0	–	95
UICC crocidolite	L 5% >5 μm	3000 f/cm³						
Chamber controls	–	–			58	0	–	78
Room controls	–	–			58	1	1 BT	95
					112	0	–	80

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intrapleural administration of rockwool and slagwool								
Intrapleural injection of rockwool and slagwool in 0.5 ml saline (SPF Sprague-Dawley rats) (Wagner et al., *1984) [sex, age unspecified]*								
Rockwool with resin (Sweden)	L 70% <5 μm D 52% <0.6 μm	20	1	Lifetime	48	3	3 M	–
Rockwool, resin removed	L 70% <5 μm D 58% <0.6 μm	20	1		48	2	2 M	–
Slagwool with resin (F.R. Germany)	L 67% <5 μm D 42% <0.6 μm	20	1		48	0	–	–
Slagwool, resin removed	L 80% <5 μm D 62% <0.6 μm	20	1		48	0	–	–
UICC African chrysotile		20	1		48	6	6 M	–
Saline	–	–	1		24	0	–	–

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal administration of rockwool and slagwool								
Intraperitoneal injection of slagwool in 2 ml saline (female Wistar rats, 15 weeks old) (Pott et al., *1987)*								
Slagwool, coarser (F.R. Germany)	L 50% <26 μm D 50% <2.6 μm	40	2	158 weeks	99	6	(M, S, Ca)	111
Slagwool, finer (F.R. Germany)	L 50% <14 μm D 50% <1.5 μm	40	2	155 weeks	96	2	(M, S, Ca)	107
Saline	–	–	2	150 weeks	48	0	–	101
Intraperitoneal injection of various kinds of rockwool in 2 ml saline (female Wistar rats, 8 weeks old) (Pott et al., *1987)*								
Rockwool (F.R. Germany)	L 50% <20 μm D 50% <1.8 μm	75	5	Preliminary results 28 months after first injection	53	32	(M, S, Ca)[c]	79
Actinolite (F.R. Germany)	L 50% <1.9 μm D 50% <0.17 μm	0.25	1		36	20	(M, S, Ca)[c]	90
UICC chrysotile B	L 50% >0.9 μm D 50% >0.11 μm	1	1		36	31	(M, S, Ca)[c]	63
Titanium dioxide	Granular	100	5		53	5	(M, S, Ca)[c]	109
Saline	–	–	5		102	2	(M, S, Ca)[c]	111
Intraperitoneal injection of various kinds of rockwool in 2 ml saline (female Sprague-Dawley rats, 8 weeks old) (Pott et al., *1987)*								
Rockwool (Sweden)	L 50% <23.0 μm D 50% <1.9 μm	75	3	134 weeks	63	45	(M, S, Ca)	77
Rockwool, fine (Sweden)	L 50% <4.1 μm D 50% <0.64 μm	10	1	134 weeks	45	6	(M, S, Ca)	97
Volcanic ash, Mount St Helen's	Granular	40	2	134 weeks	54	3	(M, S, Ca)	93
Saline	–	–	2	134 weeks	54	3	(M, S, Ca)	94

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Cumulative exposure [mg/m³× h]	Duration of exposure					
Inhalation exposure to ceramic fibres								
Inhalation exposure to total dust concentration of 20–120 mg/m³ (rat) (Pigott & Ishmael, 1982) [strain, sex, age unspecified]								
Ceramic fibre	D median, 3.3 um	6 700 resp. f	18 months	Lifetime (to 85%	32	0	–	–
'Aged' fibre		7 400 resp. f		mortality)	38	0	–	–
UICC chrysotile A		13 800 resp. f			39	9	5 A, 1 AdCa, 3 ScCa	–
Clean air	–	–			34	0	–	–
Inhalation exposure to respirable dust concentrations of 10 mg/m³ (AF/Han Wistar rats, 12 weeks old) (Davis, 1984) [sex unspecified]								
Ceramic fibres (aluminium silicate glass)	L ~90% <3 μm D ~90% <0.3 μm		7 h/day, 5 days/week, 12 months (224 days)	32 months (4 animals, 12 months; 4 animals, 18 months)	48	7	1 A, 3 Ca, 4 malignant unspecified	–
Controls	–	–			39	0	–	–
Nose-only inhalation exposure to dust clouds of ceramic fibres (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
Ceramic fibres	L g. mean, 25 μm D g. mean, 0.9 μm	10.8 mg/m³ (200 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	55	0	–	100
UICC crocidolite	L 5% ⩾5 μm	3000 f/cm³			57	3	1 M, 2 BT	109
Chamber controls	–	–			59	0	–	108
Room controls	–	–			125	0	–	103
Nose-only inhalation exposure to dust clouds of ceramic fibres (male Syrian golden hamsters, 100 days old) (Smith et al., *1987)*								
Ceramic fibres	L g. mean, 25 μm D g. mean, 0.9 μm	10.8 mg/m³ (200 f/cm³)	6 h/day, 5 days/week, 2 years	Lifetime	70	1	1 M	96
UICC crocidolite	L 9% ⩾5 μm	3000 f/cm³			58	0	–	79
Chamber controls	–	–			58	1	1 BT	95
Room controls	–	–			112	0	–	80

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intratracheal administration of ceramic fibres								
Intratracheal instillation of ceramic fibres in 0.2 ml saline (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
Ceramic fibres	L g. mean, 25 μm D g. mean, 0.9 μm	10	5 (weekly)	Lifetime	22	0	–	100
UICC crocidolite	L 5% ⩾0.5 μm	10	5 (weekly)		25	2	2 BT	91
Saline	–	–	5 (weekly)		25	0	–	98
No treatment	–	–			125	0	–	103
Intratracheal instillation of ceramic fibres in 0.2 ml saline (female Syrian golden hamsters, 100 days old) (Smith et al., *1987)*								
Ceramic fibres	L g. mean, 25 μm D g. mean, 0.9 μm	10	5 (weekly)	Lifetime	25	0	–	64
UICC crocidolite	L 5% ⩾0.5 μm	10	5 (weekly)		27	20	20 BT (13 benign, 7 malignant)	85
Saline	–	–	5 (weekly)		24	0	–	81
No treatment	–	–			112	0	–	80
Intrapleural administration of ceramic fibres								
Intrapleural injection of ceramic fibres in 0.4 ml saline (SPF Wistar rats, twice as many males as females, 13 weeks old) (Wagner et al., *1973)*								
Ceramic fibres (aluminium silicate)	D 0.5–1 μm	20	1	Lifetime	31	3	3 M	105
SFA chrysotile		20	1		36	23	23 M	81
SFA chrysotile		20	1		32	21	21 M	91
Aluminium oxide	Granular, D <10 μm	20	1		35	1	1 M	101
Intrapleural injection of ceramic fibres in saline (rats, equal numbers of females and males) (Pigott & Ishmael, 1982) [strain, age unspecified]								
Ceramic fibres	D median, 3.3 μm	20	1	Lifetime	48	0	–	–
'Aged' fibres		20	1		48	0	–	–
UICC chrysotile A		20	1		48	7	7 M	–
Saline	–	–	1		48	0	–	–

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal administration of ceramic fibres								
Intraperitoneal injection of ceramic fibres in 2 ml saline (AF/Han Wistar rats) (Davis et al., *1984) [sex, age unspecified]*								
Ceramic fibres (aluminium silicate glass)	L ~90% <3 μm D ~90% <0.3 μm	25	1	–	32	3	1 M, 2 FS?	–
Controls	–	–			39	2	2 M (peritoneum or digestive system)	–
Intraperitoneal injection of ceramic fibres in 2 ml saline (female Wistar rats, 8 weeks old) (Pott et al., *1987)*								
Ceramic wool	L 50% <8.3 μm D 50% <0.91 μm	45	5	Preliminary results 28 months after first injection	47	32	(M, S, Ca)[c]	51
	L 50% <6.9 μm D 50% <1.1 μm	75	5		54	12	(M, S, Ca)[c]	91
Actinolite (F.R. Germany)	L 50% <1.9 μm D 50% <0.17 μm	0.25	1		36	20	(M, S, Ca)[c]	90
UICC chrysotile B	L 50% >0.9 μm D 50% >0.11 μm	1	1		36	31	(M, S, Ca)[c]	63
Titanium dioxide	Granular	100	5		53	5	(M, S, Ca)[c]	109
Saline	–	–	5		102	2	(M, S, Ca)[c]	111

Table 41 (contd)

Substance	Fibre dimensions: length (L), diameter (D)	Dosing schedule		Length of observation	No. of animals examined	No. of animals with tumours[a]	Histological type[b]	Median or average survival time [weeks]
		Total dose [mg]	No. of applications					
Intraperitoneal injection of ceramic fibres in 0.5 ml saline (female Osborne-Mendel rats, 100 days old) (Smith et al., *1987)*								
Ceramic fibres	L g. mean, 25 μm D g. mean, 0.9 μm	25	1	Lifetime	23	19	19 M	69
Saline	–	–	1		25	0	–	106
No treatment	–	–			125	0	–	103
Intraperitoneal injection of ceramic fibres in 0.5 ml saline (male Syrian golden hamsters, 100 days old) (Smith et al., *1987)*								
Ceramic fibres	L g. mean, 25 μm	25	1	Lifetime	15	2	2 M	66
	D g. mean, 0.9 μm	25	1		21	5	5 M	70
Saline	–	–	1		25	0	–	72
No treatment	–	–			112	0	–	72

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

[b]A, adenoma; AdCa, adenocarcinoma; BT, bronchoalveolar tumour; Ca, relatively undifferentiated epidermoid carcinoma; FS?, somewhat similar to a fibrosarcoma; M, mesothelioma; (M, S), mesothelioma and/or sarcoma; (M, S, Ca), mesothelioma, sarcoma and/or carcinoma (rarely) in the abdominal cavity, excluding tumours of the uterus; P, papilloma; ScCa, squamous-cell carcinoma; T, other lung tumours (fibrosarcoma, lymphosarcoma, lung metastases)

[c]Partly macroscopic diagnosis only

–, not applicable

3.2 Other relevant data

(*a*) *Experimental systems*

(i) *Deposition, retention and clearance*

A number of mechanisms result in the deposition of inhaled particles, both fibrous and nonfibrous, in the respiratory tract (Lippmann *et al.*, 1980). Deposition in the naso-pharyngeal region occurs mainly by inertial impaction due to the high velocity and abrupt changes in direction of the airstream. Deposition in the tracheobronchial region is determined by inertial impaction and by gravitational settling. A disproportionate amount of deposition in this region of both nonfibrous (Lippmann & Schlesinger, 1984) and fibrous (Morgan *et al.*, 1975) particles occurs at airway bifurcations. On the basis of studies on humans, the estimated deposition of monodisperse particles in the pulmonary region peaks for mouth-breathing subjects at an aerodynamic equivalent diameter of ~3 μm and for nose-breathing subjects at ~2.5 μm (Lippmann *et al.*, 1980). [The aerodynamic equivalent diameter of a particle is the diameter of a spherical particle of unit density which has the same falling speed.] Particles of this size are deposited mainly by sedimentation, but, for submicron particles, deposition by diffusion prevails. Other mechanisms are also important for fibrous materials: interception is important when the length of fibres becomes a significant fraction of the airway diameter; however, when fine, straight fibres are inhaled they tend to align themselves along the axes of airways due to the aerodynamic forces acting upon them so that they can penetrate effectively to the pulmonary region (Lippmann *et al.*, 1980). The electrostatic enhancement of lung deposition of fibrous aerosols was reviewed by Vincent (1985), who suggested that it is important for polydisperse fine fibres.

Rats were exposed by nose-only inhalation for 30 min to glass microfibres and to UICC standard reference samples of asbestos, and deposition was measured using a radioactive tracer technique. The amount of fibre respired was calculated from the aerosol concentration, exposure time and minute volume (Hammad *et al.*, 1982). For glass microfibre and anthophyllite, which had activity median aerodynamic diameters of 2.3 and 2.0 μm, respectively, measured with the Cascade Centripeter, ~70% of the respired glass fibre was deposited throughout the respiratory tract, compared with less than half of the chrysotile and of the finer amphibole fibres (activity median aerodynamic diameters, 1.2–1.5 μm). [The activity mean aerodynamic diameter of an aerosol is determined from the distribution of radioactivity on the stages of a size-classifying sample previously calibrated with spherical particles of unit density. If the radioactivity is homogeneously distributed within the material, which is likely to be the case in the experiments described above, the activity mean aerodynamic diameter and mass median aerodynamic diameter will be identical.] Deposition in the alveolar region was relatively unaffected by activity median aerodynamic diameter and averaged about 11% (Morgan *et al.*, 1977). In later studies by the same workers, rats were exposed to sized glass fibres with nominal diameters of 1.5 and 3 μm and lengths ranging from 5 to 60 μm. A similar radioactive tracer technique was used. All of the respired longer (≥30 μm), 1.5-μm diameter fibre was deposited, mainly in the upper respiratory tract; the same applied to thick fibres (diameter, 3 μm) ≥10 μm in length.

Deposition of these materials in the alveolar region was negligible and, in rats, appeared to peak at an aerodynamic diameter of ~2 μm, which is less than that in humans (Morgan *et al.*, 1980).

Rats were exposed by nose-only inhalation for six days to unsized man-made mineral fibres. The fibres had a count median diameter of ~1 μm and a count median length of ~10 μm. They were recovered from lungs using a low-temperature ashing technique, and the fibre content of lung tissue was compared, for different size categories, with the estimated number of fibres respired. The retention of fibres with diameters <0.5 μm reached a peak of 8% at a fibre length of 21 μm; the retention of fibres with diameters >0.5 μm was <1% for all fibre lengths. A correlation of retention with calculated aerodynamic equivalent diameter confirmed that fibres with an aerodynamic equivalent diameter of >3.5 μm were not found in the lung (i.e., were not respirable) (Hammad *et al.*, 1982). These results, combined with those obtained using sized man-made mineral fibres, indicate that deposition in the alveolar region of rat lung must fall rapidly from a maximum at an aerodynamic equivalent diameter of 2 μm to effectively zero at about 3.5 μm.

Rats were exposed chronically to 'microfibre' glasswool (JM 100) and to thicker glass- and rockwool fibres at a concentration of 10 mg/m^3 on five days per week for periods of up to one year. The count median diameter of the glass microfibre (<0.5 μm) was less than that of either the rockwool (0.5–1 μm) or of the thicker glasswool (~1 μm). After one year's exposure, the weights in the lung were 4.45 mg microfibre, 0.94 mg thicker glasswool and 3.11 mg rockwool, indicating that the microfibre was more respirable. No fibre longer than 30 μm was found in the lungs, although they were present in the airborne dust cloud (Wagner *et al.*, 1984). In a similar study, Le Bouffant *et al.* (1987) exposed rats to the same microfibre (JM 100) and to aerosols of different samples of thicker glass- and rockwool for periods of up to two years. In this study also, larger quantities of microfibre than of the thicker glasswool or the rockwool were found in the lungs. [The Working Group noted that, in the case of chronic inhalation exposure to fibres, it is difficult to derive accurate data on deposition, as clearance takes place simultaneously.]

After exposure of rats and hamsters by inhalation to monodisperse particles, the deposited material is not distributed evenly between the lung lobes: the apical region of the right lung receives a higher relative concentration, and the diaphragmatic regions receive less (Raabe *et al.*, 1977). Similar observations have been made for glass fibres (Morgan *et al.*, 1980) and for ceramic fibres; the disproportion of fibres between lobes increased with aerodynamic diameter (Rowhani & Hammad, 1984).

The physical clearance of particles deposited in the alveolar region of the lung is thought to be mediated by pulmonary alveolar macrophages (Morgan, A. *et al.*, 1982). These phagocytic cells are found both in the interstitium and free in the alveolar spaces. Count median diameters of rat pulmonary alveolar macrophages range from 11 to 12 μm (Sykes *et al.*, 1983a). Fibres that can be encompassed in their entirety by pulmonary alveolar macrophages can be mobilized and transported to the terminal bronchioles, from where they are cleared from the lung by mucociliary action. Fibres that are too long to be engulfed by a single cell may remain at the site of deposition or penetrate into the interstitium

(Morgan, 1979). Similar size considerations apply to the clearance of fibres through lymph nodes associated with the lung (Le Bouffant *et al.*, 1987).

Sized glass fibres (0.5 and 1 mg) were administered to rats by intratracheal instillation. Animals were killed serially, the lungs digested with sodium hypochlorite (Morgan & Holmes, 1984a) and the number of fibres determined by optical microscopy. Of the 5 × 1.5 μm (diameter) fibres and the 10 × 1.5 μm fibres present in the lung immediately after instillation, only 10% and 20%, respectively, remained in the lung after one year. With 30 × 1.5 μm fibres and 60 × 1.5 μm fibres, there was no evidence of clearance over the same period, suggesting that the critical length of fibres for removal from the lung is between 10 and 30 μm (Morgan, A. *et al.*, 1982). In studies with some of the same sized glass fibres, a radioactive tracer method (^{65}Zn; half-life, 245 days) was used to quantify the clearance of 5 × 1.5 μm and 60 × 1.5 μm fibres from the lung of rats. In contrast to the results of the previous study, it was reported that there was relatively rapid clearance of both types of fibre (half-life, one month) and that the clearance curves did not differ significantly (Bernstein *et al.*, 1980). [The Working Group noted that observations that are based on the removal of a radioactive constituent of the fibre from the lung do not enable physical clearance to be distinguished from dissolution and may give misleading results.]

In the same study, only short glass fibres were found in regional lymph nodes 18 months after intratracheal instillation (Bernstein *et al.*, 1984). At various times after exposure of rats by inhalation to glass microfibres (JM 100) and to thicker glass- and rockwool fibres, much greater numbers of the thin microfibres than of either glass- or rockwool were transported to the tracheobronchial lymph nodes. With all of these materials, the fibres in the lymph nodes were shorter than those in the lungs, with very few fibres >10 μm in length (Le Bouffant *et al.*, 1987).

Following injection of glasswool (JM 104; count median length, 6 μm; count median diameter, 0.23 μm) or asbestos into the pleural cavity of rats, translocation of glass fibre (in terms of number concentration) to the mediastinal lymph nodes was less than that of the asbestiform minerals; however, in mass terms, they were equivalent. Less than 1% of the injected fibres was transported to the lung, but, as ascertained by transmission electron microscopy, the mean length of fibres recovered from lung increased with time. Fibres at concentrations of 10^6–10^7 fibres/g of tissue were detected in a range of organs, including lung, spleen, kidney, liver and brain; in the intrathoracic lymph nodes, the concentration was ten- to 100-fold higher. These figures suggest that migration occurred *via* the bloodstream (Monchaux *et al.*, 1982).

Solubility in vivo: The solubility of man-made mineral fibres and asbestos fibres, both *in vivo* and *in vitro*, has been reviewed (Morgan & Holmes, 1986).

In a study of lung clearance using sized glass fibres, Morgan, A. *et al.* (1982) noted that short (≤10 μm) fibres dissolved quite slowly and uniformly in rat lung. Fibres of ≥30 μm in length dissolved much more rapidly and less uniformly; after 18 months, some had become so thin that they had fragmented, while the diameters of others were relatively unchanged. These observed variations in solubility were attributed to differences in physiological pH; for example, the intracellular pH of pulmonary alveolar macrophages is lower than that of the general lung environment (Laman *et al.*, 1981). Following administration of the same

fibres to rats, the fibres in lung sections were characterized using scanning electron microscopy. It was noted that long fibres that had been engulfed by pulmonary alveolar macrophages had dissolved more extensively than those lying free in the alveolar spaces (Bernstein *et al.*, 1984). In both of these investigations, it was noted that the ends of long glass fibres dissolved more rapidly than the middle. In a later, analogous study, dimensional changes of sized rockwool fibres (count median length, 27 μm; count median diameter, 1.1 μm) in rat lung were characterized following their administration by intratracheal instillation. After 18 months, there was no change in the median diameter at the middle of the fibres, but it was observed qualitatively that fibres were becoming thinner at their ends. The authors concluded that the rockwool sample tested was much less soluble *in vivo* than the glasswool tested previously (Morgan & Holmes, 1984b).

Rats were exposed by inhalation to 'microfibre' glasswool (JM 100), to a thicker glasswool and to rockwool fibres for one or two years. At the end of the dusting period, gravimetric measurements showed that much greater quantities of the microfibre had been retained; however, after a further 16 months without dusting, the concentration of glass microfibres in the lung had been reduced to an extent similar to that of the thicker glasswool and rockwool fibres, indicating either a more rapid clearance or more rapid dissolution. Scanning electron microscopy of glass- and rockwool fibres isolated from the lung by low-temperature ashing showed that their surfaces were eroded; examination under the analytical electron microscope revealed that certain constituents of these fibres (mainly sodium and calcium) had been lost (Le Bouffant *et al.*, 1987). Glass microfibres removed from rat lung following chronic exposure and examined by transmission electron microscopy appeared to be more susceptible to surface etching (irregularities in their outlines, loss of electron density, appearance of pits along their edges) than either thicker glasswool or rockwool fibres (Johnson *et al.*, 1984).

The durability of some man-made mineral fibres, including various glasswool, rockwool and ceramic fibres, was studied in rat lung over a period of two years following intratracheal instillation. Both the number of fibres and the size distribution of fibres remaining in the lung were determined by transmission electron microscopy. Count median diameters ranged from 0.1–0.2 μm for glass microfibres to 1.8 μm for rockwool. In all cases, fibres <5 μm in length were removed from the lung more rapidly than longer fibres; however, there was a wide variation in the clearance rates of the latter. Acid-treated JM 104 E glass microfibre was cleared very rapidly, apparently by dissolution; untreated microfibre (JM 104/Tempstran 475) was scarcely cleared at all, but some leaching of sodium and calcium was detected. Of the other fibres, the ceramic fibre had the longest residence time (half-life, 780 days for fibres >5 μm in length), compared with 280 days for rockwool fibres >5 μm in length and for thicker glasswool. The authors concluded that fibres with a high calcium content dissolve most rapidly *in vivo* and that calcium content is a more important determinant of solubility than sodium or potassium content (Bellmann *et al.*, 1987).

Solubility in vitro: A number of studies have been made of the solubility of man-made mineral fibres *in vitro*, using both static and continuous-flow systems. [The Working Group noted that the latter approximates more closely to the situation *in vivo*.] The dissolution of

specific constituents has been quantified by analysis of the leachate (Förster, 1984; Klingholz & Steinkopf, 1984).

Man-made mineral fibres were quite stable in water at 37°C, but their solubilities increased in simulated extracellular fluid: with Gamble's solution, fibres dissolved more rapidly in continuous-flow than in static systems; it was reported in one study that slagwool dissolved more rapidly than glasswool, which dissolved more rapidly than rockwool (Klingholz & Steinkopf, 1984). [The Working Group noted that only single samples of each type of fibre were tested.] With glass fibres, the square root of the weight of individual undissolved fibres decreased linearly with leaching time, and glass composition appeared to be a major determinant of the rate of dissolution (Leineweber, 1984), as also appeared to be the case *in vivo* (Bellmann *et al.*, 1987). In a study of dissolution in physiological media, precipitation of alkali earth carbonates occurred at higher than physiological temperatures (60°C). Rates of dissolution of 10 ng/cm^2 per hour or higher were measured at 37°C, indicating that fine fibres (diameter, <1 μm) could dissolve completely after one year in a continuous-flow system (Förster, 1984). The surface layers of leached fibres were converted to colloidal shells (Förster, 1984; Klingholz & Steinkopf, 1984).

The dissolution of silica from a range of industrial man-made mineral fibres (including glasswool, rockwool, slagwool and ceramic fibres) was compared *in vitro* with that of natural amphibole fibres, using a solution with a similar composition to Gamble's. The man-made mineral fibres showed a variety of calculated dissolution velocities, ranging from 0.2 to 3.5 nm/day. The corresponding value for natural amphibole fibre was <0.01 nm/day. Dissolution velocities for glass fibres showed a 15-fold variation: the samples of rockwool and slagwool had intermediate solubility among the fibres tested, and the solubility of the ceramic fibres was generally at the lower end of the range (Scholze & Conradt, 1987). Leineweber (1984) also found great variability in the solubility of glass fibres; one ceramic fibre was found to be highly insoluble.

[The Working Group noted that it is important to attempt to predict in-vivo solubility when estimating the possible biological effects of man-made mineral fibres; however, it is difficult to reproduce *in vitro* the varying conditions of pH and concentrations of complexing agents which fibres encounter in the intra- and extracellular environments of the lung. Furthermore, no overall generalization regarding the absolute or relative solubilities of the main families of such fibres can be made on the basis of the results of the studies reported. For example, while most samples of glasswool studied have proved to be relatively soluble and ceramic fibres relatively insoluble, there have been exceptions — at least one sample of glass fibre was extremely durable and one sample of ceramic fibres relatively soluble (Leineweber, 1984).]

(ii) *Toxic effects*

The toxic effects of man-made mineral fibres *in vivo* and *in vitro* have been reviewed (Hill, 1978; Konzen, 1980; Davis, 1986).

Toxicity in vivo: All 20 hamsters that received an intratracheal instillation of 7 mg of glass microfibre (median diameter, 0.1 μm) died within 30 days; the lungs were haemorrhagic and oedematous. In contrast, only 3/20 animals instilled with a thicker

microfibre (median diameter, 0.2 μm) died during this period, and no animal died in groups injected with three types of glass fibre used for insulation purposes (median diameters, 2.3–4.1 μm) (Pickrell *et al.*, 1983). Similar acute deaths were reported in rats following intratracheal instillation of 3–70 mg of very finely-ground (particulate) ceramic fibre (mean size, 0.04 μm), but not following instillation of another preparation of the same material containing coarser dusts (mean size, 0.7–0.8 μm) (Gross *et al.*, 1956). Acute deaths from haemorrhagic peritonitis also occurred in two groups of hamsters (21/36 and 15/36) that received intraperitoneal injections of 25 mg of refractory ceramic fibre (median diameter, 1.8 μm) (Smith *et al.*, 1987).

No toxic effect was found in cats that had inhaled finely-ground rockwool dust (average particle size, 2.2 μm) for two months (total dust levels, 50–900 mg/m^3) (Fairhall *et al.*, 1935).

Decreases in haemoglobin levels and erythrocyte counts, coupled with an increase in reticulocyte count, were reported in rats that had inhaled lead silicate glass fibres at a dose level of 100 mg/m^3 for 4.5 months (Azova *et al.*, 1971).

Rubbing of the shaved skin of guinea-pigs with a tampon of glasswool produced erythema and, rarely, punctiform haemorrhages. Glass fibres were found embedded only in the epithelial layers of the skin (Pellerat & Condert, 1946).

Pulmonary inflammation: Glass fibre (1 mg; nominal diameter, 1.5 μm) was administered to rats by intratracheal instillation. One or seven days after instillation, the number of neutrophil leucocytes in the cell population (obtained by bronchoalveolar lavage) was increased by at least ten-fold over that in controls administered saline. Levels of lactate dehydrogenase in the lavage fluid were raised at seven days, although not at one day, following instillation (Sykes *et al.*, 1983b).

Rats were exposed by inhalation to US glasswool (JM 102; diameter, 0.1–0.6 μm) for six months; cell populations (obtained by bronchoalveolar lavage) contained 5–10% of lymphocytes and many multinucleate giant cells (10–20% of the cell population). In culture, more macrophages from the treated animals formed erythrocyte aggregating rosettes than did control macrophages (Miller, 1980).

Interaction with cells: Short, thin man-made mineral fibres deposited in lung tissue are rapidly phagocytosed by macrophages (Davis *et al.*, 1970). Long fibres cannot be engulfed completely by single macrophages and so protrude from them (Miller, 1980). Complete engulfment of long fibres is accomplished by the formation of multinucleated giant cells (Schepers, 1955; Sethi *et al.*, 1975; Miller, 1980).

Deposition of various man-made mineral fibres in lung tissues produces ferruginous bodies, most of which appear to form in relation to giant cells (Davis *et al.*, 1970; Botham & Holt, 1971). Factors such as fibre length and thickness which predispose fibres to become coated have been discussed by Morgan and Holmes (1985).

After exposure of guinea-pigs by inhalation to glass fibres, a very thin coating was detected (using Perls' stain) within 48 h on some of the glass fibres inside macrophages. After one month, the typical golden-yellow coating could be seen on fibres by phase-contrast microscopy; it was continuous initially and became segmented with time. By 18 months, the

fibres had a beaded appearance and were fragmenting. In hamsters exposed by intratracheal administration to sized glass fibres (3 μm in diameter), partially coated fibres were detected after one month with 60- and 100-μm-long fibres and after two months with 10- and 30-μm — a similar time scale to that observed by Botham and Holt (1971) in guinea-pigs. Fibres <10 μm in length did not become coated (Holmes *et al.*, 1983). The first signs of coating of rockwool fibres in hamsters were detected after about two months; coating occurred only on fibres <2 μm in diameter and was often discontinuous on the longer fibres and did not appear to inhibit their dissolution (Morgan & Holmes, 1984b).

It is likely that all the man-made mineral fibres considered produce ferruginous bodies (Davis *et al.*, 1970).

Alveolar lipoproteinosis: Rats and hamsters exposed for 90 days to 400 mg/m³ of mainly short glass fibres (<2 μm) developed areas in the lung where alveoli were filled with granular material (lipoproteinosis), which regressed during a one-year period following termination of dusting. Guinea-pigs treated similarly developed very little alveolar lipoproteinosis (Lee *et al.*, 1979). Rats and hamsters treated with glass fibre developed alveolar lipoproteinosis, but those treated with ceramic fibres (potassium octatitanate, pigmentary potassium titanate) or amosite asbestos did not (Lee & Reinhardt, 1984).

Rats exposed for one year to 10 mg/m³ respirable ceramic fibre dust (90% fibres with diameter <3 μm) developed alveolar lipoproteinosis. While most of the mass of the ceramic fibre dust cloud consisted of relatively thick fibres (diameter, 2–3 μm), many extremely small nonfibrous particles of ceramic material were also present (Davis *et al.*, 1984).

Fibrosis: Rats and guinea-pigs exposed by inhalation to dust from glasswool and then to 'glass cotton' ([fibre length not given] fibre diameter, 3-6 μm) at a dose level of 4 mg/m³ for two to four years developed no fibrosis; minor areas of focal atelectasis and proliferation of alveolar epithelial cells occurred close to the terminal bronchioles (Schepers, 1955; Schepers & Delahant, 1955).

Rats and hamsters were exposed by inhalation to 100 mg/m³ glass fibre (average length, 10 μm; average diameter, 0.5 μm) for 24 months; in most cases only a normal 'dust reaction' was seen in lung tissue; however, a few of the oldest rats showed some foci of 'septal collagenous fibrosis' (Gross *et al.*, 1970a).

Exposure of rats by inhalation to high doses (1200 mg/m³) of 'microfibre' glasswool (JM 102; all fibres <1 μm in diameter) for eight weeks produced no pulmonary fibrosis during the subsequent four weeks; lung tissue showed only a 'dust reaction' (Hardy, 1979). Exposure of rats, hamsters and guinea-pigs to glass fibre dust (average diameter, 1.2 μm; only 15% with aspect ratio, >3:1; concentration of fibres >5 μm in length, approximately 700/cm³) at a dose level of 400 mg/m³ for 90 days produced no significant fibrosis during the subsequent two years (Lee *et al.*, 1979).

Rats were treated in two studies with dust clouds of 'microfibre' glasswool (JM 100; mean diameter, 0.2–0.5 μm) and, in one study, with a thicker glasswool (median diameter, 1–2 μm). During the subsequent 24 months, the animals developed a pulmonary reaction

described as 'minimal interstitial cellular reaction to the dust with no evidence of fibrosis' (McConnell *et al.*, 1984; Wagner *et al.*, 1984).

Rats and hamsters were treated by nose-only inhalation to dust clouds of four types of glasswool with mean diameters ranging from 0.45–6.1 μm at dose levels of up to 3000 fibres/cm³ for 24 months. Levels of pulmonary fibrosis were extremely low (Smith *et al.*, 1984, 1987).

'Microfibre' glasswool (JM 100; diameter, <1 μm) and one thicker sample of glasswool (diameter, 1–3 μm) were administered to rats by inhalation at dose levels of 5 mg/m³ for up to 24 months. 'Slight septal fibrosis' was reported with all three dusts, which tended to diminish after dusting had stopped (Le Bouffant *et al.*, 1987).

Baboons exposed to 7.5 mg/m³ glasswool JM 102 and 104 (median diameter, 0.5–1.0 μm) for up to 30 months showed limited pulmonary fibrosis (Goldstein *et al.*, 1983, 1984).

Rockwool (Wagner *et al.*, 1984; Le Bouffant *et al.*, 1987), slagwool and ceramic fibre (Smith *et al.*, 1987) produced similar low levels of pulmonary fibrosis in rats and hamsters exposed by inhalation at doses of 0.5–10 mg/m³. In contrast, in another study, rats exposed by inhalation to ceramic fibre dust at a similar dose level (10 mg/m³) for one year developed significant levels of pulmonary interstitial fibrosis by the age of 2.5–3 years (Davis *et al.*, 1984).

Fibres of potassium octatitanate (a ceramic fibre, 3–15 μm in length) produced pulmonary fibrosis in guinea-pigs, hamsters and particularly rats following long-term inhalation at dose levels between 3000 and 40 000 fibres/cm³ for three months. By two years after exposure, the lesions were well collagenized but were less frequent and less developed than those produced by asbestos (Lee *et al.*, 1981; Lee & Reinhardt, 1984).

One year after intratracheal instillation of 50 mg glasswool dust (length, 20–50 μm) into guinea-pigs, focal areas of pneumonitis were reported but no pulmonary fibrosis (Vorwald *et al.*, 1951). Focal atelectasis but no fibrosis was also reported after administration of two samples of glasswool fibres (diameter, ≤3 μm) to guinea-pigs by intratracheal instillation of three doses of 25 mg (Schepers & Delahant, 1955). These studies were later expanded to include rats, rabbits and monkeys (Schepers, 1976). Sometimes, a severe tissue reaction occurred in response to the injected dust, but this did not progress to lasting fibrosis.

Intratracheal instillation of 10.5 mg of fine glass fibre (average diameter, 1 μm) in rats and hamsters produced inflammatory lesions that were no longer present one year later (Gross *et al.*, 1970a,b). In contrast, intratracheal instillation of 50 mg of glass fibre dust (diameter, 3 μm; length, 5–8 μm) into rats produced a proliferation of fibroblasts in the pulmonary interstitium and a progressive fibrosis (Wenzel *et al.*, 1969).

Definite areas of pulmonary fibrosis were found in guinea-pigs that received intratracheal instillations (total dose, 12 mg) of long glass fibres (50% or 92% longer than 10 μm) but not in those given short fibres (length, <5 μm or 93% <10 μm; total dose, 25 mg) (Wright & Kuschner, 1977).

After intratracheal instillation of ceramic fibre to rats at a dose of 10.5 mg, dust deposits in the lung tissue were surrounded by inflammatory cells (Gross *et al.*, 1970b).

The effects of binders, coating agents and plastic dust on the pathogenicity of glass fibres has been examined by inhalation and by intratracheal instillation in several species. No effect on the fibrogenicity of the mineral fibres was observed (Schepers *et al.*, 1958; Schepers, 1959, 1961; Gross *et al.*, 1970a). However, glass fibres in combination with styrene were reported to cause more cuboidal metaplasia of the bronchiolar lining cells in mice than styrene alone (Morisset *et al.*, 1979).

Intraperitoneal injection of 10 mg glass fibre (mean diameter, 0.05–0.1 μm or 2.5–4 μm) and three samples of 'man-made insulation fibres' (median diameter, 4–10 μm) into mice caused cellular granulomata, which eventually became collagenized. The degree of cellular response and subsequent fibrosis depended on the fibre length of the dust preparations, finely ground material being much less effective than dust containing long fibres (Davis, 1972).

Toxicity in vitro: Treatment of guinea-pig alveolar and peritoneal macrophages with glass fibre (diameter, 0.25–1 μm; length, 1–20 μm) at a dose of 75 μg/ 10^6 cells increased cell membrane permeability, as determined by erythrosin staining of cells and liberation of lactic acid dehydrogenase, but did not affect overall cell metabolism, as measured by lactic acid production (Beck *et al.*, 1972; Beck & Bruch, 1974; Bruch, 1974; Beck, 1976a,b).

Fine glass fibre preparations (nominal diameters, 0.05–0.1 μm) caused greater release of both lactic dehydrogenase and β-glucuronidase in mouse peritoneal macrophages *in vitro* than thicker samples (1.5–2.5 μm). When respirable fractions of each sample were tested, only that of the thicker glass fibre increased cytotoxicity over that induced by the bulk sample (Brown *et al.*, 1979a; Davies, 1980).

For each of three pairs of long and short glass fibre preparations, long-fibred dust at a dose level of 100 μg/ 10^6 cells produced more toxicity to rat and guinea-pig alveolar macrophages, as measured by release of lactic dehydrogenase and β-glucuronidase than short-fibred preparations; short fibres showed some toxicity if their diameter was small enough (Tilkes & Beck, 1983a). Long fibres (⩾4 μm) produced a greater release of both prostaglandins and β-glucuronidase in rat alveolar macrophages than did short fibres (<3 μm) (Forget *et al.*, 1986). Long glass fibre increased the membrane permeability of L-cells (fibroblasts), causing release of lactic dehydrogenase. This effect was absent when the fibre was finely ground (Beck *et al.*, 1971). Of four glass fibre preparations, an ultrafine preparation (mean diameter, 0.19 μm) caused a greater reduction in cell numbers and in cellular uptake of ^{3}H-thymidine by phagocytic ascites tumour cells than did three thicker specimens (mean diameters, 0.2–0.43 μm). The toxicity of the fibres increased with increasing length and dose (Tilkes & Beck, 1980, 1983b).

Potassium octatitanate fibres (ceramic fibres) caused marked release of both lactic dehydrogenase and β-glucuronidase in mouse peritoneal macrophages (Chamberlain *et al.*, 1979). The viability of $P388D_1$ cells (permanent line of macrophage-like cells) up to 48 h appeared to be unaffected by 50 μg ceramic fibre dust/ml solution containing 10^5 cells (Davis *et al.*, 1985; Wright *et al.*, 1986).

Very fine glass fibres (JM 100) were much more active than thicker fibres (JM 110) in reducing the cloning efficiency of Chinese hamster V79/4 cells and in increasing the mean

cell diameter in A549 cells (transformed human type II pneumocytes). Glass powder (crushed bulk glass) showed little activity (Chamberlain & Brown, 1978). Respirable fractions of JM 100 fibres had similar activity to manually crushed material; the respirable fraction of JM 110 fibres showed activity approaching that of JM 100 preparations (Brown *et al.*, 1979a). Commercial samples of unspecified glasswool, rockwool and slagwool reduced the cloning efficiency of V79/4 cells but were much less active than crocidolite asbestos. Removal of the resin binder slightly increased their activity, and they increased the diameter of A549 cells under these conditions (Brown *et al.*, 1979b). A sample of potassium octatitanate fibres was very active in both the A549 and V79/4 assays (Chamberlain *et al.*, 1979).

In lung fibroblast cultures, glass fibre induced only a slight increase in collagen production, in contrast to chrysotile asbestos (Richards & Morris, 1973).

Long (unmilled) glass fibres (JM 100; diameter, 0.2 μm; length, 15 μm) were more toxic than short (milled) fibres (diameter, 0.2 μm; length, 2 μm) in a dye exclusion test and in a colony-forming assay with a permanent cell line of rat tracheal epithelial cells (Ririe *et al.*, 1985). Similar cultures of hamster tracheal epithelial cells showed greater production of ornithine decarboxylase when treated with long glass fibres (JM 100) than with glass particles (Marsh *et al.*, 1985).

Neither 'small' nor 'large' glass fibres (JM 100 and JM 110) inhibited blastoid transformation or β_2-microglobulin production in cultures of human peripheral blood lymphocytes. Similarly, natural killer cell activity and antibody-dependent cell-mediated cytotoxicity were unaffected. Chrysotile asbestos proved very active in these test systems (Nakatani, 1983).

(iii) *Effects on reproduction and prenatal toxicity*

No adequate data were available to the Working Group.

(iv) *Genetic and related effects*

After treatment of C3H 10T1/2 cells with potassium octatitanate (Fybex®; 0–250 μg/ml), no DNA damage was observed, as measured by sensitivity to S_1 nuclease, whereas crocidolite and erionite gave positive results when tested at a dose of 250 μg/ml (Poole *et al.*, 1986).

Fine and coarse glass fibres (JM 100 and 110; mean particle lengths, 2.7 μm and 26.0 μm; mean particle diameters, 0.12 μm and 1.9 μm, respectively) did not induce mutation in *Escherichia coli* strains B/r, WP2, WP2 *uvrA* and WP2 *uvrA polA* or in *Salmonella typhimurium* strains TA1535 and TA1538, either in the presence or absence of rat liver microsomal enzymes (S9 mix). Both types of glass fibre were tested over a wide range of concentrations (1–5000 μg/plate) (Chamberlain & Tarmy, 1977).

Glasswool (JM 100 and 110) did not increase sister chromatid exchange in Chinese hamster ovary (CHO-K1) cells *in vitro* at doses of 0.001–0.05 mg/ml or in human fibroblasts or lymphoblastoid cells after treatment of the cells with 0.01 mg/ml (Casey, 1983).

Exposure of CHO-K1 cells to 0.01 mg/ml glass fibres (nominal diameters, 1.5–2.5 μm; >60% longer than 20 μm; Wagner *et al.*, 1973) for 48 h or five days did not increase the

frequency of chromosomal aberrations or polyploid cells (Sincock & Seabright, 1975). [The Working Group noted that only one dose level was tested.]

In a preliminary study, an increase in chromosomal aberrations was observed in CHO-K1 cells after treatment with JM 100 glasswool (0.01 mg/ml), but not with the same dose of JM 110 (Sincock, 1977). This finding was confirmed in a later study in which JM 100 (0.01 mg/ml) induced chromosomal breaks and rearrangements in CHO-K1 cells, while JM 110 glass fibres had no effect; some increase in polyploidy was observed with both fibres (Sincock *et al.*, 1982).

Statistically significant increases in numerical chromosomal changes (aneuploidy and tetraploidy) as well as in the number of binucleated and micronucleated cells were observed after treatment of Syrian hamster embryo cells with JM 100 glasswool (2 μg/cm^2; diameter, 0.2–0,2 μm). A slight increase, which was not statistically significant, was also noted in the number of chromosomal aberrations. JM 110 glasswool (average diameter, 0.8 μm) was much less potent in inducing cytogenetic damage at the same dose level; a significant increase was observed only in the number of binucleated cells. Milling of JM 100 glasswool abolished its ability to induce cytogenetic effects (Oshimura *et al.*, 1984).

JM 110 glasswool (0.02 mg/ml; nominal diameter, 1.5–2.5 μm) was applied to Chinese hamster V79-4 cells as both total material and respirable fraction. Only the respirable fraction increased chromosomal breaks and fragments significantly (Brown *et al.*, 1979a).

No increase in chromosomal damage or polyploidy was observed in primary human fibroblasts or in human lymphoblastoid cells after exposure of the cells to 0.01 mg/ml JM 100 or JM 110 glasswool (Sincock *et al.*, 1982). [The Working Group noted that only one dose level was tested.] It was reported in an abstract that a slight increase in chromosomal breaks was noted in cultured human primary mesothelial cells after treatment with glass fibres (Linnainmaa *et al.*, 1986).

Both JM 100 and JM 110 glasswool induced a linear, dose-dependent increase in the frequency of transformed colonies of Syrian hamster embryo cells in culture (dose range, 0.1–10 μg/cm^2) after a single treatment of the cells. Thin glass fibres (JM 100; average diameter, 0.1–0.2 μm) were as active as asbestos. When compared on a per-weight basis, thick glass fibres (average diameter, 0.8 μm) were 20-fold less potent than thin fibres (average diameter, 0.13 μm) in inducing cell transformation. When the average length of thin glass fibres was reduced from 9.5 to 1.7 μm by milling, there was a ten-fold decrease in transforming activity; there was no activity when the average fibre length was reduced to 0.95 μm. The cytotoxicity of the glass fibre dusts was found to correlate with their transforming potency (Hesterberg & Barrett, 1984). As reported in an abstract, in similar studies in the same cell systems, JM 100 wool, but not JM 110, increased the frequency of cell transformation (Mikalsen *et al.*, 1987).

Ceramic fibres (potassium octatitanate, Fybex®) at 6.2 and 12.5 μg/ml caused low levels of transformation of C3H 10T1/2 cells *in vitro* (Poole *et al.*, 1986).

(*b*) *Humans*

(i) *Deposition, retention and clearance*

There are no experimental data on the effects of fibre dimensions on the deposition of man-made mineral fibres in humans. Studies of the falling speeds of fibres indicated that they are probably respirable only if their actual diameter is <3.5 μm (Timbrell, 1965). This hypothesis was confirmed subsequently by an examination of fibres extracted from the lungs of Finnish anthophyllite miners. The mean value for the maximum diameter of fibres from various lung lobes was 3.4 μm (Timbrell, 1982). A rough approximation of the aerodynamic equivalent diameter of a fibre may be obtained by multiplying its *actual* diameter by 3 (Timbrell, 1965). However, the precise relationship depends upon fibre length, shape and density.

A number of studies of asbestos fibres in humans confirm that short fibres are cleared preferentially from the lung (e.g., Morgan & Holmes, 1982). From studies in mine workers, the critical length above which fibres cannot be cleared from the lung has been estimated to be ~17 μm (Timbrell, 1982). [The Working Group noted that this value falls within the range of 10–30 μm reported by Morgan, A. *et al.* (1982) on the basis of animal studies with sized glass fibres.]

(ii) *Toxic effects*

Lung: Epidemiological evidence for pulmonary effects in humans exposed to man-made mineral fibre has been reviewed (Saracci, 1985, 1986). Few consistent pulmonary effects have been noted in populations exposed industrially to glass and other man-made mineral fibres. No abnormality was observed in chest radiographs of 935 employees in a factory manufacturing glass fibre who had been exposed to dust for at least ten years (Wright, 1968). Chest radiographs of 2028 male workers in the glass fibre manufacturing industry, two-thirds of whom had been employed for more than ten years, showed eight cases of 'micronodular' opacities, one of pinpoint nodularity and 17 with questionable 'nodularity' (Nasr *et al.*, 1971). Among 232 glass fibre workers in the Federal Republic of Germany, nine were found to have small rounded opacities on chest radiographs in the category range of 0/1–1/1. In addition, 30 of these workers had irregular opacities of category 0/1–1/1 (Valentin *et al.*, 1977). In a population of 1028 workers from seven glass fibre and mineral wool plants (mean employment period, 18 years), 25 men had a profusion of small rounded opacities of grade 1/0 and six men had grade 1/1. The occurrence of small rounded opacities was more frequent among smokers, and it was concluded that, although their presence may have been related to glass fibre exposure as well, it was unlikely that they represented pulmonary fibrosis (Weill *et al.*, 1983, 1984). Of a population of 340 workers (275 with over ten years' employment) in a plant manufacturing man-made mineral fibres, 11% showed small rounded opacities of category 1/0 or more. However, no relation was detected between the prevalence of these opacities and the duration and intensity of exposure to fibres (Hill *et al.*, 1973, 1984).

Pathological and mineralogical studies of 20 glass fibre workers (employment period, 16–32 years) showed no pulmonary change that could be associated with dust exposure. In

addition, the number of mineral fibres/g of dried lung tissue was not higher than in a control population (Gross *et al.*, 1971).

In contrast, four of seven workers with prolonged exposure to glass fibre during manufacture showed parenchymal involvement of lung tissue, three had evidence of pulmonary fibrosis and one showed both (Tomasini *et al.*, 1986). Chiappino *et al.* (1981) reported that three workers who had been exposed for nine to 17 years to glass fibre during manufacture showed signs of respiratory distress. The only radiographic abnormality was slight pleural thickening in one case. The authors reported that haemorrhagic alveolitis was present, as determined by the presence of siderocytes in the sputum. [The Working Group noted that, while these cells may indicate pulmonary haemorrhage, they are also common following exposure to many dust types, when inhaled particles within macrophages become surrounded by haemosiderin pigment.]

Six workers exposed to glasswool and rockwool for periods of eight to 29 years showed no abnormality in vital capacity, pulmonary compliance or pulmonary diffusion capacity, whereas eight asbestos workers showed a marked restriction in dynamic lung function and reduced diffusing capacity (Bjure *et al.*, 1964). No evidence of small airways dysfunction or resting ventilatory impairment was found in six nonsmoking sheet-metal workers who had been exposed to glass fibre (Sixt *et al.*, 1983). In a group of British workers exposed to glass fibre, forced expiratory volume (FEV_1) and forced vital capacity (FVC) were lower than predicted; however, results in controls from the same town were equally low (Hill *et al.*, 1973, 1984). Twenty-one workers exposed to rockwool for an average of 18 years showed no abnormality in lung function compared to 43 controls during a series of detailed physiological tests (Malmberg *et al.*, 1984). In a group of over 150 workers exposed to rockwool, no difference in FVC, FEV_1 or maximum expiratory flow rate could be attributed to exposure to man-made mineral fibres (Skurić & Stahuljak-Beritić, 1984).

Upper respiratory tract: Early reports indicated that heavy exposure to man-made mineral fibres caused irritation and inflammation of the nasopharyngeal region and of the upper respiratory tract (Champeix, 1945; Roche, 1947; Cirla, 1948; Mungo, 1960).

Rhinitis, sinusitis, pharyngitis and laryngitis were all found in a series of 66 cases exposed to fibrous glass reported over periods of only 1.5 years and one year (Milby & Wolf, 1969). Both Müller *et al.* (1980) and Maggioni *et al.* (1980) reported that nasopharyngeal irritation was found more frequently in workers exposed to glass fibre than in controls.

While irritation of the nasopharyngeal region obviously predominates, increased frequency of bronchitis was reported among 135 000 construction workers, which appeared to be related directly to their levels of exposure to man-made mineral fibres (Engholm & von Schmalensee, 1982).

Skin: The development of skin irritation following occupational exposure to man-made mineral fibres has been reviewed. Irritation can be mild, disappearing in a few days, or more severe, when it can be follicular or papulopustular in character (Fisher, 1982). The occurrence of this condition was reported by Sulzberger and Baer (1942) and confirmed in numerous subsequent publications (Schwartz & Botvinick, 1943; Champeix, 1945; Pellerat & Condert, 1946; Pellerat, 1947; Cirla, 1948). While skin over large areas may be involved, paronychia and interdigital maceration have been observed (McKenna *et al.*, 1958).

Skin reactions induced by glass fibre are not confined to occupational exposures, but may result from contamination of clothing washed with fabrics manufactured from glass fibre (Peachey, 1967; Fisher & Warkentin, 1969; Lechner & Hartmann, 1979). There is also evidence that fibre contamination of the atmosphere of buildings insulated with glass fibre products can result in skin lesions (Farkas, 1983).

In contrast to pulmonary pathology, it has been demonstrated that thick fibres are the most harmful, very fine fibres causing no skin lesions at all. It has been suggested that fibres <4 μm in diameter do not cause a skin reaction (Heisel & Hunt, 1968; Possick *et al.*, 1970).

Eye: Corneal irritation has also been reported after occupational exposure to man-made mineral fibres (Longley & Jones, 1966).

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Mutagenicity and chromosomal effects*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

Two major studies have comprehensively addressed the cancer experience of workers in glasswool, glass filament, rockwool and slagwool production.

Enterline and others (Enterline *et al.*, 1983; Enterline & Marsh, 1984) reported the findings of a follow-up (through 1977) mortality study of 16 730 white male workers at 17 plants in the USA on which partial reports had previously been issued (Enterline & Marsh, 1979, 1980). The 17 plants included 11 fibrous glass plants: six producing glasswool (dates of starting, 1946–1952), three producing glass filament (dates of starting, 1941–1951) and two producing both (dates of starting, 1938 and 1950) (Enterline *et al.*, 1984), with a total worker population of 14 884 men, and six plants producing rockwool and slagwool (dates of starting, 1929–1948), with a workforce of 1846 persons. The workers had been employed in production or maintenance for one year or more during the years 1945–1963, except for men working in two plants producing small-diameter fibres (<1.5 μm), for which the criterion of inclusion was six months' or more experience. Across these factories, overall mean worker exposures to respirable fibres (length, >5 μm; diameter, <3 μm) ranged from 0.003 to 0.427 fibres/cm^3, based on exposure estimates for each worker over his working lifetime in the industry. Most average values were below 0.5 fibres/cm^3 (Esmen *et al.*, 1979a). The most recent follow-up (through 1982) of this cohort has been reported by Enterline *et al.* (1987).

Saracci *et al.* (1984a,b) reported the findings of a mortality and cancer registration follow-up study (through 1977) of 25 146 workers at 13 plants producing man-made mineral fibres in seven European countries (Denmark, Finland, the Federal Republic of Germany, Italy, Norway, Sweden and the UK). Results for some of the national components of this collaborative study have also been published separately (Bertazzi *et al.*, 1984; Claude & Frentzel-Beyme, 1984; Olsen & Jensen, 1984). Of the 13 factories, four produced glasswool (dates of starting, 1933–1943), seven produced rockwool and slagwool (dates of starting,

1937–1950) and two produced glass filament (dates of starting, 1946 and 1961) (Simonato *et al.*, 1986a). Workers ever employed at each plant (with at least one year of employment at one plant in the UK and in all three plants in Sweden) were included in the cohort. At 12 of the 13 factories, environmental surveys were made during the late 1970s (Ottery *et al.*, 1984); average respirable fibre concentrations of 0.02, 0.04 and 0.006 fibre/cm^3 were found for the main occupational groups in the glasswool, rock-/slagwool and continuous filament plants, respectively. [The arithmetic mean for combined individual exposures was 0.04 fibre/cm^3 (excluding secondary process 2 in one plant), and that for the six rockwool plants was 0.1 fibre/cm^3 (0.07 fibre/cm^3 without secondary process 2).]

The results of an extended follow-up of the whole cohort have been reported (Simonato, 1986a,b, 1987), together with detailed presentations of each national component (Andersen & Langmark, 1986; Bertazzi *et al.*, 1986; Claude & Frentzel-Beyme, 1986; Gardner *et al.*, 1986; Olsen *et al.*, 1986; Teppo & Kojonen, 1986; Westerholm & Bolander, 1986). The cohort excluded office workers and production workers in a non-man-made mineral fibre area of one factory that had been included in the original study, reducing the number of workers to 21 967. Mortality and cancer incidence were followed up until 1981–1983 in the various countries, and expected deaths were calculated on the basis of age- and calendar year-specific reference rates. In addition, correction factors for regional or more local lung cancer mortality levels were incorporated (Simonato *et al.*, 1986a). [On the assumption that persons for whom death certificates could not be located had died of causes that were distributed in the same way as those for whom death certificates were located, a correction can be made by dividing the SMR by 0.983.] Exposure assessment was based on a historical environmental investigation carried out by the Institute of Occupational Medicine (Edinburgh, UK) and by the International Agency for Research on Cancer, in which detailed information on production processes, raw materials, dust-suppressing agents, contaminants in the workplace and ventilation systems was collected (Cherrie & Dodgson, 1986). Airborne fibre levels were 'estimated' to be highest when dust suppressants were not used and/or batch processing was employed (called the 'early technological phase'), and lowest when oil and resin binders were in use with modern mechanized production methods (the 'late technological phase'); the remaining period was termed the 'intermediate technological phase'. This classification could not be applied to the continuous filament factories. In this extension of the study, follow-up was 95% complete (Simonato *et al.*, 1987).

Besides these two major studies, a number of other studies have been reported by investigators who examined the cancer experience of workers in specific types of fibre production.

(*a*) *Glasswool*

A number of studies have been reported covering workers involved in the production of glasswool and glass filament in US plants (Enterline & Henderson, 1975; Bayliss *et al.*, 1976a,b; Enterline *et al.*, 1983; Enterline & Marsh, 1984; Morgan *et al.*, 1984; Enterline *et al.*, 1987). The results given in the most recent report (Enterline *et al.*, 1987) largely cover the populations studied in the earlier reports, except for a number of plants described

by Morgan *et al.* (1984) and a case-control report of Bayliss *et al.* (1976a,b) which covers lung cancer cases not included by Enterline *et al.* (1987).

Bayliss *et al.* (1976a,b) carried out a 'case-control within a cohort' study of glasswool plant workers to examine whether exposure to small-diameter fibres (1–3 μm) was associated with respiratory disease. Workers who died of respiratory cancer were matched sequentially for date of birth (plus or minus six months), race and sex with an alphabetized list of other workers at the plant. A total of 16 cases and 16 matched controls were studied. Cases and controls were classified according to whether they had worked in at least one of several 'pilot plants' that produced small-diameter fibres: four of the cases and none of the controls had worked in a pilot plant. [The Working Group noted that there has been reconsideration of the criteria for exposure in this study, and the reported findings are thus tentative.]

Morgan *et al.* (1984) reported the mortality experience of 4399 men who had worked for a minimum of ten years in fibrous glass production and who were employed at some time during 1968–1977 at one or more fibrous glass plants owned by a single US company. [One of the major plants investigated in this study was later examined by Enterline *et al.* (1987).] Deaths were followed up for the period 1968–1977. Only men in 'exposed' jobs were included in the study. For respiratory cancer, the standardized mortality ratio (SMR) was 136 (39 observed) in the total cohort and 177 (11 observed) in the subcohort of men with 20 years' or more employment and with first exposure dating back 30 years or more. When mortality was examined by job category, the only findings of interest were SMRs for lung cancer of 181 (seven observed) in textile forming and 132 (20 observed) in wool forming and fabrication. [This is a revision of an earlier report (Morgan *et al.*, 1981) in which there were some problems (Morton, 1982; Morgan, R.W. *et al.*, 1982): glasswool production was not separated from glass filament production, and no environmental data were reported.]

Enterline *et al.* (1987) reported a study of eight US plants that produced glasswool (six produced glasswool only and two produced both glasswool and continuous filament). The cohort consisted of 11 380 white male workers with one year or more of experience in production or maintenance during the years 1945–1963, except for men working in two plants that produced small-diameter fibres, for which the criterion was six months' or more experience; 97% were traced, and death certificates were located for 97.5% of those who were believed to have died. In the analyses presented, expected deaths were based on both US and local county, white, male, age- and time-specific mortality rates. The authors concluded that the latter were the more relevant. On the assumption that persons for whom death certificates could not be located had died of causes that were distributed in the same way as those for whom death certificates were located, a correction can be made by dividing the SMR by 0.975. [The Working Group considered that it was, in general, appropriate to make this correction. However, doing so did not alter the category of the p value (>0.05 to <0.05, and <0.05 to <0.01) obtained from any test of the statistical significance of a raised SMR.] The SMRs for respiratory cancer for the period 1946–1982 were calculated to be (US) 116 and (local) 109, based on 267 deaths. For workers with fewer than 20 years since first exposure, the SMRs were (US) 95 and (local) 105 (60 observed), and those for workers with 20 years or more since first exposure, (US) 124 ($p < 0.01$ [95% confidence interval,

103–148]) and (local) 111 (207 observed). SMRs for respiratory cancer increased with time since first exposure, but the trend was less steep when local rates were used; there was no relationship with duration of exposure or with a time-weighted measure of exposure expressed as fibres/cm^3-months. Fibre exposure levels for each of the eight glasswool plants were estimated to range from 0.005 fibre/cm^3 in one plant to 0.293 fibre/cm^3 in a plant that produced small-diameter fibres. The highest individual average fibre exposure level estimated for any member of this cohort was 1.5 fibres/cm^3.

A separate analysis was made by Enterline *et al.* (1987) of 7586 workers in four plants where small-diameter fibres (<3 μm in diameter) were produced. For 1015 workers ever exposed during the production of small-diameter fibres, the SMRs for respiratory cancer were (US) 133 and (local) 124 (22 observed), and for those never exposed, (US) 115 and (local) 105 (183 observed). SMRs were higher for workers exposed during the production of small-diameter fibres than for those not exposed in each of the three plants at which deaths were observed. Of the 22 deaths, eight occurred during the period 1946–1977 and 14 during the period 1978–1982. During the period 1978–1982, SMRs were (US) 264 ($p < 0.05$) and (local) 198. SMRs for respiratory cancer increased with time since first exposure to small-diameter fibres. Death certificates for the men indicated two mesotheliomas, one of which occurred in a plant that produced small-diameter fibres. Slides obtained for the other case (exposure unknown) were submitted to the US Mesothelioma Reference Panel of the UICC; no one on the panel considered that mesothelioma was a reasonable diagnosis.

Enterline *et al.* (1987) carried out a 'case-control within a cohort' study of these glasswool workers, adjusting for cigarette smoking. All white men in their cohort study of 11 glasswool and glass filament plants who had died of respiratory cancer between 1950 and 1982 were compared with a 4% stratified (by plant and year of birth), random sample of workers, selected from the cohort of glass workers who had reached the age of 43 prior to 1983. In total, 330 cases and 529 controls were initially selected. Smoking histories for 73% of cases and 73% of controls, and details as to age at which smoking had started and stopped for 64% of cases and 71% of controls, were obtained by telephone interviews with the worker or a knowledgeable informant. [The Working Group considered that the results of this case-control study may have been affected by differences in the methods of collecting information on smoking, since smoking histories for most cases were obtained from surrogate respondents, whereas those for the majority of the controls were obtained from the respondents themselves, leading to the possibility of bias.] Data were analysed by the method of logistic regression in which age at exit from the study, year of birth, cumulative exposure to respirable glass fibres (expressed as fibres/cm^3-months) and a term reflecting interaction between smoking and exposure to fibrous glass were considered as explanatory variables. Age at exit, year of birth and smoking were statistically significant ($p < 0.05$), but cumulative exposure to glasswool was not. A second analysis was carried out in which smoking was expressed as duration of smoking and time since starting smoking; however, the fit of the model was poor and the results uninformative.

In the European cohort study (Simonato *et al.*, 1987), there was an excess of lung cancer among glasswool workers (93 observed, 73.3 expected; SMR, 127; $p < 0.05$) when compared to national rates; but after local mortality correction factors were applied, the expected

number of deaths was increased to 91, giving an SMR of 103. [The Working Group considered that the use of local rates was more appropriate, since one of the glasswool plants, which contributed 76% of the total lung cancer deaths, was located in an area where mortality rates for lung cancer were some 20% higher than the national rates.] For the glasswool workers, there was an increasing trend in SMRs for lung cancer with time since first employment, which was not statistically significant. There was no evidence of a relationship between lung cancer mortality and duration of employment, nor with technological phase. Similar analyses of lung cancer incidence data in the Nordic countries tend to confirm the mortality patterns, although there were slightly lower ratios of observed to expected numbers of cases. One case of mesothelioma was found, but the specific exposure was not given.

Shannon *et al.* (1987) reported the mortality experience of 2557 men who had worked 90 days or more in a glasswool plant in Canada and who were employed in 1955–1977, following an earlier report (Shannon *et al.*, 1984). They traced 97% of the men, and the cohort was followed for deaths to the end of 1984. The cohort was divided into three groups of workers: plant only, office only and 'mixed exposure'. For the plant-only group, the SMR for lung cancer based on provincial rates was 199 ($p < 0.05$; 19 deaths). In the office-only and mixed-exposure groups together, there were two lung cancer deaths compared to 2.4 expected (SMR, 83). For plant-only workers who had been exposed for five years or more and with ten or more years since first exposure, there were 13 deaths from lung cancer (SMR, 182). The authors examined lung cancer deaths by duration of exposure and time since first exposure and found no increasing relationship. Historical exposure data were not available, but samples taken since 1978 suggested that fibre concentrations were rarely >0.2 fibre/cm^3, mean levels in most areas being <0.1 fibre/cm^3.

Moulin *et al.* (1986) examined 1374 male workers at a French glass-fibre production factory who were employed at any time during 1975–1984 and who had worked at the factory for at least one year. Occurrence of cancer during these years was ascertained from company records, and the diagnoses were obtained from various medical sources. This study was set up because 'an industrial physician had noticed an excess of cancers in the upper respiratory and alimentary tracts' in the factory. [The Working Group noted that this study could therefore be considered essentially a confirmation of a case report and that the authors did not include oesophagus in the upper alimentary tract.] Expected numbers of cases were calculated using age-specific incidence rates from the combined data of three regional cancer registries covering the period 1975–1981, but which did not include the population of the particular region where the factory was located. To confirm the suitability of the reference incidence rates, the cancer mortality rates in the regions of the three cancer registries were checked against those in the region of the factory. [The Working Group noted that the authors gave no figures from the data used for the check.] For 'upper respiratory and alimentary tract' cancers, 19 cases were observed compared to 8.7 expected (standardized incidence ratio [SIR], 218; 95% confidence interval, 131–341); for lung cancers, five cases were observed compared to 6.8 expected (SIR, 74; 24–172); and, for other cancers, 17 cases (including one case of oesophageal cancer) were reported with 22.1 expected (SIR, 77; 45–124). The expected number of lung cancer cases in workers with

20 years of exposure since first employment was only 1.8 (one observed), indicating the low power of this study. The excess for 'upper respiratory and alimentary tract' cancers was divided among larynx (5 observed, 2.2 expected), pharynx (5 observed, 3.6 expected) and buccal cavity (9 observed, 3.0 expected). The excess was limited to production workers, and among them there was a nonsignificant increasing trend in incidence ratio with increasing duration of employment. These features were not true of lung and other cancers. A survey of cigarette smoking habits among the 1983 workforce indicated slightly lower levels than in France nationally. The authors suspected an etiological role of glass fibre, possibly including fibres both inside and outside the respirable range, because of the sites for which cancer incidence was raised. [The Working Group noted that the paper does not report whether any case of 'upper respiratory and alimentary tract' cancer was later ascertained in addition to the index cases, and that the expected number of lung cancer cases in workers with 20 years of exposure since first employment was very small.]

(*b*) *Glass filament*

Enterline *et al.* (1987) reported a cohort study of 3435 white male workers from three US plants that produced continuous glass filament, but not glasswool, who had had one year or more work experience in production or maintenance during the years 1945–1963 and who were followed for deaths to the end of 1982; 97.1% were traced, and death certificates were located for 97% of those who were believed to have died. Expected deaths were based on both US and local age- and time-specific mortality rates. For the period 1946–1982, the SMRs for respiratory cancer were (US) 95 and (local) 92 (64 observed) in the three plants. There was no clear relationship with time since first exposure, nor with duration of exposure nor with a cumulative measure of exposure expressed as fibres/cm^3-months. Estimated mean fibre exposure was low — 0.021, 0.003 and 0.005 fibre/cm^3 for the three plants, respectively (average, 0.01); the highest individual average fibre exposure level estimated for any member of the cohort was 0.093 fibre/cm^3.

In the European study (Simonato *et al.*, 1987), 15 lung cancer deaths were observed among continuous filament workers, compared to 12.5 expected from national rates (SMR, 120) and 15.4 expected from local figures (SMR, 97) — a change similar to that for glasswool workers, which corresponds generally to higher lung cancer rates among the mainly urban populations in the areas of the factories. There was no trend in SMRs for lung cancer with time since first employment, but the number of expected deaths (2.4 on the basis of local rates) more than 20 years after first exposure was very small. There was no evidence of a relationship between lung cancer mortality or incidence and duration of employment in the glass filament industry. An analysis by technological phase could not be carried out, as the separation into distinct technological phases does not apply to the continuous filament production process.

(*c*) *Rockwool and slagwool*

Enterline *et al.* (1987) studied a cohort of 1846 white male workers from six US plants that produced slagwool or rock-/slagwool. Workers had had one year or more of experience in production or maintenance during the years 1945–1963 and were followed for deaths until

1982; 97% were traced, and death certificates were located for 95% of workers believed to have died. Expected deaths were based on both US and local age- and time-specific mortality rates. For the period 1946–1982, the SMRs for respiratory cancer were (US) 148 and (local) 134 (60 observed; $p < 0.01$ and $p < 0.05$) for the six plants, (US) 156 and (local) 143 (15 observed) for workers with fewer than 20 years since first exposure, and (US) 146 ($p < 0.05$) and (local) 131 (45 observed) for those with 20 years or more since first exposure. The correction factor used for those who had died of unknown causes (see p. 139) was 0.946. SMRs for respiratory cancer were not related to time since first exposure or to duration of exposure. There was a decreasing trend in lung cancer SMR with a time-weighted measure of fibre exposure expressed as fibres/cm^3-months, although this was not statistically significant. SMRs for respiratory cancer were highest for workers first employed most recently. For workers who started during 1950–1959, for example, the SMRs were (US) 216 and (local) 198 (19 observed; both $p < 0.01$). One pleural mesothelioma was recorded on the death certificate of a worker with unknown detailed employment history, but the case was not submitted for confirmation to the Mesothelioma Panel. Mean fibre exposure in these plants was estimated to be approximately ten times that in glasswool plants (except for one plant producing small-diameter fibres) and ranged from 0.195 to 0.427 fibre/cm^3. The highest individual average fibre exposure level estimated for any member of this cohort was 1.41 fibres/cm^3.

Enterline *et al.* (1987) carried out 'a case-control within a cohort' study of the rockwool and slagwool workers, adjusting for cigarette smoking. All white men in their cohort study of six slagwool or rock-/slagwool plants who had died of respiratory cancer between 1950 and 1982 were compared with a 4% stratified (by plant and year of birth), random sample of workers, selected from the cohort of workers who had reached the age of 43 prior to 1983. In total, 60 cases and 67 controls were initially selected. Smoking histories for 75% of cases and 73% of controls, and details as to age at which smoking had started and stopped for 63% of cases and 64% of controls, were obtained by telephone interviews with the worker or a knowledgeable informant. Data were analysed by the method of logistic regression in which age at exit from the study, year of birth, cumulative exposure to respirable rock-/slagwool (expressed as fibres/cm^3-months) and a term reflecting interaction between smoking and exposure to rock-/slagwool were considered as explanatory variables. Only smoking was statistically significant ($p < 0.05$). In a further analysis, in which smoking was expressed as duration of smoking and time since starting smoking, the term representing exposure was positive and statistically significant ($p < 0.01$). Terms relating to smoking were also statistically significant, and the model appeared to be a good fit to the data set ($p = 0.75$). In an attempt to explain the discrepancy between these two analyses and the cohort study, the authors point out that (a) smoking is a powerful variable in this study; (b) smoking multiplies any effect of fibre exposure in the logistic model; and (c) the prevalence of smokers in the cumulative exposure groups varies, ranging from 75% 'ever' smokers in a low category to 54% 'ever' smokers in the highest. Thus, the highest cumulative exposure group in their study consists mainly of US men born in 1900–1909, while the lowest exposure group tends to consist of men born after 1920; and these two cohorts have different smoking patterns. [The Working Group was concerned that the apparent downward trend for lung

cancer against cumulative exposure in the cohort analysis had changed to a positive coefficient for cumulative exposure in the case-control study. The results of this case-control study may have been affected by differences in the methods of collecting information on smoking, since smoking histories for most cases were obtained from surrogate respondents, whereas those for the majority of controls were obtained from the respondents themselves. As a result, it is possible that the effects of smoking were not fully controlled, and the variable for time-weighted cumulative exposure to fibres may have been improperly corrected for the effects of smoking. These factors made the results of the analysis difficult to interpret. In view of the major effect of smoking on the incidence of lung cancer, any uncertainty regarding smoking histories makes it impossible to disentagle, with any confidence, by statistical analysis, any effect of estimated amounts of cumulative exposure.]

In the European cohort (Simonato *et al.*, 1987), among rock-/slagwool workers, 81 deaths from lung cancer were observed compared to 65.4 expected from national rates; the expected number remained unchanged after application of local correction factors, giving an SMR of 124 [95% confidence interval, 98–154]. There were increasing trends in the SMRs for lung cancer with time since first employment, which were not statistically significant.

In terms of lung cancer mortality and technological phase, a statistically significant decreasing trend in SMR was observed for rock-/slagwool workers from early to intermediate to late phases, independent of whether the comparison was with national or local corrected rates. The highest SMRs for lung cancer were seen among workers employed during the early phase and followed up for more than 20 years; ten deaths were observed compared to 4.0 expected from national rates [SMR, 250; 120–460] and 3.3 expected from local rates [SMR, 303; 145–557]. The decreasing trend in SMR by technological phase was also observed after follow-up for more than 20 years after first employment, and it reached statistical significance with both national (χ^2, 6.5 [one degree of freedom; $p < 0.05$]) and local (χ^2, 9.8 [one degree of freedom; $p < 0.01$]) reference rates. Similar analyses of lung cancer incidence in Nordic countries tended to confirm the mortality patterns, although there were slightly lower ratios of observed to expected numbers of cases. There was no evidence of a relationship between lung cancer mortality or incidence and duration of employment in the rock-/slagwool industry (Simonato *et al.*, 1987).

Lung cancer mortality was examined in relation to other workplace conditions on the basis of the historical environmental investigation (Cherrie & Dodgson, 1986). Neither the presence of asbestos in some products, the use of bitumen and pitch as a binder nor exposure to formaldehyde appeared to explain the lung cancer excesses (Simonato *et al.*, 1987). [The Working Group noted that other potential exposures, such as to silica and chromium, were not taken into account.]

The use of slag as raw material is associated with excess mortality from lung cancer in rock-/slagwool production. This finding was, however, difficult to interpret, as the periods during which slag was in use include the entire early technological phase, in which the estimated fibre levels were highest and which contributes most of the lung cancer excess. In later phases, when the estimated fibre levels had been reduced, the use of slag was associated with an SMR of 146 (13 observed, 8.9 expected) (Simonato *et al.*, 1987).

The authors concluded that, since respirable fibres were a significant component of the pollution within the workplace, it is plausible that fibre exposure during the early phase of rock-/slagwool production, alone or in combination with other factors, may have contributed to the observed lung cancer excess. Cigarette smoking was considered unlikely to account for the more than a two-fold excess of lung cancer. There was no evidence of an increase in the incidence of pleural tumours or of nonmalignant respiratory diseases (Simonato *et al.*, 1987).

(*d*) *Cancer at sites other than the lung*

In the European study (Simonato *et al.*, 1987), bladder was the only site for which there was a statistically significant increasing trend in cancer mortality with time since first exposure; this trend was limited to rock-/slagwool workers, but there was no relationship to technological phase, in contrast to lung cancer. [The Working Group noted that this comparison was one of a large number carried out, and the result may be a chance finding.]

There was a small excess of mortality from cancers of the buccal cavity and pharynx (13 observed, 10.6 expected from national rates; SMR, 123) (Simonato *et al.*, 1987), but there was a statistically significant excess in incidence among rock-/slagwool workers (22 observed, 12.2 expected from national rates; SIR, 181; 95% confidence interval, 113–274), as compared, for example, to glasswool workers (4 observed, 4.9 expected; SIR, 83) (Simonato *et al.*, 1986a). The study by Moulin *et al.* (1986) of French glasswool production workers was set up because of the observation by an industrial physician of an excess of cancers of the pharynx, larynx and buccal cavity. [The Working Group commented earlier (p. 142) on the limited nature of this study.]

A small excess of cancer of the larynx (4 observed, 2.1 expected from local rates; SMR, 188) was observed by Bertazzi *et al.* (1984, 1986) in the Italian glasswool/glass filament subcohort of the European study. No parallel finding emerged from the other subcohorts.

An increase in mortality from cancer of the digestive tract was reported in one of the US rock-/slagwool plants (Robinson *et al.*, 1982) in a study of 596 workers. The SMR was 130 (15 observed); SMRs increased with time since first exposure and with duration of exposure. The excess was not related to any particular site in the digestive tract. Claude and Frentzel-Beyme (1986) reported an increase in stomach cancer mortality with time since first exposure, based on small numbers (8 observed, 4.5 expected for $\geqslant$20 years' exposure) in the Federal Republic of Germany subcohort of the European study.

[The Working Group could not regard any of these associations as established due to their relatively weak strength, lack of consistency and to the unaccounted role of exposures such as alcohol and tobacco smoking.]

(*e*) *Overview of results of major epidemiological studies of production workers*

Table 42 gives the main findings from the US and European epidemiological studies of glasswool, glass filament and rock-/slagwool plants, both individually and in combination, where appropriate. There is a notable similarity between the outcomes of the two large investigations when comparable analyses were made. Findings from the Canadian study of glasswool workers are footnoted.

Table 42. Mortality from lung cancer and mesothelioma in the major US and European epidemiological studies of man-made mineral fibre production workers[a]

Feature	Study	Glass filament		Glasswool	Rock-/slagwool
No. of workers in study	US	3435		11 380	1846
Person-years of follow-up			385 924		48 188
Lung cancer deaths		64		267	60
No. of workers in study	European	3566		8286	10 115
Person-years of follow-up		56 332		148 203	160 066
Lung cancer deaths		15		93	81
SMRs from lung cancer compared to local reference rates					
Lung cancer mortality	US	92		109	134 ($p < 0.05$)
	European	97		103	124
	[Combined	93		108	128 ($p < 0.01$)]
Time since first exposure (<10/10–19/20–29/30+ years)	US	104/53/119/80		92/108/108/114	90/157/127/135
	European	176/76/0/0		68/113/100/138	104/122/124/185
	[Combined	138/61/111/79		77/110/106/116	102/130/125/148]
Duration of exposure (<20/20+ years)	US		106/110		145/111
	European	–		118/60	143/141
>20 years since first exposure	[Combined		112/100		129/121]
Cumulative exposure	US				
In increasing intervals of fibre/cm³-months	cohort	96/51/109/63		120/109/81/108	185/164/119/104
Two models adjusting for smoking	case-control[b]	–		No trend, no trend	No trend, increase ($p < 0.01$)
Technological phase	European				
Early/intermediate/late		–		92/111/77	257 ($p < 0.05$)/141/111
By time since first exposure (early phase only, <10, 10-19, 20-29, 30+ years)		–		108/70/80/121	0/0/317/295 ($p < 0.05$)
Small-diameter fibres	US				
Ever/never exposed		–		124/105	–
By time since first exposure (Ever exposed only, <10/10–19/20–29/30+ years)		–		61/128/105/198	–
Mesothelioma		No case		No excess	No excess
Statistically significant results[c]		None		None (but see footnote)	Yes (as shown)

Table 42 (contd)

Feature	Study	Glass filament	Glasswool	Rock-/slagwool
Estimated concentrations of respirable fibres	US	Lower	Intermediate (higher concentration of small-diameter fibres than large)	Higher

[a]From Enterline *et al.* (1987); Simonato *et al.* (1987). In a much smaller cohort study of glasswool workers in Canada, the overall lung cancer SMR compared to local rates was 199 (19 observed, $p < 0.05$), but there was no increasing relationship with time since first exposure (<10 years, SMR = 241; 10+ years, SMR = 195) nor with duration of exposure (<5 years, SMR = 291; 5+ years, SMR = 174) (Shannon *et al.*, 1987).

[b]Reservations about the interpretation of this study are expressed in the text.

[c]These relate to SMRs themselves where only one or two are shown, otherwise the statistical tests used examined for linear trends.

Table 43 summarizes results from the US study and shows a relationship between estimated fibre concentrations and observed SMRs for the total cohorts and for workers 20 years after first exposure.

Table 43. Respiratory cancer in man-made mineral fibre production workers in the major US epidemiological study[a]

Fibre type	Estimated average concentration (fibre/cm³)[b]	SMR[c]	
		Total	20 years' latency
Glass filament	0.01	92 (64)	105 (49)
Glasswool	0.06	109 (267)	111 (207)
Small-diameter	0.1	124 (22)	146 (14)
Rock-/slagwool	0.35	134* (60)	131 (45)

[a]From Enterline *et al.* (1987)

[b]Fibres <3 μm in diameter and >5 μm in length

[c]Number of deaths in parentheses; expected deaths based on local mortality rates

*$p < 0.05$

(f) Users with mixed exposure

A report by Engholm *et al.* (1987) gave results of an extended follow-up of Swedish construction workers to December 1982 for lung cancer registration and to December 1983 for mortality. Of a total of 135 037 Swedish male construction workers, 135 026 were followed up from 1971–1974, when they were first examined medically. Exposure to man-made mineral fibres (mixed categories), exposure to asbestos, occupation, cigarette

smoking habits and other information were determined by questions during the medical examination (Engholm *et al.*, 1984). The numbers of lung cancer cases were 440 observed and 483 expected, giving an SIR of 91 (95% confidence interval, 83–100). A nested case-control study was carried out to examine the relationship between lung cancer and exposures to man-made mineral fibres and to asbestos, classified on the basis of a combination of job category and self-reported information. The authors suggested that these construction workers had had exposure to asbestos because of the occurrence of 23 cases of pleural mesothelioma, with 11 expected; an analysis within the paper suggests that heavy exposure to asbestos was underreported by the workers. Using a revised classification of heavy exposure and adjusting for smoking habits and population density of area of residence in a logistic regression analysis, the authors reported a relative risk of 1.21 (95% confidence interval, 0.60–2.47) for exposure to man-made mineral fibres (adjusted for asbestos exposure) and a relative risk of 2.53 (0.77–8.32) for exposure to asbestos (adjusted for man-made mineral fibre exposure). The authors discuss the difficulty caused in the analysis by the large overlapping of reported exposures to asbestos and man-made mineral fibres.