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I DISTRIBUTION OF FIBRE

	No. of fibres sized
>25	
0.1	—
2	345
2	336

reference samples.

INHALATION AND INJECTION EXPERIMENTS IN RATS TO TEST THE CARCINOGENICITY OF MMMF

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Abstract—In parallel inhalation and intraperitoneal injection (i.p.) experiments with rats, the glass fibre JM 104, Tempstran 475, exemplifying a very thin and durable man-made mineral fibre (MMMF), was compared with crocidolite (South Africa) and chrysotile (California, Calidria RG 144). Aerosol concentrations were 2.2–6 mg m^{-3}. Exposures lasted 1 yr. No significant tumour rate was found in the inhalation test nor from an exposure combination of 100 ppm SO_2 and glass fibres. In 74% of the animals exposed to crocidolite bronchiolo-alveolar hyperplasia was detected. Intraperitoneal injection of 0.5 mg of the three different fibre types showed a tumour rate of 17% for glass fibre JM 104, 55% for crocidolite and only 6% for Calidria chrysotile. Calidria chrysotile seems to be much less persistent than other chrysotile samples. The long persistence of JM 104 475 in the lung (half-time of lung clearance about 600 days) and the carcinogenic effects of these fibres after intraperitoneal injection indicate that after inhalation of thin (< 1 μm), long and durable MMMF the suspicion of a carcinogenic potency of these fibres is still well-founded.

INTRODUCTION

VARIOUS inhalation studies on rats with man-made mineral fibres (MMMF) have not shown any evidence of lung tumours (LEE *et al.*, 1981; GOLDSTEIN *et al.*, 1983; MCCONNELL *et al.*, 1984; SMITH *et al.*, 1984; WAGNER *et al.*, 1984), whereas many injection experiments showed positive results. A review was recently published by DAVIS (1986). Inhalation of UICC chrysotile (Zimbabwe and Canada) and some other specimens of chrysotile induced malignant and benign lung tumours in 20–40%. Crocidolite and amosite with relatively short fibres caused only relatively low tumour rates (WAGNER *et al.*, 1974; DAVIS *et al.*, 1978); long amosite was much more effective (DAVIS, 1985).

Various causes may be responsible for these findings, such as the higher fibre concentration of chrysotile used in the different experiments. Moreover, the fibre number for chrysotile can increase inside the body by splitting.

It has been supposed that lesions of the bronchial epithelium caused by infections or by inhalation of irritants, e.g. cigarette smoke, enhance the chance of penetration and long-lasting residence of fibres in the bronchial wall. The settling and persistence of fibres in this susceptible tissue might be the precondition for the induction of tumours. This patho-mechanism gives one of the possible explanations for the syncarcinogenic effect of asbestos and cigarette smoke (POTT *et al.*, 1984).

In our inhalation experiment with rats, the carcinogenic effects of very thin glass fibres, of crocidolite with a greater length than in the UICC sample, of chrysotile from the Calidria mine in California and of the combination of glass fibres with SO_2 inhalation have been tested.

The design of the inhalation study was based mostly on the results of WAGNER *et al.* (1974) and of DAVIS *et al.* (1978). From the studies of WAGNER *et al.* (1974) in which

tumours were produced after an exposure of only 1 day, we expected a significant tumour rate following crocidolite and chrysotile inhalation. More recent results of MCCONNELL *et al.* (1984) and WAGNER *et al.* (1984), which were published after the beginning of this study, showed lower tumour rates.

For comparison with the inhalation study, the carcinogenicity of the three fibre specimens was also determined in intraperitoneal tests. The *in vivo* persistence of MMMF was investigated in a parallel study (BELLMANN *et al.*, 1987, this symposium). The studies were funded by different sponsors, so partly different fibre types and fibre treatments were used.

MATERIALS AND METHODS

Origin of dusts

Glass wool (Code 104, Tempstran 475) was supplied by Manville Corp., Denver, Colorado, U.S.A. A detailed description of this very thin and durable fibre is given in the paper by BELLMANN *et al.* (1987). Fibres were shortened by a 50-min treatment in a knife mill.

Sufficient amounts of UICC asbestos samples for the inhalation study were not available. Therefore, another crocidolite sample from South Africa was used which was slightly longer than UICC crocidolite (preparation by Dr Rendall). In accordance with a recommendation in a report from NIOSH (1979), chrysotile from the mine Idria in California, U.S.A. (=Calidria chrysotile) was used. It was provided as commercially processed powder. NIOSH (1979) characterized this specimen of chrysotile as follows: contaminants included mica (less than 2%), quartz (less than 2%), carbonates (less than 3%), other unidentified mineral (less than 1%) and hematite. Approximately 1% of the sample consisted of metallic fragments, probably introduced during mining and processing of the crude asbestos ore. The sample displayed good fibrous structure; no non-fibrous serpentine minerals were detected. The fibres existed in bundles with rounded ends. Bundles ranged from 0.5 to 68 μm in width and 1 300 μm in length. The material as received was treated in a knife mill for 1 min before it was fed into the aerosol generator. The Calidria chrysotile samples for the inhalation experiment and for the injection study came from different distributors. The material used for the injection study was pre-treated by the producer for manufacturing asbestos paper; this resulted in smaller fibre diameter (see Tables 1 and 3). For the intraperitoneal test, UICC-chrysotile B (Canada) and titanium dioxide (P 25 Degussa) as a granular control dust were used additionally.

Inhalation study

The fibre aerosol was generated by a vibrating-bed aerosol generator (SPURNY *et al.*, 1975; SPURNY, 1980). The size distribution of the fibres in the exposure box is listed in Table 1. This is different from the distribution of the initial material, because the aerosol generation process favours the aerosolization of thicker fibres. Monthly samples were taken on a Nuclepore filter. The size distribution of about 200 fibres was determined with a scanning electron microscope. The detection limit was 0.05 μm. Fibres were discharged by a Krypton-85 source. The fibre aerosol concentrations are listed in Table 1. Chrysotile fibres consisted of relatively thick bundles, therefore the

fibre number appeared low. Further investigations showed that bundles of Calidria chrysotile split into thinner fibres in the lung.

Female Wistar rats (Ivanovas, Kisslegg im Allgäu, F.R.G., age 12 weeks at start) were exposed, nose only, in tubes which were constructed similarly to those described by SMITH *et al.* (1981). The inhalation box could house up to 60 rats. Exposure lasted 5 h 4 times a week. After each treatment, the animals were put into Makrolon® cages. The total exposure period lasted 1 yr.

Because of the high solubility of SO_2 in water, exposure leads to an impairment of the upper airways. LAMB and REID (1968) and REID (1970) exposed rats to 400 ppm for 3 h a day, 5 days per week for up to 6 weeks. Tracheal goblet cells increased in number and size. On the basis of this information, a few hours after the fibre exposure we exposed rats to 100 ppm SO_2 in a 6 m^3 stainless steel exposure box for 5 h, 5 days per week for 1 yr. During exposure animals were housed in wire-mesh cages.

Total exposure lasted 1000 h, so the cumulative mass exposure was 3000, 2200 and 6000 mg h m^{-3} for glass fibres, crocidolite and chrysotile, respectively.

The numbers of fibres retained in the lungs were measured after low-temperature ashing, by scanning or transmission electron microscopy 6, 12 and 24 months after the start of exposure. For chrysotile samples at 24 months a transmission electron microscope was used.

Table 2 gives the total numbers of animals in the exposed groups with the numbers sacrificed early for the investigation of fibre retention. Animal numbers in the glass fibre groups were about doubled, because low effects were expected.

Injection study

Female Wistar rats (from Ivanovas, Kisslegg im Allgäu, F.R.G., aged 5 weeks at start) were treated by means of a single intraperitoneal injection with one of the dusts mentioned, suspended in 1 ml saline (for doses see Table 8). The details of animal housing and examinations are described by POTT *et al.* (1976, 1987). The distributions of the fibre sizes are listed in Table 3. Fibres were sonicated for about 1 min. Calidria chrysotile consisted partly of fibrils and partly of large bundles, therefore sizing of these fibres was very difficult and resulting numbers and fibre dimensions are not very reliable.

RESULTS

Inhalation study

Table 4(a) shows the numbers of retained fibres after 6, 12 and 24 months of the study. The size distribution of fibres is shown in Table 4(b). Size distribution of chrysotile fibres was investigated by SEM and TEM after 12 months' exposure. Results showed that the SEM was capable of detecting fibre size distribution correctly. At 24 months (1 yr post treatment) fibre splitting was marked for chrysotile. The median fibre diameter decreased from 0.2 to 0.08 μm. The median fibre lengths of glass fibres and crocidolite were about the same. The gravimetric fibre contents of lungs were calculated from the data of Tables 4(a) and (b). These are listed in Table 5. The retained chrysotile mass decreased by a factor of 10 in the 12 months of the post-treatment observation period. For glass fibre and crocidolite fibre mass was determined additionally by analysing the silicon content of lungs after 12 months' exposure. The

TABLE 1. SIZE DISTRIBUTIONS AND CONCENTRATIONS OF FIBRES IN THE EXPOSURE BOX. FIBRE ANALYSES BY SEM. DETECTION LIMIT 0.05 μm

	Size distributions					
	Fibre length (μm)			Fibre diameter (μm)		
Fibre type	10% <	50% <	90% <	10% <	50% <	90% <
Glass fibres (JM 104 475)	2.0	4.8	12.4	0.23	0.42	0.80
Crocidolite (South Africa)	0.72	1.5	4.5	0.17	0.27	0.46
Chrysotile (Calidria)	2.0	6.0	14.0	0.28	0.67	1.6

	Concentrations: means and standard deviations		
	Fibre mass	Number of fibres (f_{SEM} ml^{-1})	
Fibre type	(mg m^{-3})	All fibres	Fibres ($l > 5$ μm)
Glass fibres (JM 104 475)	3.0 ± 1.8	576 ± 473	252 ± 139
Crocidolite (South Africa)	2.2 ± 1.3	2011 ± 835	162 ± 74
Chrysotile (Calidria)	6.0 ± 5.9	241 ± 165	131 ± 72

TABLE 2. EXPOSURE GROUPS AND ANIMAL NUMBERS IN THE INHALATION STUDY

	Number of experimental animals	
Exposure group	For tumour investigation	For investigation of fibre retention
Glass fibres	108	12
Glass fibres + SO_2	108	12
Crocidolite	50	10
Chrysotile (Calidria)	50	10
SO_2	50	10
Clean air (nose only)	55	5
Clean air (without treatment)	50	10
Total	471	69

TABLE 3. SIZE DISTRIBUTIONS OF THE FIBROUS MATERIALS USED IN THE INTRAPERITONEAL TESTS

	Fibre length (μm)				Fibre diameter (μm)		
Fibre type	10% <	50% <	90% <	Per cent >5 μm	10% <	50% <	90% <
Glass fibres*	1.4	3.2	8.4	28	0.09	0.18	0.40
Crocidolite*	0.9	2.1	7.7	21	0.09	0.20	0.36
Chrysotile (Calidria)†	0.4	1.2	5.9	12	0.02	0.03	0.10
UICC-chrysotile (Canada)‡	0.3	0.9	3.6	3	0.08	0.11	0.18

*Analysis by SEM.
†Fibres exist to some extent as bundles analysis by TEM.
‡According to TIMBRELL (1970).

TABLE 4. FIBRES (SEM) RETAINED IN LUNGS AT VARIOUS TIMES FROM THE START OF THE INHALATION STUDY
(a) NUMBER OF FIBRES PER LUNG, ALL SIZES AND, IN PARENTHESIS, FIBRES LONGER THAN 5 μm

Exposure group	Fibres per lung (10^6) 6 Months mean ± SD	N	12 Months (i) mean ± SD	N	24 Months (ii) mean ± SD	N
Glass fibre	183 ± 28 (49 ± 11)	4	310 ± 165 (70 ± 26)	4	187 ± 32 (25 ± 3)	4
Glass fibre + SO_2	208 ± 43 (40 ± 11)	3	340 ± 104 (61 ± 25)	4	233 ± 118 (40 ± 18)	4
Crocidolite	403 ± 84 (66 ± 15)	3	557 ± 269 (56 ± 31)	3	229 ± 82 (52 ± 2)	2
Chrysotile	398 ± 134 (39 ± 9)	3	347 ± 129 (33 ± 16)	3	223 ± 26* (11 ± 7)	3

(b) SIZE DISTRIBUTION OF FIBRES RETAINED IN LUNGS AFTER INHALATION (FROM SEM-PHOTOS)

Exposure group	Months	Distribution of fibre length (μm) 10% <	50% <	90% <	Per cent > 5 μm	Distribution of fibre diameter (μm) 10% <	50% <	90% <
Glass fibres	6	0.98	2.7	8.2	26	0.09	0.23	0.52
	12 (i)	0.86	2.5	8.7	23	0.08	0.20	0.52
	24 (ii)	0.92	1.9	5.8	13	0.10	0.23	0.49
Glass fibres + SO_2	6	0.82	2.4	7.1	21	0.09	0.22	0.52
	12 (i)	0.64	1.9	7.3	17	0.08	0.18	0.55
	24 (ii)	0.91	2.2	6.6	17	0.10	0.25	0.55
Crocidolite (slightly longer than UICC)	6	0.78	1.8	7.3	17	0.16	0.26	0.49
	12 (i)	0.71	1.7	4.9	10	0.15	0.25	0.44
	24 (ii)	0.96	2.7	9.3	24	0.12	0.22	0.43
Chrysotile (Calidria)	6	1.0	2.2	5.0	10	0.11	0.19	0.41
	12 (i)	0.9	2.3	5.1	12	0.11	0.20	0.54
	24* (ii)	0.8	1.5	3.4	5.1	0.04	0.08	0.12

(i) End of exposure; (ii) 12 months post exposure.
*Analysis by TEM.

TABLE 5. FIBRE MASS RETAINED IN LUNGS DURING AND AFTER INHALATION EXPOSURE (MEAN AND SD)

Exposure group	Calculated fibre mass per lung (mg) After 6 months	After 12 months	After 24 months	Chemically determined mass per lung (mg) 12 months	N
Glass fibres	0.42 ± 0.09	0.56 ± 0.23	0.20 ± 0.05	0.53 ± 0.14	4
Glass fibres SO_2	0.40 ± 0.16	0.52 ± 0.16	0.21 ± 0.05	n.d.	
Crocidolite	0.59 ± 0.16	0.67 ± 0.54	0.39 ± 0.01	1.2 ± 0.5	3
Chrysotile (Calidria)	0.31 ± 0.13	0.29 ± 0.12	0.03 ± 0.03	n.d.	

n.d. = not determined.

values are also listed in Table 5. At the end of the inhalation period of 12 months the lung burden was about 0.3–1 mg, corresponding to 200–600 million fibres per lung for the three different fibre types. The half-times of fibre clearance during the 12 months after exposure were calculated on the assumption of first order clearance kinetics. The standard error range of the half-time was calculated from the clearance constant k and the standard error

$$(t_{1/2})_{low} = \frac{\ln 2}{k - k_{SE}} \qquad (t_{1/2})_{high} = \frac{\ln 2}{k - k_{SE}}.$$

Results are presented in Table 6. In the inhalation experiment the clearance half-time of fibres (all sizes) was 300 days for crocidolite and about 600 days for glass fibres. The additional treatment with SO_2 during the exposure period did not influence the half-time of glass fibre clearance. For chrysotile, the half-time is influenced by fibre splitting (see below) and therefore appears high. Table 6 also lists, for comparison, the corresponding half-time of fibre clearance after intratracheal instillations found in a companion study by BELLMANN *et al.* (1987, this symposium).

TABLE 6. CLEARANCE HALF-TIMES OF FIBRES AFTER INHALATION AND, FOR COMPARISON, AFTER INTRATRACHEAL INSTILLATION (BELLMANN *et al.*, 1987). ALL FIBRE-SIZES

	Half-time (days)					
	Fibre inhalation[a]			Intratracheal instillation[a]		
Fibre type	Low	Mean	High	Low	Mean	High
Glass fibre (JM 104/475)	376	597	892	539	745	1205
Glass fibre (JM 104 475) $+SO_2$	347	573	1642			
Crocidolite (UICC)				135	185	289
Crocidolite (slightly longer than UICC)	201	301	603			
Chrysotile A (UICC)				2655[b]	−824[c]	−488[b]
Chrysotile (Calidria)	402	617	1333			

[a]Results of BELLMANN *et al.* (1987).
[b]Calculated half-times and standard error range.
[c]Negative values due to increase of fibre number splitting of fibre bundles.

The results of histopathological examination are given in Table 7. The median life-times of the exposure groups were 106–111 weeks. Only the SO_2-treated group had a shorter life-time of 99 weeks. The last surviving animals were sacrificed at 140 weeks from the onset of the exposure. Three primary lung tumours were detected: one adenocarcinoma (crocidolite), one squamous cell carcinoma (glass fibres) and one adenoma (glass fibre + SO_2). One squamous metaplasia was found after crocidolite inhalation and one after glass fibre inhalation.

Septal thickening, which can be caused by interstitial fibrosis and/or interstitial inflammation, was detected in about 30–40% of the animals in all fibre-exposed groups. The degree of fibrosis appeared to be slight to medium. A high incidence of bronchiolo-alveolar hyperplasia was detected after crocidolite exposure.

The tracheae of rats sacrificed after 6 months or 1 yr of exposure to SO_2 were

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TABLE 7. HISTOPATHOLOGICAL CHANGES IN THE LUNGS OF RATS EXPOSED TO VARIOUS FIBRES (INHALATION STUDY)

Exposure group	Number of rats examined	Septal thicking (interst. fibrosis, interst. inflammation) Total (%)	Bronchiolo-alveolar hyperplasia Total (%)	Squamous metaplasia	Primary tumour	Median life-time (weeks)
Glass fibre	107	41 (38%)	12 (11%)	1	1 (SC)	110
Glass fibre $+SO_2$	108	30 (29%)	17*(16%)	0	1 (A)	106
Crocidolite	50	18 (36%)	37*(74%)	1	1 (AC)	111
Chrysotile	50	21 (42%)	6 (12%)	0	0	109
SO_2	50	5 (10%)	1 (2%)	0	0	99
Control (nose only-tubes)	55	6 (11%)	4 (7%)	0	0	108
Control (without treatment)	50	12 (24%)	3 (6%)	0	0	108

*Significantly different in χ^2-test from combined control groups. $P<0.05$.
SC: Squamous cell carcinoma.
A: Adenoma.
AC: Adenocarcinoma.

examined by scanning electron microscopy. Compared with controls, the SO_2-exposed rats showed basically a normal respiratory epithelium.

Injection study

The tumour rates in the injection study are given in Table 8. Incidences after treatment with saline, TiO_2 and Calidria chrysotile are low.

TABLE 8. RESULTS OF THE INTRAPERITONEAL TEST: TUMOURS IN THE ABDOMINAL CAVITY EXCLUDING UTERUS TUMOURS

Fibre type	Number of rats examined	Dose i.p. (mg)	Tumour rates (%)	Median life-time (weeks)
Glass fibre (JM 104 475)	30	0.5	17*	116
Crocidolite (South Africa)	32	0.5	55*	109
Chrysotile (Calidria)	32	0.5	6	116
Chrysotile (Canada)	32	1.0	84*	51
TiO_2	32	10	0	130
Saline	32	1 ml	6	120

*Significantly different in χ^2-test from combined control groups. $P<0.05$.

DISCUSSION

In the inhalation experiment, no significant tumour rate was obtained for any of the fibres investigated. First of all, this result was surprising for the positive control crocidolite for which the carcinogenicity in man is evident, as results by WAGNER *et al.*

(1960) have shown. Although WAGNER *et al.* (1974) demonstrated the carcinogenic effect of crocidolite in rats, DAVIS *et al.* (1978) established only very low tumour rates after crocidolite inhalation in rats, as in this study. The lung burden of crocidolite in both those studies was about 9 mg after 12 months of exposure. The crocidolite lung burden in our study was about 1 mg, but WAGNER *et al.* (1974) reported a significant tumour rate for a comparable dust retention. The high rate of bronchiolo-alveolar hyperplasia of 74%, one case of squamous metaplasia and one adenocarcinoma may indicate a tendency of the crocidolite fibres used to induce neoplasms.

In the intraperitoneal test, crocidolite showed a very strong carcinogenic effect. An injection of only 0.5 mg led to 55% malignant tumours in rats, compared with a tumour rate of 0 6% in controls. In regard to the Calidria chrysotile which was recommended by NIOSH (1979) as a reference material for chrysotile, we do not know of any carcinogenicity studies. When we started our inhalation experiment, we expected a carcinogenic potency comparable to that of the UICC-chrysotile samples. However, a significant tumour rate was found neither in the inhalation experiment nor after intraperitoneal injection of 0.5 mg. An injection of 1 mg UICC-chrysotile, Canada, led to a tumour rate of 84%. BOLTON *et al.* (1984) showed that even an injection of 0.05 mg of this material induced tumours. For Calidria chrysotile, a considerable decrease of the fibre diameter was observed for fibres retained in lungs compared with fibres in the inhalation chamber. This is due to the higher deposition rate of thicker fibres in the upper airways and splitting of fibres in the lung. There seems to be a considerable difference in persistence between the UICC-chrysotile samples and the Calidria chrysotile. BELLMANN *et al.*, (1987, this symposium) found a strong increase in the numbers of very thin UICC chrysotile fibres longer than 5 μm 2 yr after intratracheal instillation. In contrast to these results, the fibre number of Calidria chrysotile decreased after the exposure period. In Table 6 the fibre clearance after inhalation and intratracheal instillation is compared. Comparable half-times were found for the glass fibre 104 475 and crocidolite, based on all fibres retained in lungs. If the clearance of glass fibres > 5 μm is similarly calculated at 12 and 24 months, the half-time after glass fibre treatment is 254 days and 547 days after glass fibre + SO_2 treatment, respectively. These values are less than the ones for all fibres as listed in Table 6 and also much less than the value of 3500 days for clearance of glass fibre JM 104 475 for fibres > 5 μm reported by BELLMANN *et al.* (1987) after intratracheal instillation. The main reasons for these differences are the relatively low number of fibres > 5 μm counted in the inhalation test and the difficulties of sizing fibres exactly. Therefore, in Table 6 the half-times for all fibres are calculated, which are more reliable.

Considering the low tumour rate of crocidolite and Calidria chrysotile in our inhalation experiment, it is not surprising that the investigated glass fibre showed no significant carcinogenic effects either. After combining SO_2-inhalation and glass fibre exposure, tumours could have been expected in the upper respiratory tract if any tumours had occurred. However, the impairment of ciliated airways by SO_2 was relatively low, therefore these results do not disprove the hypothesis that after impairment of the bronchial epithelium the carcinogenic risk of inhaled fibres is elevated.

It is concluded that the low carcinogenic potency which may be associated with thin and durable MMMF is very difficult to demonstrate in an inhalation study. However, the long persistence of JM 104 475 in the lung and the carcinogenic effects of these

fibres after intraperitoneal injection indicate that after inhalation of thin (< 1 μm), long and durable MMMF the suspicion of a carcinogenic potency of these fibres is still well founded.

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REFERENCES

BELLMANN, B., MUHLE, H., POTT, F., KÖNIG, H., KLÖPPEL, H. and SPURNY, K. (1987) Persistence of man-made mineral fibres (MMMF) and asbestos in rat lungs. *Ann. occup. Hyg.* **31**, 693–709.

BOLTON, R. E., DAVIS, J. M. G., MILLER, B., DONALDSON, K. and WRIGHT, A. (1984) The effect of dose of asbestos on mesothelioma production in the laboratory rat. In *VIth International Pneumoconiosis Conference* 1983, Vol. 2, pp. 1028–1046. Bergbau-Berufsgenossenschaft, Verlag für neue Wissenschaft, Bremerhaven.

DAVIS, J. M. G. (1985) Review of animal experiments. In: *Proceedings of the Fifth International Colloquium on Dust Measuring Technique and Strategy*, Johannesburg, S.A., 29–31 October 1984 (Edited by BAUNACH, F.), pp. 25–38. SAAPAC, Johannesburg.

DAVIS, J. M. G. (1986) A review of experimental evidence for the carcinogenicity of man-made vitreous fibres. *Scand. J. Wk Environ. Hlth* **12**, suppl. 1, 12–17.

DAVIS, J. M. G., BECKETT, S. T., BOLTON, R. E., COLLINGS, P. and MIDDLETON, A. P. (1978) Mass and number of fibres in the pathogenesis of asbestos-related lung diseases in rats. *Br. J. Cancer* **37**, 673–688.

GOLDSTEIN, B., RENDALL, R. E. G. and WEBSTER, I. (1983) A comparison of the effects of exposure of baboons to crocidolite and fibrous-glass dusts. *Environ. Res.* **32**, 344–359.

LAMB, D. and REID, L. (1968) Mitotic rates, goblet cell increase, and histochemical changes in mucus in rat bronchial during exposure to sulphur dioxide. *J. Path. Bact.* **96**, 97–111.

LEE, K. P., BARRAS, C. E., GRIFFITH, F. D., WARITZ, R. S. and LAPIN, C. A. (1981) Comparative pulmonary responses to inhaled inorganic fibres with asbestos and fiberglass. *Environ. Res.* **24**, 167–191.

MCCONNELL, E. E., WAGNER, J. C., SKIDMORE, J. M. and MOORE, J. A. (1984) A comparative study of the fibrogenic and carcinogenic effects of UICC Canadian chrysotile B asbestos and glass microfibre (JM 100). In: *Biological Effects of Man-Made Mineral Fibres*, Vol. 2, pp. 234–250. World Health Organization, Regional Office for Europe, Copenhagen.

NIOSH (1979) Publication No. 79-139 Preparation and characterization of analytical reference minerals, U.S. Department of Health, Education and Welfare, Cincinnati, U.S.A.

POTT, F., FRIEDRICHS, K.-H. and HUTH, F. (1976) Ergebnisse aus Tierversuchen zur kanzerogenen Wirkung faserförmiger Stäube und ihre Deutung im Hinblick auf die Tumorentstehung beim Menschen. *Zentbl. Bakt. Hyg., I. Abt. Orig.* **B162**, 467–505.

POTT, F., ZIEM, U. and MOHR, U. (1984) Lung carcinomas and mesotheliomas following intratracheal instillation of glass fibres and asbestos. In: *VIth International Pneumoconiosis Conference* 1983, Vol. 2, Bergbau-Berufsgenossenschaft, Verlag für neue Wissenschaft, Bremerhaven.

POTT, F., ZIEM, V., REIFFER, F.-J., HUTH, F., ERNST, H. and MOHR, V. (1987) Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. *Expl Path.* (in press).

REID, L. (1970) Evaluation of model systems for study of airway epithelium, cilia and mucus. *Archs intern. Med.* **126**, 428–434.

SMITH, D. M., ORTIZ, L. W. and ARCHULETA, R. F. (1984) Long-term exposure of Syrian hamsters and Osborne-Mendel rats to aerosolized 0.45 μm mean diameter fibrous glass. In: *Biological Effects of Man-Made Mineral Fibres*, Vol. 2, pp. 253–272. World Health Organization, Regional Office for Europe, Copenhagen.

SMITH, D. M., ORTIZ, L. W., ARCHULETA, R. F., SPALDING, J. F., TILLERY, M. I., ETTINGER, H. J. and THOMAS, R. G. (1981) A method for chronic nose-only exposure of laboratory animals to inhaled fibrous aerosols (Edited by LEONG, B. K. J.). In: *Proceedings of the Inhalation Toxicology and Technology Symposium* (Edited by LEONG, B. K. J.), pp. 89–105. Ann Arbor Science Publishers, Ann Arbor, Michigan.

SPURNY, K. (1980) Fibre generation and length classification. In: *Generation of Aerosols and Facilities for Exposure Experiments* (Edited by WILEKE, K.), pp. 257–298. Ann Arbor Science Publishers, Ann Arbor, Michigan.

SPURNY, K., BOOSE, C. and HOCHRAINER, D. (1975) Zur Zerstäubung von Asbestfasern in einem Fließbett-Aerosolgenerator. *Staub-Reinh. Luft* **35**, 440–445.

TIMBRELL, V. (1970) Characteristics of the International Union Against Cancer standard reference samples

of asbestos. In: *Pneumoconiosis, Proceedings of the International Conference*, Johannesburg, 1969 (Edited by SHAPIRO, H. A.). pp. 28–36 Oxford University Press, Cape Town.

WAGNER, J. C., BERRY, G. B., HILL, R. J., MUNDAY, D. E. and SKIDMORE, J. W. (1984) Animal experiments with MMM(V)F fibres—effects of inhalation and intrapleural inoculation in rats. In: *Biological Effects of Man-Made Mineral Fibres*. Vol. 2, pp. 209–233. World Health Organization, Regional Office for Europe, Copenhagen.

WAGNER, J. C., BERRY, G., SKIDMORE, J. W. and TIMBRELL, V. (1974) The effect of the inhalation of asbestos in rats. *Br. J. Cancer* 29, 252–269.

WAGNER, J. C., SLEGGS, C. A. and MARCHAND, P. (1960) Diffuse pleural mesothelioma and asbestos exposure in the Northwestern Cape Province. *Br. J. ind. Med.* 17, 260–271.