INHALED PARTICLES

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PART 1

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A STUDY OF THE SHORT-TERM RETENTION AND CLEARANCE OF INHALED ASBESTOS BY RATS, USING U.I.C.C. STANDARD REFERENCE SAMPLES

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Abstract—Rats have been dosed over a 6-week period with U.I.C.C. standard reference samples of amosite, crocidolite and chrysotile A, each at three concentrations: 1, 5 and 10 mg/m³. Mass concentrations in the exposure chambers were monitored daily. Because the M.R.E. gravimetric sampler was found to undersample some of these dusts at high concentrations, a sampler with a vertical elutriator was developed. Data on other physical parameters of the dust clouds were also obtained and it was found that the fibre number (>5 μm in length) *vs.* mass correlation varied markedly between asbestos varieties.

After dusting, the rats were sacrificed in five batches over a period of 4 months. The lungs of some animals were retained for pathological examination. This did not reveal any fibrosis although large numbers of fibres were visible, mostly within alveolar macrophages. The remaining lungs were analysed for their asbestos contents by a method based upon infrared spectrophotometry. The clearance data confirm earlier published reports that for rats dosed at similar mass concentrations of chrysotile and amphibole, those dosed with chrysotile retain considerably less dust in their lungs. The data also suggest that the retention and clearance of amphibole asbestos may be dose related. Some mathematical treatment of the clearance data has been undertaken.

BACKGROUND

Inhalation experiments reported by WAGNER and SKIDMORE (1965) showed that the clearance of asbestos by rats, over a period of 2 months from the end of exposure, could be described by single exponential functions. Their results suggested that chrysotile asbestos was cleared at three times the (exponential) rate of the amphibole asbestos varieties, amosite and crocidolite. As part of a programme of research* into the health hazards of asbestos dust it was decided to investigate these findings in more depth, especially as the airborne concentrations used by Wagner and Skidmore (>25 mg/m³) were very much higher than would be expected in practice. It was thought that at high concentrations fibres might bridge the nasal passages and thus prevent other fibres reaching the lungs; a further possibility was that the dust concentrations during exposure might influence the rates of retention and clearance.

METHODS AND MATERIALS

Rats were dosed with U.I.C.C. standard reference asbestos samples—chrysotile A, amosite and crocidolite (TIMBRELL *et al.*, 1968a; RENDALL, 1970)—each at three

* On behalf of the Asbestosis Research Council.

different mass concentrations. The target concentrations chosen were 1, 5 and 10 mg/m^3. These are several times lower than the concentrations used by Wagner and Skidmore but even so the lowest is an order of magnitude higher than the concentration permitted (for amosite and chrysotile) in British factories (H.M. FACTORY INSPECTORATE, 1974). The U.I.C.C. asbestos samples were used in order to facilitate comparison with results from other groups. The rats were dosed over a period of 6 weeks, during which the dust generators were run for 7 h a day, 5 days a week.

The animal exposure chambers were based upon the design of TIMBRELL *et al.* (1970b), adapted to suit the space available. The "Timbrell" dust generator (TIMBRELL *et al.*, 1968b) was used but modified to produce a more stable cloud (BECKETT, 1975). The dust was passed through a cyclone elutriator before being dispersed in the chamber. This was thought to be desirable in order to reduce the amount of non-respirable fibre, in particular the fibre flocs, in the chambers.

Mass concentrations were measured daily using the M.R.E. sampler (Casella Type 113A; DUNMORE *et al.*, 1964); this instrument had been used by WAGNER and SKIDMORE (1965). However, initial tests showed that when sampling at high chrysotile concentrations the sample filters often contained numerous balls of fibre about 1 mm in diameter, which appeared to have rolled off the end of the elutriator plates. An independent mass monitor was therefore developed using a vertical elutriator, which was incapable of blocking. This consisted of an inverted 50 mm diameter filter holder which has the same selection curve as the M.R.E. sampler elutriator, when sampling at a flow-rate of 140 cm^3/min (WALTON, 1954). Both mass monitors were used routinely to sample the dust clouds.

Estimation of the small weights of asbestos collected for the daily samples was carried out using a direct on-filter infrared absorption technique, based on that described by DODGSON and WHITTAKER (1973). Total dust in the chambers was estimated by operating the vertical elutriator sampler at a flow rate of 2 l./min.

The mass monitors were normally operated only during the 7 h of dusting. Some asbestos collected on the rat fur during the day and it was thought that this could become airborne at night. Therefore dust monitors were occasionally operated for the remaining 17 h, but no significant quantities of asbestos were detected.

The airborne dusts were also characterized in terms of fibre number concentrations, measured according to standard procedure (ASBESTOSIS RESEARCH COUNCIL, 1971); fibre length distributions were obtained by optical and scanning electron microscopy. In order to obtain fibre number ($>5\ \mu$m length) *vs.* mass correlations each chamber was sampled frequently over a period of several days, and the mean fibre numbers compared with the mass estimates from the M.R.E. and vertical elutriator samplers.

At the end of exposure, 5 days were allowed for asbestos to clear from the bronchial tract and major airways before sacrifice of the first group of eight or ten animals. Four subsequent groups, each of eight rats, were sacrificed at approximately monthly intervals. Some of the lungs were retained for pathological examination and embedded in paraffin wax for light microscope study.

The remaining lungs were analysed for their asbestos content by a method based upon infrared spectrophotometry (BECKETT *et al.*, 1975). This method of analysis was adopted because of its high sensitivity and because it is essentially non-destructive, permitting further study of the recovered residues if required. In order to calibrate the

method about 10 mg of each variety of asbestos was added to the excised lungs of unexposed rats and taken through the normal recovery process. Calibration samples were prepared by incorporating weighed amounts of this recovered asbestos into potassium bromide discs, suitable for infrared spectrophotometric analysis.

Lungs from rats dosed with amphibole asbestos were dried and ashed in a muffle furnace at 380°C for 3 days. Unoxidized carbonaceous material remaining after this treatment was removed by low temperature ashing (*ca.* 150°C), using a stream of oxygen excited by a radio-frequency discharge (see for instance, GLEIT and HOLLAND, 1962). Lung salts were removed by washing the residues with 3 ml of 0.2 M hydrochloric acid. Because chrysotile is more susceptible to alteration by heating and acid washing, the lungs from animals exposed to this type of asbestos were not muffle ashed, but instead were ashed for a longer period in the low-temperature asher, before being washed in water to remove lung salts. The residues were incorporated into potassium bromide discs for the estimation of asbestos. Aliquots were taken from some of the recovered residues for measurement of fibre lengths. The asbestos was deposited on filters and examined by scanning electron microscopy.

RESULTS

The mean mass concentrations in the chambers during exposure (estimated by the vertical elutriator sampler) are given in Table 1, together with the standard deviations of the daily measurements and the mean respirable/total dust ratios. It can be seen that the target concentrations were not achieved exactly.

TABLE 1. PHYSICAL CHARACTERISTICS OF THE DUST CLOUDS

Type of asbestos	Mean chamber concentrations (mg/m³ (S.D.))			Mean ratio of respirable to total dust	Fibres/cm³ equivalent to 1 mg/m³ (S.E.)
Amosite	1.4(0.6)	4.6(1.9)	9.4(3.9)	0.86	25(2.9)
Crocidolite	1.4(1.4)	5.9(3.5)	11.8(4.0)	0.97	48(2.0)
Chrysotile	1.2(0.8)	3.8(5.0)	7.8(2.2)	0.87	114(9.1)

For amosite and crocidolite the mass estimates from the M.R.E. and vertical elutriator samplers differed by less than 10%, but for chrysotile there was as good agreement only at lower concentrations. At higher fibre densities of chrysotile the M.R.E. sampler undersampled by an average of 30% compared to the vertical elutriator sampler. That the M.R.E. sampler rather than the vertical elutriator sampler was in error was confirmed by the fibre number *vs.* mass correlations. At lower concentrations (*ca.* 1 mg/m³) both samplers indicated that 90 fibres/cm³ (estimated by the standard fibre counting method) were equivalent to 1 mg/m³ (estimated by the gravimetric samplers). But at a concentration of *ca.* 10 mg/m³ the inverted filter sampler indicated that 110 fibres/cm³ were equivalent to 1 mg/m³ whereas the M.R.E. sampler indicated 155 fibres/cm³ equivalent to 1 mg/m³. The inverted filter sampler was there-

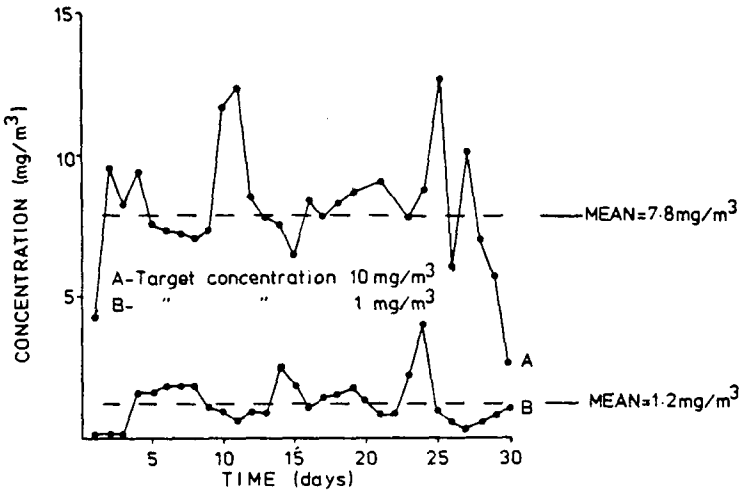

FIG. 1. Daily records of dust concentrations in two of the chrysotile exposure chambers.

fore considered to give the better estimate of mass. An indication of the daily fluctuations in concentration is given by the standard deviations quoted in Table 1, and by Fig. 1.

The fibre number *vs.* mass correlations (Table 1) show that for equal mass the fibre numbers of amosite, crocidolite and chrysotile are in the ratio 1 : 1.9 : 4.6. The large standard errors arise because the mass estimates were integrated over 7-h periods, whereas the fibre counts were derived from samples taken over periods of only a few minutes, and were susceptible to short-term fluctuations. In addition there is uncertainty due to the imprecision of fibre counting (BECKETT and ATTFIELD, 1974).

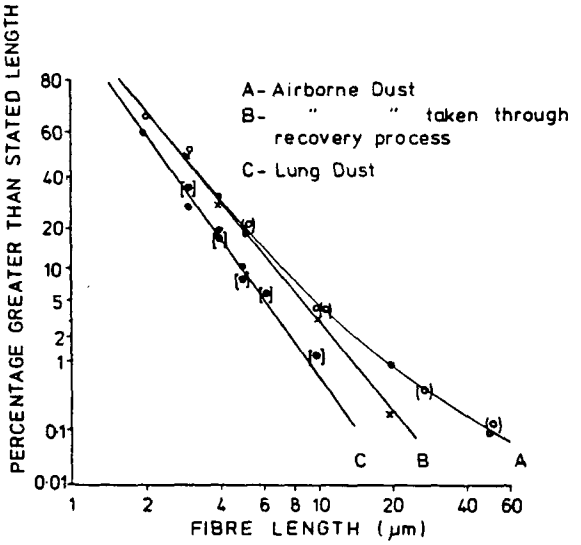

FIG. 2. Fibre length distributions of airborne and recovered amosite. Points in parentheses ( ) are data of TIMBRELL (1970a), those in parentheses [ ] are data of TIMBRELL *et al.* (1970a).

The fibre length distributions of the airborne dusts were in good agreement with those given by TIMBRELL (1970a), indicating that elutriation of the dust in the present experiment had not affected the length distribution (see Fig. 2 and BECKETT, 1975).

Some of the amosite dust obtained during sampling was added to the excised lungs of an unexposed rat and taken through the normal recovery process. When the length distribution of this dust is compared with that of the parent airborne material (Fig. 2) it is apparent that the recovery process tends to shorten some of the longer fibres. This

TABLE 2. RETENTION DATA.

Type of asbestos and dose (mg/m^3)		Asbestos recovered				
Amosite	Days after last exposure	5	32	66	102	128
1.4	μg/rat (S.E.)	78(5)	72(4)	45(8)	47(4)	38(12)
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	4.4	4.1	2.6	2.7	2.2
4.6	μg/rat (S.E.)	646(31)	309(42)	160(19)	138(20)	119(12)
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	11.1	5.3	2.8	2.4	2.1
9.4	μg/rat (S.E.)	1498(132)	605(58)	410(51)	332(21)	371(29)
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	12.6	5.1	3.5	2.8	3.1
Crocidolite	Days after last exposure	5	35	67	96	126
5.9	μg/rat (S.E.)	1012(68)	645(117)	442(48)	337(50)	268(45)
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	13.6	8.7	5.9	4.5	3.6
11.8	μg/rat (S.E.)	1338(140)	1267(188)	1114(219)	884(118)	583(68)
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	9.0	8.5	7.5	5.9	3.9
Chrysotile	Days after last exposure	5	40	70	97	125
3.8	μg/rat (S.E.)	109(17)	52(9)	42(3)	36(3)	—
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	2.3	1.1	0.9	0.8	—
7.8	μg/rat (S.E.)	245(23)	93(20)	113(16)	63(17)	55(8)
	$\frac{\mu g/\text{rat}}{\text{aggregate dose}} \times 100$	2.5	0.9	1.1	0.6	0.6

effect does not, however, seem to be sufficient to explain the considerably increased proportion of shorter fibres observed in amosite recovered from exposed rats (Fig. 2). The length distribution of the recovered dust, although biased in favour of shorter fibres, is in good agreement with the published data of TIMBRELL *et al.* (1970a).

Histological examinations of sample lungs taken at each killing date showed that almost all dust visible by light microscopy was located within alveolar macrophages, many of which contained large numbers of fibres. At the first killing date the numbers

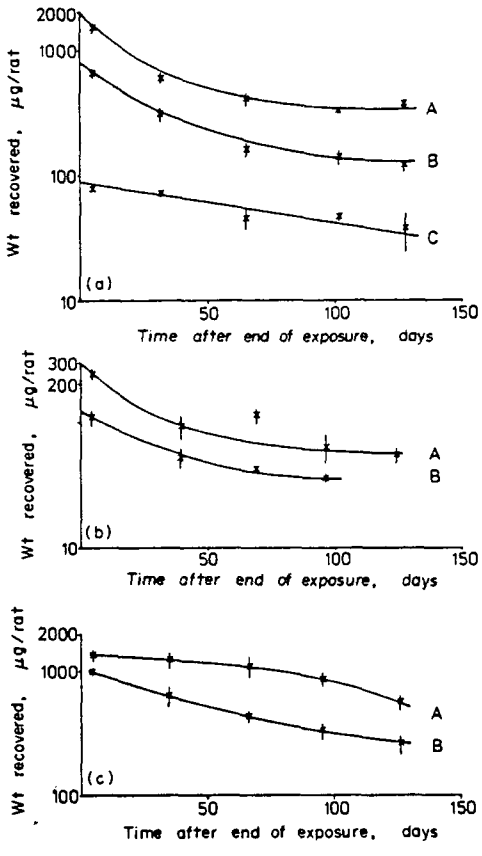

FIG. 3. (a) Retention data for rats dosed with U.I.C.C. amosite at target concentrations of A: 10 mg/m³, B: 5 mg/m³ and C: 1 mg/m³. (b) Retention data for rats dosed with U.I.C.C. chrysotile A at target concentrations of A: 10 mg/m³ and B: 5 mg/m³. (c) Retention data for rats dosed with U.I.C.C. crocidolite at target concentrations of A: 10 mg/m³, B: 5 mg/m³.

of dust-containing cells appeared to be in proportion to the concentrations during exposure. As the period between the end of exposure and sacrifice increased there was a noticeable reduction in the number of dust-containing cells in the lungs of rats dosed at the two higher concentrations. This effect was not noticeable in animals dosed at 1 mg/m³. No significant pathological changes were observed. The only anatomical alteration noted was the deposition of a very small amount of reticulin,

amongst clumps of dusted macrophages, which had aggregated close to respiratory bronchioles. Both the reticulin and the clumps of macrophages were less widespread in the lungs of animals sacrificed at the later killing dates.

The retention data are summarized in Table 2 and presented in graphical form in Fig. 3. Each point represents the average value from between seven and ten rats, analysed individually or occasionally bulked in groups of two or three. The standard errors of the means are given in the Table and are represented by bars in Fig. 3. Variation between animals was found to be considerable, sometimes exceeding 2: 1. The aggregate doses were calculated from the known exposure period and the dust

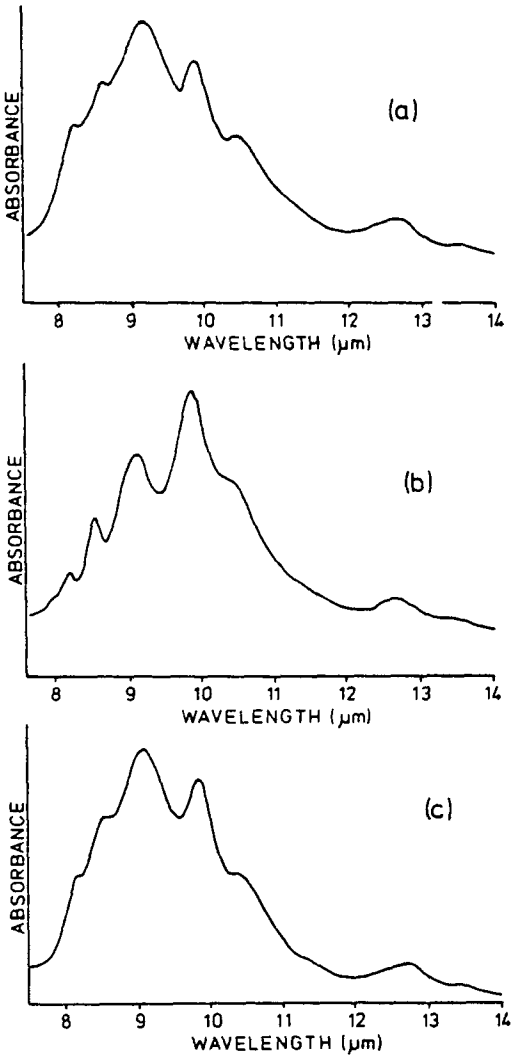

FIG. 4. Infrared spectra of U.I.C.C. crocidolite. (a) Recovered from the lungs of an exposed rat. (b) Calibraliou material, taken through the recovery process. (c) Calibration material with the addition of amorphous precipitated silica.

concentrations measured by the vertical elutriator sampler (see Table 2), assuming a minute volume of 100 cm^3.

The estimation of recovered crocidolite was complicated by the presence in the residues of material giving rise to a strong absorption band at *ca.* 9 μm and a weaker band at *ca.* 12.5 μm (Fig. 4a). This interfered with the crocidolite band at *ca.* 9.7 μm for which a calibration had been established. By making appropriate corrections this band was still useful for analysis, but estimates were based on an alternative calibration using the weaker band at *ca.* 15.4 μm. It was found that the addition of "amorphous" precipitated silica to the crocidolite used for calibration produced a sample which gave an infrared spectrum closely similar to that of the residues recovered from the lungs of the exposed rats (Fig. 4 b, c). On this evidence it is thought that the interfering material in the recovered residues is amorphous silica.

The amounts of amorphous silica present in the residues were estimated from the measured absorbance values at 12.5 μm (after making small corrections for the absorbance at this wavelength due to crocidolite in the samples). Preliminary data indicate that the weight of amorphous silica associated with a given weight of crocidolite increases with the time of residence of the dust in the lungs.*

DISCUSSION

The retention data obtained confirm the finding of WAGNER and SKIDMORE (1965) and WAGNER *et al.* (1974) that for similar airborne mass concentrations and exposure periods, the weight of chrysotile retained in the lungs of rats is considerably less than the weight of amphibole asbestos. This can be attributed in part to the more rapid (exponential) rate of clearance of chrysotile—observed by Wagner and his co-workers. However, this effect cannot wholly explain the different levels of retention and it seems likely that significantly less chrysotile than amphibole asbestos pentrates deep into the lungs in the first instance. Experiments reported by TIMBRELL (1970b) indicated that the curly nature of chrysotile fibres (as opposed to the straight, needle-like character of amphibole fibres) might be an important factor influencing fibre penetration.

The large variations in retention between identically exposed animals sacrificed at the same date confirms a result reported by DOWNS *et al.* (1967) for an inhalation experiment using uranium dioxide. WRIGHT (1957) encountered somewhat less variability between animals when rats were dosed with alumina or coal dust.

The retention data for amosite indicate a more rapid clearance (*ca.* 75%) during the first 2 months after the end of exposure, than was reported by WAGNER and SKIDMORE (1965) *ca.* 30%. The reasons for this are not clear but may be related to the absolute levels of dust burden in the lungs which even for the animals dosed at 10 mg/m^3 were only between a half and a third of the levels reported by Wagner and Skidmore.

* Since the presentation of this paper it has been established that the amorphous silica in these residues is produced by the breakdown of crocidolite during the recovery process. Similar degradation of the crocidolite ashed with lung material and used for calibration purposes was not encountered. Work is in progress to investigate these findings further. In the light of this finding it seemed appropriate to recalculate the weights of amorphous silica to "crocidolite equivalent" (assuming that crocidolite contains on average 51% by weight of silica). The figures in Table 2 represent the sum of crocidolite estimated as such and "crocidolite equivalent".

Additional factors might be the use of different (not U.I.C.C.) dust samples by Wagner and Skidmore, and the use of an elutriator in the present experiment.

The clearance curves for rats dosed with amosite at *ca.* 5 and 10 mg/m^3 can be described in terms of the sum of two exponential functions. The curves have been fitted to functions consistent with clearance from two "compartments" with biological half-times of 14 and 145 days. The animals dosed at *ca.* 1 mg/m^3 appear to show only the slower phase of clearance.

Any dust deposited on the ciliated airways would be expected to have been cleared from the lungs very quickly, either during the dusting period itself or during the 5 days between the cessation of dusting and the killing of the first batch of animals. Hence it can be presumed that all the dust involved in the present study must have been deposited in the respiratory bronchioles or in the alveoli. The similarity between these two sites makes it unlikely that the fast and slow clearance rates depend on the exact site of deposition of any asbestos fibre, although clearance from the different lung lobes may occur at different rates.

Variations in the speed of dust removal are more likely to be due to the degree of "fixation" of the deposited dust. After deposition, all dust particles not cleared within a few hours would have been phagocytosed by alveolar macrophages. The subsequent behaviour of these dusted cells probably governs clearance speed. Many dusted macrophages remain singly in their normal position on the alveolar surface and it may be these cells that are relatively easily cleared from the lung in the fast clearance phase. Other macrophages become grouped together near respiratory bronchioles becoming bound by reticulin network and some penetrate into the interstitial space. In these latter two cases, clearance might initially be impossible but with the passage of time and the occurrence of macrophage death, asbestos fibres would be released from their original sites and subsequently cleared. In addition to this, some macrophages are known to travel from the interstitial space, along lymphatics, to the lymph nodes outside the lung. These two modes of elimination could constitute the slow phase of lung clearance.

The data indicate that with clouds of only 1 mg/m^3 there was proportionately less accumulation of amosite than at the higher concentrations. Not only was the total lung load at the end of the dusting period lower in proportion than with clouds of 5 mg/m^3 and 10 mg/m^3, but also there was little clearance of the dust during the subsequent 4 months. A possible explanation is that at very low doses the normal lung clearance mechanisms can cope quickly with most of the dust deposited and that which does remain in the lung tissue becomes fixed and is only slowly cleared. At higher dose levels the clearance mechanisms may become saturated during the dusting period, allowing a greater dust build-up. After the cessation of dusting, however, much of the accumulated dust would eventually be cleared. If this were true of human beings it would indicate that a lifetime's exposure to very low doses should not result in much dust accumulation.

The retention data for crocidolite are as yet incomplete and their interpretation is complicated by the presence of the material thought to be amorphous silica.

This indication that crocidolite suffers a significant degree of leaching during residence in the lung is surprising. Reappraisal of the infrared spectra obtained from the amosite residues indicates that these may have contained small amounts of amor-

phous silica but that these were insignificant compared to the quantities associated with the crocidolite residues.

The possibility that the amorphous silica was an artefact of the recovery process was considered, but this seems unlikely as respirable crocidolite recovered from an M.R.E. sampler, added to lungs from unexposed rats and taken through the recovery process (simultaneously with lungs from exposed rats) gave no indication of extraneous material. This also suggests that it is unlikely that this material may have originated from fine dust in the rats' food supply.*

CONCLUSIONS

The need for further investigation of the influence of dose rate upon retention and subsequent clearance is indicated. In view of the considerable differences that exist in the fibre number *vs.* mass correlations for different asbestos varieties there is also a requirement to consider carefully the number as well as the mass of fibres. We are at present conducting long-term inhalation experiments which it is hoped will provide data relevant to these problems. It is also hoped to investigate further the length distributions of fibres retained in the lungs after different clearance periods.

The apparent leaching of crocidolite during its residence in the lung needs to be investigated in detail and confirmatory evidence of the presence of amorphous silica obtained.

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* See footnote to page 254.

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