

Erionite exposure and mesotheliomas in rats

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Summary Epidemiological and environmental surveys in the Cappadocian region of Turkey have linked the high incidence of pleural and peritoneal mesothelioma in the occupants of some villages with the zeolite fibres released from the locally occurring volcanic tuff. In view of the low ambient fibre concentrations and the extraordinary incidence of mesothelioma a study to test the hypothesis of high biological activity for the zeolite fibres was required.

Experimental studies using both intrapleural inoculation and inhalation techniques have been undertaken with the erionite from this region and from Oregon in the United States. Additionally a non-fibrous zeolite from Japan and a synthetic non-fibrous zeolite of similar chemical composition to erionite have been included in the experiments.

In these studies the samples from Oregon and Turkey produced a very high incidence of tumours. All the rats inoculated intrapleurally with Oregon erionite and almost all those inoculated with the Turkish fibre died with a mesothelioma. Inhalation of the Oregon erionite induced a similar effect. No other dusts we have investigated have produced this high incidence of tumours particularly following inhalation. These studies demonstrate that we now have a valuable new fibre for experimental study and a possible hazard to man in regions other than Turkey.

Following upon the report of the occurrence of a large number of mesotheliomas in the Urgup region of Cappadocia, Turkey (Baris *et al.* 1978) a study of mineral samples from the region detected volcanic tuff which included fine erionite fibre (Pooley, 1979). These fibres being of appropriate morphology were considered to be the probable cause of the tumours and the hypothesis was strengthened when long fibres were found in a small fragment of lung tissue in one of the human biopsy specimens. One of the authors of this paper (JWS) during an environmental survey confirmed that zeolite fibres made the major contribution to the fibres in the airborne dust and identified the source as a poorly consolidated rock which outcropped and, in places, formed the walls of caves still used as utility rooms and animal quarters. The rock is an incompletely formed erionite, the fibres are contained in an amorphous matrix which has the same composition as erionite. In the past some blocks of the material have been cut and used for building purposes. A sample of this rock was obtained for animal experiments in Karain, a severely affected village. It was obtained from a cave adjoining the home of a family in which several mesotheliomas had occurred. We also obtained from Professor F.H. Mumpton a sample of erionite from Oregon, USA, and Dr R.S. Taylor of Laporte Industries provided us with a sample of a synthetic non-fibrous zeolite with chemical composition identical to erionite.

Materials and methods

Dusts were prepared from the rock samples by disc milling for just long enough to permit the generation of clouds. The synthetic zeolite required no milling. Respirable dust samples were collected aerodynamically from the clouds for the inoculation experiments and the size characteristics of the contained fibres determined by transmission electron microscopy. The inhalation experiment was carried out using 1.4 m³ exposure chambers in which clouds with mean respirable dust concentrations of 10 mg m⁻³ were maintained for 7 h day⁻¹ on 5 days of each week over a period of one year. Corresponding fibre counts were determined from samples collected on nucleopore membranes and evaluated in the scanning electron microscope.

Fischer 344 barrier maintained rats were used for both treatments. For the intrapleural inoculation experiment 200 rats, 100 males and 100 females, were randomly allocated into 5 treatment groups of 20 males and 20 females. At ~60 days of age the groups were inoculated intrapleurally with 20 mg Oregon erionite, Turkish (Karain) rock fibre, non-fibrous (Japanese) zeolite, chrysotile (positive control) or saline (vehicle). After injection the rats were maintained normally until either they died or were killed when distressed.

For the inhalation experiments 4 treatment groups of rats were similarly selected for exposure to Oregon erionite, non-fibrous synthetic erionite or UICC crocidolite, the fourth group were unexposed. There were 20 male and 20 female rats in each treatment group except for the crocidolite

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group which contained 16 males and 19 females. The rats were ~57 days of age when first exposed to dust and inhalation continued for 12 months after which they were transferred to clean living quarters to live out their lives. Small numbers of rats were removed and sacrificed throughout the experiment to study the development of dust accumulation at 3, 6, 12 and 24 months after the start of exposure. There was no sacrifice of rats exposed to Oregon erionite at 24 months. Post mortem examinations were performed on all rats and the appropriate tissues fixed in formalin for histological examination.

Results

Intrapleural inoculation

The dusts were ultrasonically dispersed in physiological saline before inoculation and a sample of the dispersed zeolite dusts was examined by transmission electron microscopy to determine fibre numbers and size details; these are given in Table I. A similar range of fibre sizes was included in both samples but the proportions in the various size

ranges differed, particularly in the longer fibre ranges. The non-fibrous dust content of the samples differed and this is reflected in the number of fibres per unit mass; Oregon erionite contained the highest number of fibres.

The numbers of mesotheliomas produced are shown in Table II. All the animals inoculated with erionite died with mesothelioma whereas only 2 mesotheliomas occurred with the non fibrous zeolite. The positive control, chrysotile asbestos, gave 19 mesotheliomas and the negative control, saline, gave one. An important observation was the length of time between inoculation and death from mesothelioma; in the chrysotile group this was an average of 678 days, a period which corresponds with previous studies using asbestos. The Oregon erionite and Karain rock fibre, however, averaged 390 and 435 days respectively, a considerable shortening of the latent period.

Inhalation

The size distributions of the fibrous particles making up the clouds and the fibre counts corresponding to the mean gravimetric respirable dust concentration of 10 mg m^{-3} are given in Table III.

Table I Size distributions of inoculated fibrous zeolites

Diameter μm Length	Karain					Oregon				
	% frequency					% frequency				
	0–	0.2–	0.5–	1	Total	0–	0.2–	0.5–	1	Total
0–2	59.3	13.3	1.2	0	73.8	29.6	0.6	0	0	30.2
2–4	7.6	3.4	3.9	0.9	15.8	26.6	2.5	0	0	29.1
4–6	2.9	2.0	0.7	0.7	6.3	11.7	3.7	0.6	0	16.0
6–8	1.1	0.5	0.2	0.2	2.0	5.0	0.8	0.6	0	6.4
8–10	0.5	0	0.2	0.2	0.9	3.7	1.5	0.6	0	5.8
–10	0.4	0.4	0.4	0.2	1.4	3.7	3.5	0.6	0.4	12.5
Total	71.8	19.6	6.6	2.2	100	80.3	12.6	6.7	0.4	100
Fibres mg^{-1} respirable dust	2.4×10^8					2.9×10^7				

Table II Tumour induction from intrapleural inoculation

Material	Total no. of rats in group	No. of mesothelioma	Mean survival time (days)	No. dead from other causes	Mean survival time (days)
Oregon Erionite	40	40	390	0	—
Karain Rock Fibre	40	38	435	2	440
Non Fibrous Zeolite (Japanese)	40	2 (1 peritoneal)	715	38	780
Chrysotile	40	19	678	21	659
Saline	40	1	720	39	721

Table III Fibre size distribution of clouds. Inhalation Experiment.

Diameter μm Length	UICC Crocidolite						Oregon Erionite					
	% frequency						% frequency					
	0-	0.2-	0.4-	0.6-	1.0-	Total	0-	0.2-	0.4-	0.6-	1.0-	Total
3-5	17.1	25.8	3.7	0.7	—	47.3	27.3	16.2	6.9	5.0	0.6	56.0
5-10	3.7	23.5	12.1	1.8	—	41.1	12.9	10.3	6.5	2.6	4.3	36.6
10-20	1.1	5.8	2.4	1.4	0.2	11.0	1.4	2.8	0.4	10	1.0	6.6
20	—	0.2	0.2	—	0.1	0.5	0.2	0.2	0.2	—	0.2	0.8
Total	21.9	55.4	18.4	7.6	0.3	99.9	41.8	29.5	14.0	8.6	6.1	100
F/ml $5\mu\text{m}$ 10mg m^{-3}	1630						354					

Table IV Tumour induction and deaths after 12 months. Inhalation experiment.

Material	Total no. of rats in group	No. of tumours	Mean survival time (days)	No. dead from other causes	Mean survival time (days)
Oregon Erionite	28	27 mesothelioma	580	1	504
Crocidolite	28	1 sq. carcinoma	917	27	718
Synthetic Non-Fibrous Erionite	28	1 mesothelioma 1 adenocarcinoma	784	26	797
Unexposed Control	28	0	—	28	738

A similar range of fibre sizes was contained in the crocidolite and erionite clouds. Fewer isometric particles and more fibres were contained in the crocidolite cloud. The synthetic non-fibrous erionite cloud contained 10.4×10^3 particles ($>0.5\mu\text{m}$) ml^{-1} .

The tumours induced by the treatments are given in Table IV. Mesothelioma was induced in 27 of the 28 animals exposed to Oregon erionite and allowed to survive for more than 12 months; 12 animals had been sacrificed previously to study dust accumulation. These tumours occurred between 385 and 800 days, an average of 580 days. The incidence of tumours in the other groups was low. One mesothelioma and one adenocarcinoma occurred in the rats exposed to the synthetic non fibrous zeolite. No mesothelioma occurred in the positive control, crocidolite, group, but one squamous carcinoma of the lung was observed.

Discussion

Preliminary reports on these experiments have been presented by Wagner (1982, 1983). The enhanced potential for mesothelioma induction of the erionite compared with the crocidolite, since a similar range

Table V Previous inhalation study (Wagner *et al.*, 1974)

Type of asbestos	No. of animals alive after 12 months	Mesothelioma
Amosite	134	1
Anthophyllite	133	2
Crocidolite	124	4
Chrysotile Canadian	125	4
Chrysotile Zimbabwe	132	0
	648	11

of fibre lengths and diameters were present in both dusts, indicates additional properties of the erionite worthy of further investigation. The magnitude of this enhancement is apparent not only in terms of number but also in terms of the time required for the development of the tumour. In a larger inhalation experiment reported in 1974 (Wagner *et al.*, 1974) and summarised in Table V, 11 mesotheliomas were induced when 648 rats were exposed to various types of asbestos, the tumours being induced 600 days or more after first exposure to dust. In this zeolite experiment virtually all the

animals died with mesothelioma with induction periods ranging from less than 400 days.

Further inhalation and other studies are in progress. Already a colleague has stated that

“Oregon erionite is the only fibrous dust we have so far examined which gives reproducible and unequivocal positive results in *in vitro* assays designed to detect genotoxicity” (Poole *et al.*, 1983).

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