

their fur to become clear of asbestos before transferring them to a clean environment for the remainder of their lives. At transfer some rats, generally 2 or 3 of each sex, were killed and the lungs removed for histological examination and determination of dust content.

As each type of asbestos has a considerable silicate content (about 50%) the amount of dust in the lungs was determined by first assessing its silica content and referring this value to that of the respirable dust to which the rat had been exposed. This method was used in previous experiments (Morris *et al.*, 1967).

After exposure the rats were caged in threes or fours isolated in a special unit supplied with filtered air. The inhalation chambers were also in this unit but in a separate room. They were fed on a proprietary brand of autoclaved cubes, and water *ad libitum*. Except for the scheduled killings, each rat was allowed to live until it died or appeared to be distressed, and a full necropsy examination was carried out.

Histological preparation, staining and microscopic methods.—For the scheduled killings, animals were killed by chloroform anaesthesia and following exsanguination the thorax opened and the lungs removed. The left lung was air inflated and suspended in formalin. Representative portions of the right lung were taken for electron microscopic examination and the remainder of the lung dilated with neutral buffered formalin. Slices were taken from both lungs for histological examination. After the histological sections had been cut, the embedded tissue and the trimmings were dewaxed and added to the remainder of the lungs which were used for the chemical estimates.

For other animals, at post mortem the lungs were dilated with neutral buffered formalin and after fixation were sliced sagittally, routine sections being taken of the whole left lung and the upper and lower lobes of the right lung. In addition, any other suspicious lesions from the lungs or other organs were taken for histological examination.

In all cases, sections were stained with haematoxylin and eosin and the lung sections were stained for elastin, reticulin and collagen. Special stains were used in some cases as an aid to diagnosis of the tumours.

Assessment of the severity of asbestosis.—

Sections of both lungs were examined without knowledge of the duration or type of asbestos exposure and with the animals in random order. The sections were observed on a viewing screen of a Projectina Microscope 4013 BK using a $\times 7$ objective. At this magnification a large proportion of the lung could be assessed in a single field and, as it was not possible to observe asbestos fibres at this magnification, the results were not biased by knowing the type of asbestos to which the animal had been exposed.

Experiments

In both experiments and for all doses there were groups which were exposed to all 5 reference samples; there were also control groups which were not exposed. Rats were allocated to treatments at random. At the start of the exposure the majority of the rats were between 5 and 7 weeks old, a few being slightly older or younger, and there were approximately equal numbers of males and females.

Experiment 1.—There were 2 time intervals of exposure: in the first, rats were exposed for 3 months starting in May 1967 and in the second, groups of rats were exposed for one day only in August 1967. In addition to the killings at the end of exposure, in the 3-month group there were also intermediate sacrifices at 5, 8 and 10 weeks.

Experiment 2.—There were 3 time intervals of exposure—6 months, 12 months and 24 months. The experiment started in January 1969 and after 6 months half of the rats were removed from the cabinets. They were replaced and a year later these replacement animals were in turn removed. They were replaced by animals to be used for special electron microscopy examinations which will not be reported in this paper. The remaining animals were removed in January 1971 after 2 years' exposure. In the 6-month groups, in addition to the killings at the end of exposure, rats were also killed after 2 years, *i.e.* 18 months after removal from exposure.

The numbers of rats are given in Table I. In the 24-month groups, overcrowding amongst the males tended to occur due to increase in size after about a year, and some were removed prematurely after 13½ months. For analysis these rats have been