THE PULMONARY TOXICITY OF MIXED DUST IS NOT ONLY RELATED TO ITS MINERALOGICAL COMPOSITION

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INTRODUCTION

Studying the relationships between the physico-chemical characteristics of some dust particles and the activity that these particles may exhibit when in contact with various biological systems is a fascinating area of research. Our understanding of the question, however, is quite limited. We know that exposure to asbestos dust may lead to asbestosis, free silica to silicosis and coal mine dust to coal worker pneumoconiosis. But we do not know which parameter at the level of the particles will trigger the relevant biological mechanisms. Several hypotheses have been made, but no satisfactory theory has emerged from the many studies on the subject.

The problem is even more complicated when dealing with mixed dust, such as coal mine dust. In addition to coal from various rank, coal mine dust generally contains free silica and clay minerals. Each component may play a role in the pathogenesis of the disease. For example, studies in rats by inhalation and intratracheal injection revealed that quartz in coal mine dust exhibited less activity than expected.[8] This phenomenon was attributed to the release of aluminum from clay minerals present, especially from illite.[6] It led to the hypothesis that the biological activity of quartz in mixed dust was depending to the ability for accompanying minerals to mask the potential toxicity of quartz.[6,7,10] The toxicity of coal mine dust would more depend on the overall mineralogical composition rather than on the quartz content alone.[12,9]

In order to explore this hypothesis, we tested in the rat two samples of coal dust having the same bulk mineralogical composition in terms of coal, quartz, illite and kaolin. They did not exhibit the same pulmonary activity.

METHODS

Sample #1 was obtained by finely grinding some coal materials extracted from the Aumance coal mine in France. The final product contained 35% coal, 17% quartz, 31% illite and 17% kaolin. Mineralogical determinations were made using a combination of X-ray diffraction and infrared spectroscopy. Sample #2 was a reconstituted mixture of fine particles of "pure" coal and minerals from other origin (illite from Le Puy, kaolin from Cornwall and quartz from Madagascar). The two samples had the same final mineralogical composition by weight. Particles in each sample were examined by Analytical Transmission Electron Microscopy (ATEM) and their number size distributions were established.

Three groups of 40 female Wistar rats were used for this study. Each animal in the exposed groups received a single intratracheal injection of 60 mg of fine particles suspended in 1 ml of saline. Some animals were killed 12 and 24 months later.

The lungs and the tracheobronchial lymph nodes were removed and weighed. Left lobes were used for histopathological examination and ATEM analysis of retained dust particles. Left lobes were perfused under 25 cm H_2O pressure and fixed in 10% neutral buffered formalin. Sections stained by hematoxylin eosine and Picrosirius were examined at three different locations under crossed polaroid filters.[5] The intensity and profusion of the lesions were scored, each on a 0–4 scale. Criteria used for intensity grading are indicated in Table I. A final histopathological score was obtained by multiplying the intensity score and the profusion score.[1] Lung tissue was then extracted from the remaining block by dewaxing in hot toluene. After digestion of the tissue in sodium hypochlorite, retained particles were concentrated by filtration on Polycarbonate membrane and analysed by ATEM.

For each group, right lungs and remaining tissue fragments not used for histology were dried and pooled. The pool was analysed for collagen by the method of Stegeman,[13] for total dust by the formamide method of Thomas,[14] for quartz by X-ray diffraction and for total Al in dust by X-ray fluorescence.

Similar methods were used to prepare and analyse lymph nodes.

RESULTS

For exposed animals, mean weight of fresh lung and mean collagen content of the lung were both above corresponding control values (Table II). The highest figures were measured at month 24 in the group of animals injected with the reconstituted mixture. In particular, the collagen content of the lung was more than three times higher with the reconstituted mixture than with the Aumance coal dust.

No histological changes were noticed in the lung of control animals, but lesions were present in the lung of exposed animals. Histopathological scores are presented in Figure 2.

Table I

Criteria Used for Scoring Intensity of Lung Lesions

Grade 0	Normal histology.
Grade 1	Focal accumulation of dust-laden macrophages without any fibrotic organisation.
Grade 2	Early fibrotic organisation with few thin collagen III fibers peripheraly to the granulomas, or intersperced throughout (green color with Picrosirius stain under cross polaroid filters).
Grade 3	Fibrotic organisation of the granulomas, with thick bundles of collagen I, in addition to collagen III (yellow orange or red color with Picrosirius stain).
Grade 4	Massive fibrotic reaction located around the main bronchus and vessels.

Table II

Weight of Fresh Lung, Weight of Lymph Nodes and Pulmonary Collagen

Month 12			Month 24		
Controls	Aumance	Mixture	Controls	Aumance	Mixture
Mean weight of fresh lungs (g/rat)					
1.3	1.8	1.8	1.3	2.1	3.6
Mean weight of lymph nodes (mg)					
--	0.08	0.28	--	0.36	1.1
Mean collagen content of the lung (mg/rat)					
29.4	47.5	52.8	31.3	52.5	167.3

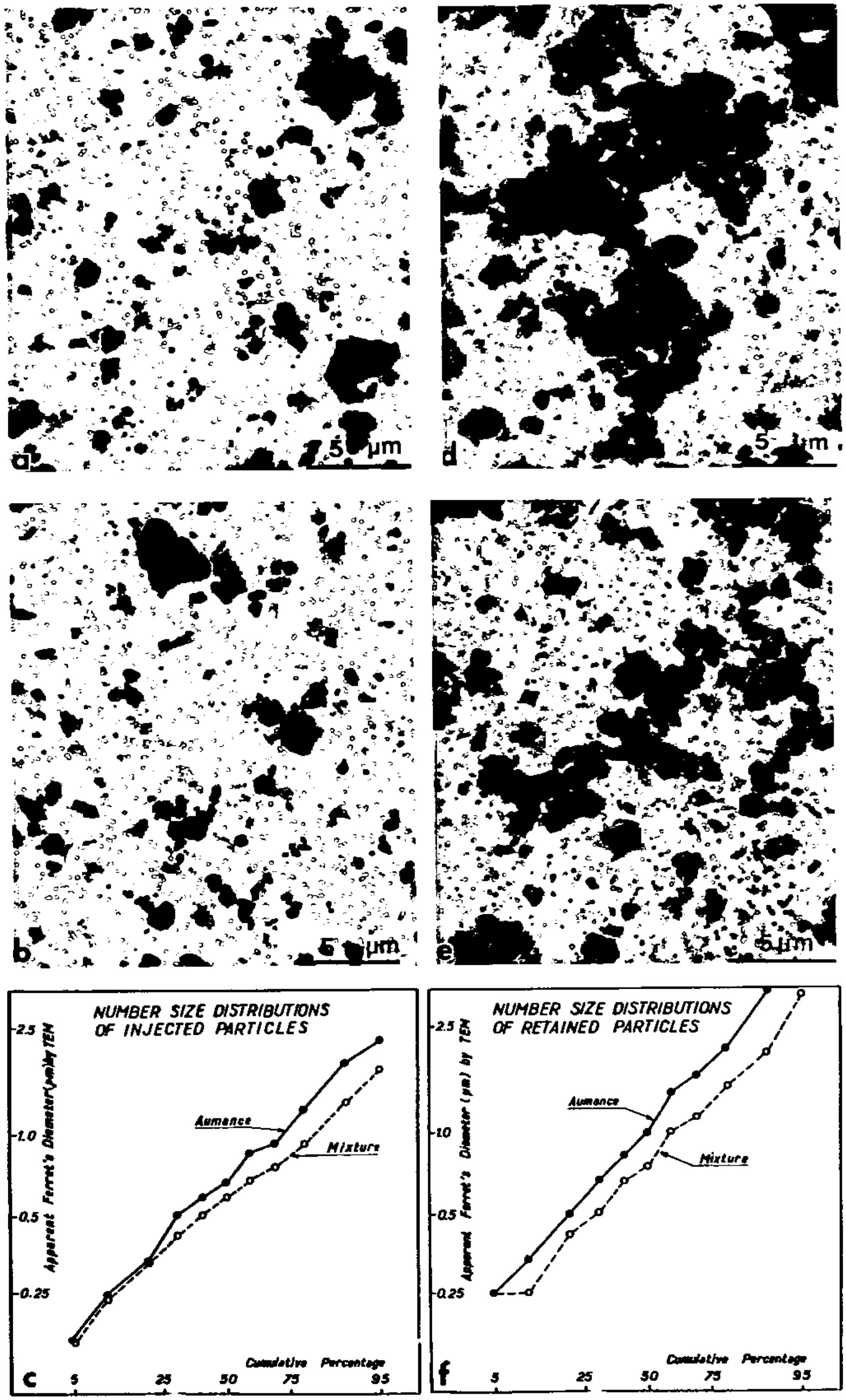

Figure 1. Analysis by transmission electron microscopy of dust particles injected, and extracted from the lung at month 24.

a. Aumance, particles injected
b. Mixture, particles injected
c. Number size distribution of injected particles
d. Aumance, particles retained in the lung
e. Mixture, particles retained in the lung
f. Number size distribution of particles retained in the lungs

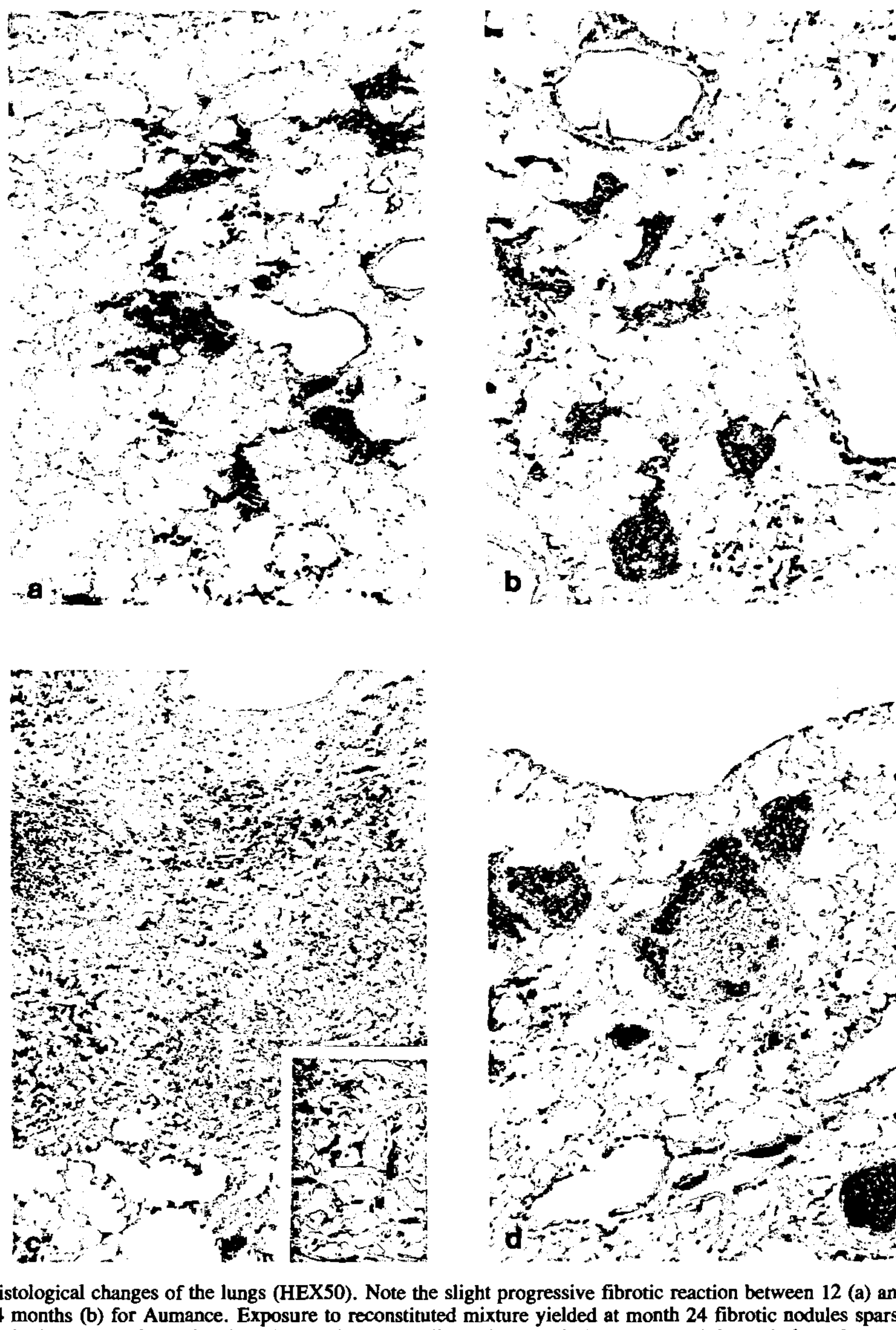

Figure 2. Histological changes of the lungs (HEX50). Note the slight progressive fibrotic reaction between 12 (a) and 24 months (b) for Aumance. Exposure to reconstituted mixture yielded at month 24 fibrotic nodules sparse in the lung (c) and massive fibrotic reaction (see collagen bundles in insert) around the main bronchus and vessels (d).

In each exposed group, the mean score was significantly higher at month 24. At this date, histopathological changes were significantly more pronounced with the reconstituted mixture. At month 12, pulmonary lesions were quite similar in the two groups but the fibrotic reaction of the tracheobronchial lymph nodes was much more intense with the mixture.

Mineral contents of the pooled lung tissue and lymph nodes are reported in Table III. A very high proportion of the injected dust was still present in the lung at month 12 and at month 24. Figures for total dust, quartz and Aluminum were systematically higher in the Aumance group. Quartz accounted for 17% of the injected dust, but overall, the proportions of quartz in the lung dust were less. At month 12, proportions of quartz and proportions of Aluminum in the lung dusts did not differ between the two exposure groups. The lung dusts were richer in quartz at month 24. The quartz contents of tracheobronchial lymph nodes were quite similar in both groups (Table III).

ATEM analysis of dust used for injection indicated the presence of very fine particles in both samples. Most of the particles observed by ATEM were less than 2.5 µm in apparent Ferret's diameter. The number size distributions were similar in the two samples, although the particles were somewhat finer in the mixture (Figure 1). Particles extracted from the lung at month 24 were either isolated or grouped into large agglomerates. Such agglomerates were not detected in the injected dust.

Detailed mineralogical analysis of the lung dust by ATEM is still in progress. Preliminary observations suggest that the clay contents of the lung were different for the two groups, with apparently more illite retained at month 24 by animals exposed to the Aumance dust.

DISCUSSION

The model used was able to produce a fibrotic reaction progressing over the two years of the experiment, and to document different activities of the two dust samples tested.

These experiments illustrate once more how complex are the mechanisms of biological action of mixed dust. The two mixed dust samples with the same bulk mineralogical composition yielded different fibrotic pulmonary responses.

Table III

Mineral Content of the Lung and of the Tracheobronchial Lymph Nodes

Month 12		Month 24	
Aumance	Mixture	Aumance	Mixture
Total mineral dust in the lung (mg/rat)			
47.8	35.5	41.7	35.5
Quartz in the lung (mg/rat)			
5.6	4.2	7.4	4.9
Percentage of quartz in lung dust			
11.7	11.8	17.7	13.8
Aluminum in lung dust (mg/rat)			
1.8	1.3	2.5	1.1
Percentage of Aluminum in lung dust			
3.7	3.7	5.9	3.1
Quartz in the lymph nodes (mg)			
1.0	1.4	1.5	1.5

The bulk analysis of lung dust did not provide any convincing explanation for this difference. May be that the microscopical analysis in progress will bring interesting information. It is conceivable that the particles be differently assembled in the natural and in the reconstituted dust. Preliminary observations of clays in lung dust do support this hypothesis. Surface analysis of injected and retained dust may also be informative.[3]

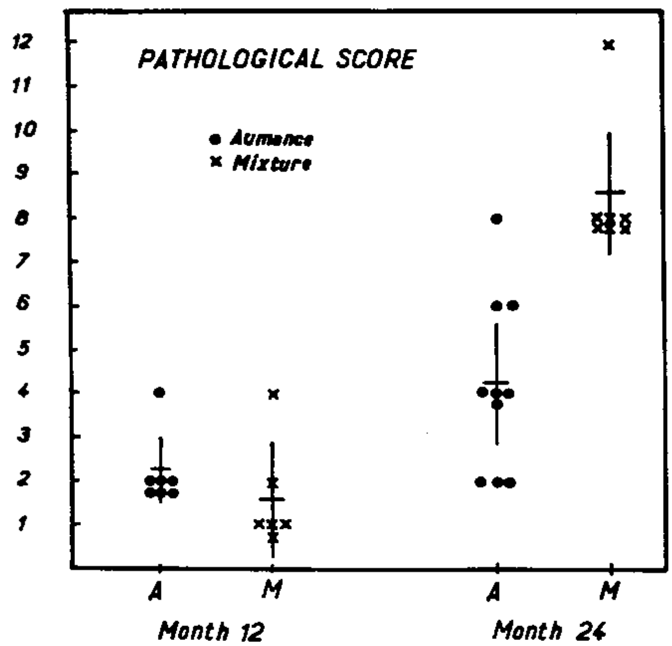

Figure 3. Pathological scores (see text for explanation) at month 12 and month 24 for the two groups of exposed animals.

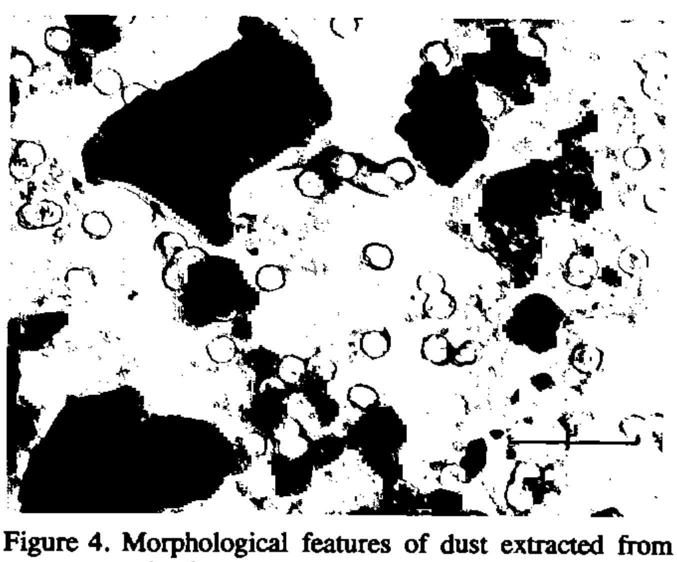

Figure 4. Morphological features of dust extracted from animals exposed to the Aumance coal dust. Note the presence of disolving illite particles.

Whatever the explanation may be, it is clear from these and other data,[2,4,9,11] that the toxicity of coal mine dust and probably of other mixed dust is not only related to its mineralogical composition (as usually determined). More subtle properties of the dust particles may also play a role. This put into question the usefulness of incorporating expensive mineralogical analyses in routine dust measuring programs.

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