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NON-FIBROUS INORGANIC PARTICLES IN HUMAN BRONCHOALVEOLAR LAVAGE FLUIDS

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Abstract

Bronchoalveolar lavage (BAL) is a simple and non-invasive sampling technique of the deep lung. Analytical electron microscopy was used for the identification and quantification of non-fibrous inorganic particles recovered in BAL fluid samples from 51 subjects with various occupational exposures (silica, silicates, metals and alloys, metallic oxides, precious and hard metals, abrasives). Around 4750 particles were analysed. More than sixty different compounds were identified, among which silica, kaolinite, illite, mica, Fe oxides and hydroxides, appeared to be ubiquitous. Feldspar, talc, chlorite, Al oxide, Ti oxide, tungsten carbide, stainless steel, carbonaceous compounds and flyash were also frequently encountered. From 1 to 21 compounds were identified in each sample. Repeated BAL samples obtained for 2 subjects did not show significant differences. Particles characteristic of the occupational exposure were found in BAL up to 21 years after cessation. BAL content can also reflect mixed occupational exposures. Absolute particle concentrations measured in twelve samples ranged between 0.1 and 9.9×10^6 particles/ml BAL fluid and mean particle diameter ranged between 0.5 and 1.2 μm .

Mineralogical analysis of non-fibrous particles in BAL can be a useful tool to investigate occupational exposures. It allows, in most cases, a better characterization of the exposure than medical questioning. It may be helpful in identifying pathogenic particles, however it must be kept in mind that a positive result is only a proof of exposure and never a proof of disease. The main limitations of this technique are difficulties in sampling severely diseased subjects and inaccuracy in detecting easily soluble compounds and particles with a high rate of alveolar clearance.

Key Words : inhaled particles, occupational dust exposure, analytical electron microscopy, bronchoalveolar lavage, energy dispersive X-ray analysis, lung pathology.

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Introduction

Airborne inorganic particles are generated by environmental and occupational emission sources as well as by outdoor and indoor air pollution. According to their aerodynamic diameter some of these particles can be inhaled and may reach the deep lung. After deposition they become subject to various fates and interaction may occur with alveolar fluids and cells. Lung clearance mechanisms include both elimination by means of the mucociliary escalator or transport through lymphatics and storage in lymph nodes. Clearance rates may be affected by cigarette smoking, drugs and environmental pollutants [22]. Depending on their chemical composition particles may also be completely or partially dissolved (e.g., sulphates, nitrates). Finally, a fraction of the inhaled particles which is not cleared remain trapped in the alveolar macrophages or in the interstitial lung tissue for very long times.

According to their nature and physical characteristics and their concentration in lung tissue, some particles may induce several types of reactions in the lung. They are recognised as the causal agents of lung fibrosis [26], granulomatosis [15, 26], alveolar proteinosis [24], asthma [10], small airways lesions [6] and as co-factors in the development of bronchial carcinoma [5, 26]. Other particles, although considered as "inert" [26] may nevertheless act as carriers for biologically active substances adsorbed (benzo-*a*-pyrene) [25] or condensed onto their surface (heavy metals or flyash) [21]. Once in the lung these substances may be released by desorption or leaching [21] from particle surfaces at sites where they can induce local toxic or irritant actions.

These are the reasons why research is needed to improve the knowledge about inhaled particles characteristics and burdens in the lungs for various occupational or environmental settings, as well as in control groups. Mineralogical analysis techniques routinely used for the characterization of small particles, principally analytical electron microscopy (scanning or transmission), may be applied to characterize and count the particles contained in lung tissue, lymph nodes or bronchoalveolar lavage (BAL) fluid. Analysis can be performed *in situ* [1, 3, 20] on tissue sections or cells, or on particles

collected from digested samples [5, 7, 12, 13, 15, 18, 19, 24, 27, 29, 30]. Actually, very few systematic data about the non-fibrous particles contained in these indicator samples are available when compared with the amount of data about asbestos fibers in the literature.

BAL is a non-invasive technique used to sample *in vivo* cells and metabolites from the deep lung [9]. Inhaled particles present on these alveolar surfaces, free or phagocytised in macrophages, are sampled at the same time. Asbestos body contents of BAL samples have been investigated in our laboratory for several years and have proved to be useful in occupational [14] and environmental [11] asbestos related diseases and exposures. We report in this paper our experience with the analysis of non-fibrous inorganic particles in BAL fluids of 51 subjects.

Materials and Methods

Subject data

BAL fluids were obtained from 48 male and 3 female subjects with various exposures to non-fibrous inorganic particles. Samples were collected in our department or in external institutions. Table 1 gives an overview of subject occupations and exposures obtained from medical questioning or from data of the Belgian Occupational Disease Fund. Subject age ranges from 25 to 75 years (mean \pm SD : 48.4 \pm 12.2). Data about smoking habits, exposure duration and delay since end of exposure are not available for all of the subjects and consequently not presented in this study. Repeated BALs were obtained for subjects 9 (4 samples) and 33 (2 samples). Delays after the first BAL sample were, respectively, 15, 17, 27 and 42 months. More detailed descriptions of subject 9 exhibiting aluminum induced lung granulomatosis and subjects 40 to 45 exhibiting talc pneumoconiosis were reported previously [15, 13].

BAL sampling and preparation

Bronchoalveolar lavage is performed in a segmental or subsegmental bronchus, usually in the right middle lobe. Aliquots of 50 ml of normal saline are injected through the fiberoptic bronchoscope. BAL fluid is recovered by aspiration after each instillation.

A sample of 10 to 40 ml of the fluid recovered from the second or third fraction is used for particle analysis. The processing technique for these samples is basically the same as the one used for asbestos fiber and body recovery [16]. Briefly, organic material (cells, mucus) contained in the BAL fluid is digested with Na hypochlorite (commercial bleach). All the fluids used in this processing are firstly filtered on membrane filters in order to avoid contamination. Filtering funnels are cleaned ultrasonically and rinsed with filtered water before use. Inorganic particles are collected on cellulose esters membrane filters (Milipore®) with a pore size of 0.45 μ m. After drying, the filters are clarified and fixed on glass slides by fusion in acetone vapors.

At this step, samples may be examined with a petrographic light microscope under various illumination modes (transmitted, incident and

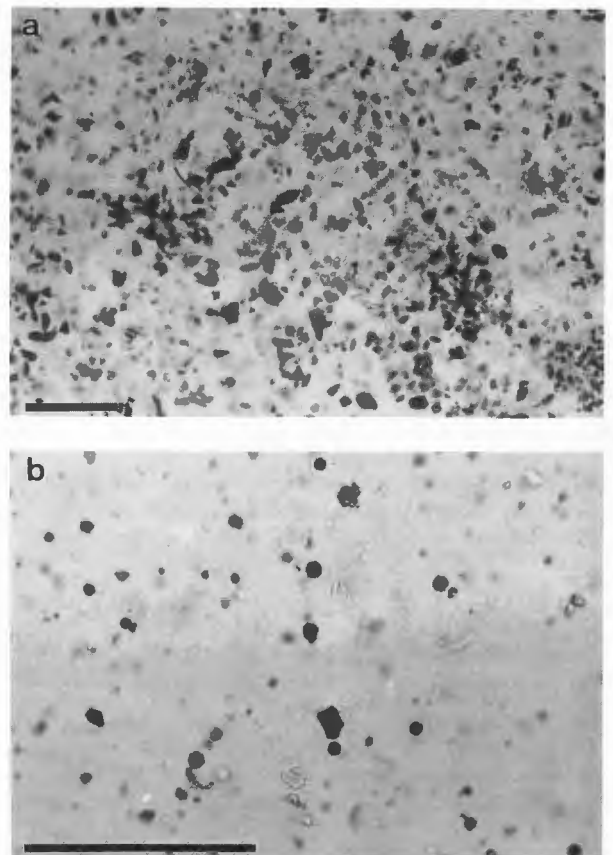


Figure 1. Light microscopy view of particles collected from the BAL of : a) a coal miner (subject 14) showing numerous coal particles, b) a welder (subject 51) showing abundant spheres of iron oxide. This spherical morphology is characteristic of high temperature formation processes. Bar = 10 μ m.

polarized light, phase contrast) and magnifications. This allows the assessment of the quality of the sample (uniformity of particle distribution and suitable filter loading) and the collection of an initial set of data about the inorganic particles (Fig. 1). Information about their optical characteristics (transparency, color, refractive index, reflectivity, luster, anisotropy) and morphology may be useful complements to analytical electron microscopy data for the identification of particles species.

To transfer the particles deposited on the filter surface to electron microscopy grids, small pieces of filter are cut out and removed from the glass slide. After carbon coating of the top surface they are deposited on the grids in a modified Jaffe washer. The filter material is removed with acetone leaving the particles embedded in an electron transparent carbon film. Copper grids are usually used as sample support, but when this element is specifically searched they are replaced by gold ones.

Analytical electron microscopy

Particles are examined with a Philips EM 400T transmission electron microscope (TEM) fitted

with a scanning (STEM) attachment. The microscope is operated at 80 kV high tension and 10 to 20 nA beam current. Chemical analysis of individual particles is performed with an EDAX PV9900 energy dispersive X-ray analyser (EDS) which allows the detection of all elements with an atomic number equal to or higher than 11 (Na). Each particle with a diameter of at least 0.1 μm which is encountered during a systematic screening at 22,000X magnification is taken into account. Morphology, electron transparency, chemical composition and qualitative information about the selected area diffraction pattern are recorded. For the most common particle species encountered, especially silicates, chemical spectra can be compared with a set of reference ones obtained in the same analytical conditions from a collection of standards. Combined with light microscopy observations this allows, in most cases, the unambiguous identification of the main particles species present in a sample. However, problems remain in distinguishing between exogenous and endogenous iron and calcium compounds [1] or between compounds that may contain low atomic number elements impossible to detect with our equipment (e.g. : silicon carbide versus silica). All the particles containing P associated with Fe, Ca or both and containing Ca alone (probably oxalates) were classified as endogenous. All others were considered as exogenous. However, exogenous particles coated with endogenous material were observed in some occurrences (e.g., subject 5). If the chemical spectra of the exogenous core can still be identified those particles were classified in the exogenous category corresponding to the nature of this core, otherwise they are classified as "combined exogenous-endogenous particles". Due to their morphological similarities and their frequent association in nature, we chose to group chlorite with talc in the presentation of the results. Illite (a clay mineral) and micas were also grouped because of their similarities in qualitative chemical composition and the existence of a wide range of compounds with intermediate composition. Stainless steel was specifically used to define particles containing Fe as a major constituent with approximately 20% of Cr and 10% of Ni. Fe oxides or hydroxides either pure or with traces of various metallic elements (Ti, Cr, Mn, Ni, Zn) were grouped as Fe compounds. Although Al hydroxides or even metallic Al cannot be ruled out in some cases, corundum (Al oxide) must be considered as the main Al compound. Carbonaceous compounds, which indiscriminantly group all particles without signal and diffraction patterns, are mainly coal and soot.

Digital imaging combined with elemental mapping capabilities of the EDAX system may also be used to resolve aggregated or heterogeneous particles or to document the most abundant phases of a sample (Fig. 2).

Around 4750 particles were analysed for this study. Depending on the complexity of the samples, from 45 to 150 (mean \pm SD : 84 \pm 21) consecutive particles were analysed. Semi-quantitative information concerning the relative concentrations in percent of the different

particle types is thus obtained for all the samples. More accurate quantitative data about particle absolute concentrations and sizes were collected for 12 subjects (3, 4, 7, 18, 19, 21, 22, 25, 27, 47, 49 and 50). For particle size, circular area equivalent diameter was visually estimated by comparing the particle image with calibrated circles inscribed on the TEM screen. To evaluate particle absolute concentrations, all the particles present in one grid square or at least 100 particles were counted. Results are expressed in number of particles per ml BAL fluid.

Results

The first statement that must be made after examination of Table 1 is that analyses are asked for a wide variety of particle types. There is generally little precise information available about the particles involved (e.g., "silicates", "abrasives",...). The main requests concern silica, silicates, metallic compounds (Fe, Al, Co, Ni, Cr, Mn, Cu, Mo,...), hard metals and abrasives.

Table 2 gives an overview of the relative concentrations of the main non-fibrous inorganic particles present in the BAL of each subject. More than sixty different compounds were identified. If we exclude subjects 40 to 45 (talc millers), BAL particulate content is rather complex. From 7 to 21 compounds (mean \pm SD : 14 \pm 4) are identified in each sample.

BAL of talc millers contains almost exclusively talc (subjects 40 and 41) or about 60% talc and 40% chlorite (subjects 42 to 45). This reflects the difference existing in the composition of the raw material these subjects were exposed to, French talc being known to contain large amounts of chlorite. For subject 42, exposure had ceased 21 years before BAL was performed. To clarify the following presentation of the results these 6 talc millers shall be omitted. The frequencies and relative concentration ranges of the main non-fibrous inorganic particles encountered in the BAL of the 45 remaining subjects are presented in Table 3. Among them 20 subjects have silicate concentrations higher than 50%. This does not appear to be specific to a particular occupation or type of exposure. Since variable concentrations of silica and Fe compounds were present in all the samples, those particles must be considered as ubiquitous. Kaolinite, illite and micas also seem to be ubiquitous, all of the BALs being positive for at least one of those compounds.

Silica concentrations higher than 50% were recorded only for subjects 18 (flint quarryman) (Fig. 2) and 34 (sand blaster) who have major occupational exposure to this mineral.

K feldspar was the main type of feldspar encountered, but plagioclase (Na-Ca feldspar) were also detected. Subject 6 was the only one with a feldspar concentration higher than 20%. He has used arkose (a sandstone containing at least 25% feldspar) as abrasive material.

Flyash spheres and crystals of mullite, both formed during high temperature processes, were

Table 1. Subject primary occupations and suspected or referred particle exposure.

| Subject # | Occupation | Exposure |
|-----------|---|--|
| 1 | Al polisher | Al, Cr, Cu |
| 2 | Al polisher | Al |
| 3 | Al polisher + Al powder production worker | Al, mica |
| 4 | Bank employee (white collar) | no specific exposure |
| 5 | Bank employee (coin sorting) | Metallic alloys |
| 6 | Blade sharpener | Abrasives |
| 7 | Boiler maker | Silicates, refractory material |
| 8 | Cable manufacture worker | Talc |
| 9 | Catalyst production plant chemist | Al, V, Cr, Fe, Co, Ni, Cu, Zn, Mo |
| 10 | Cement plant (painter) | Silica, silicates |
| 11 | Cement plant storekeeper | Silicates |
| 12 | Ceramist | Silicates |
| 13 | Coach builder | Silica |
| 14 | Coal miner | Silicates, coal |
| 15 | Diamond cutter | Hard metals, cobalt |
| 16 | Diamond cutter | Hard metals, cobalt |
| 17 | Docker | Al, Co |
| 18 | Flint quarryman | Silica, silicates |
| 19 | Foundry foreman | P, As, Cr, Fe, Cd, Pb, coal |
| 20 | Foundry worker | Bentonite |
| 21 | Glass wool production worker | Amorphous silica |
| 22 | Hard metal worker (powder preparation) | Hard metals, Co |
| 23 | Hard metal worker (powder preparation) | Hard metals, Co |
| 24 | Hard metal worker (recovery) | Hard metals, Co |
| 25 | Hard metal worker (recovery) + foundry worker | Hard metals, Co |
| 26 | Jewel designer | Alloys, Cr, Ni, Cu, Ag, Pt |
| 27 | Joiner | Zn pigments |
| 28 | Decarbonization worker | Metals, hard metals |
| 29 | Pharmacist | Talc, silica |
| 30 | Pipe manufacture worker | Metals |
| 31 | Polisher | Al, Al ₂ O ₃ , kaolinite, K feldspar |
| 32 | Resin preparator | Co, Ni |
| 33 | Sand blaster | Silica, silicates |
| 34 | Sand blaster | Silica, silicates |
| 35 | Steel worker (slag mill) | P, V, Mn, silicates |
| 36 | Steel worker (slag mill) | Mn |
| 37 | Steel worker (rolling mill) | Co |
| 38 | Steel worker | Cr, Mn, Fe, Ni, Mo |
| 39 | Steel worker | Al, Si, Cr, Mn, Fe, Ni, W |
| 40 | Talc miller (USA+Australian talc) | Talc |
| 41 | Talc miller (USA+Australian talc) | Talc |
| 42 | Talc miller (French talc) | Talc |
| 43 | Talc miller (French talc) | Talc |
| 44 | Talc miller (French talc) | Talc |
| 45 | Talc miller (French talc) | Talc |
| 46 | Tiller | Silicates |
| 47 | Tool sharpener | Hard metals, Cr, Ni, Co |
| 48 | Tool sharpener | Hard metals |
| 49 | Tungsten miner + coal miner | Silicates, W, coal |
| 50 | Tungsten miner + iron miner | Silicates, W, Fe oxide |
| 51 | Welder | Metals |

frequently observed in BAL samples. Spherical aggregates of mullite crystals resulting from the *in vivo* dissolution of a glassy siliceous matrix reported by Golden et al. [19] were also encountered in the present study (Fig. 3).

The other types of silicates occasionally found in BAL are siliceous glass, fayalite, zircon, sphene and wollastonite.

Fe compounds were found in concentrations higher than 50% in 4 subjects. An occupational

exposure to metals was reported for subjects 28 and 30, to abrasives for subject 31 and to iron mine dust for subject 50. However, the exposure of subject 31 was not correctly identified by medical questioning. He was not expected to have been exposed to abrasive iron oxide [26], but to other abrasive agents which were not recorded in his BAL. For subject 50 exposure to iron mine dust had ceased 15 years before BAL was performed.

Non-fibrous Particles in BAL Fluids

Table 2. Relative abundance of the main inorganic particle species identified in BAL fluid samples.

| S u b j e c t # | S | F | T | K | I | F | A | T | W | S | C | Miscellaneous |
|--|----------------|----------------------------------|----------------------|--|----------------------------------|---|--|------------------------------|---|-----------------------|---|------------------------|
| | il lic a | el l d s p a r | al cl + Chl | al lo l i n i t e | il l + M i c a | el l c o m p o u n d | al lo c o m p o u n d | al lo x i d e | W e i b e r i d e | S t e e l | C a r b o n a c e o u s i n g | |
| 1 | ● | ● | | ● | ● | ★ | ● | ★ | | ● | | silic glass ●, Cu ox ● |
| 2 | ● | ● | | ● | ● | ● | ● | ● | | | | |
| 3 | ★ | ● | | ● | ● | ● | ● | ● | | | | |
| 4 | ● | ● | | ● | ★ | ● | ● | | | | | |
| 5 | ● | ● | | ● | ● | ● | ● | | | ● | | FeCr alloy ● |
| 6 | ● | ● | | ● | ★ | ● | ● | | | | | |
| 7 | ● | ● | | ● | ● | ● | ● | | | | | flyash★ |
| 8 | ● | ● | | ★ | ★ | ● | ● | | | | | flyash★ |
| 9 | ★ | ● | | ● | ● | ● | ● | | | | | |
| 10 | ● | ● | | ★ | ★ | ● | ● | | | | | fayalite★ |
| 11 | ● | ● | ★ | ★ | ★ | ★ | ★ | | | ● | | |
| 12 | ● | ● | | ● | ● | ★ | ● | | | | | |
| 13 | ★ | ● | | ● | ● | ★ | ● | ● | | | | Cr ox ● |
| 14 | ● | ● | | ● | ● | ● | ● | | | | | |
| 15 | ● | ● | | ● | ● | ★ | ● | | | | | diam★, Co comp★ |
| 16 | ● | ● | | ● | ● | ● | ● | | | | | diam●, Co comp● |
| 17 | ● | ● | | ● | ● | ● | ● | | | | | endog★ |
| 18 | ★ | ● | | ★ | ★ | ● | ● | | | | | |
| 19 | ★ | ● | | ★ | ★ | ● | ● | | | ● | | |
| 20 | ● | ● | | ★ | ● | ● | ● | | | | | Ag comp ●, flyash ● |
| 21 | ● | ● | | ● | ● | ● | ★ | | | | | silic glass●, flyash ● |
| 22 | ● | ● | | ● | ★ | ● | ● | ● | | | | Cr ox ● |
| 23 | ● | ● | | ● | ★ | ● | ● | | | | | |
| 24 | ★ | ● | | ● | ★ | ● | ● | ★ | | | | |
| 25 | ★ | ● | | ● | ● | ● | ● | ● | | | | |
| 26 | ★ | ● | | ● | ★ | ★ | ★ | | | | | Au ●, Cr ox★ |
| 27 | ● | ● | | ● | ★ | ★ | ● | | | | | |
| 28 | ● | ● | | ● | ● | ★ | ● | | | | | FeCr alloy ● |
| 29 | ● | ★ | | ★ | ● | ● | ● | | | | | flyash ● |
| 30 | ● | ● | | ★ | ● | ★ | ● | | | | | |
| 31 | ● | ● | | ● | ● | ★ | ● | | | | | |
| 32 | ● | ● | | ● | ● | ● | ● | | | | | |
| 33 | ● | ● | | ● | ● | ● | ● | | | ● | | chromite ●, zircon ● |
| 34 | ★ | ● | | ● | ★ | ★ | ● | | | | | |
| 35 | ★ | ● | | ● | ● | ● | ● | | | | | |
| 36 | ● | ● | | ● | ★ | ● | ● | | | | | |
| 37 | ★ | ● | | ● | ● | ● | ● | | | | | Pb ●, Sn ●, PbSn ● |
| 38 | ● | ● | | ● | ★ | ● | ● | | | | | flyash ● |
| 39 | ★ | ● | | ★ | ● | ● | ● | | | ● | | complex alloys ● |
| 40 | | | | | | | | | | | | |
| 41 | | | ★ | | | | | | | | | |
| 42 | | | ★ | | | | | | | | | |
| 43 | | | ★ | | | | | | | | | |
| 44 | | | ★ | | | | | | | | | |
| 45 | | | ★ | | | | | | | | | |
| 46 | ● | ● | | ★ | ★ | ● | ● | | | | | flyash★ |
| 47 | ★ | ● | | ● | ● | ★ | ● | ★ | | | | flyash ● |
| 48 | ● | ● | | ● | ● | ● | ● | | | | | Ni★ |
| 49 | ● | ★ | | ● | ● | ● | ● | | | | | |
| 50 | ★ | ● | | ● | ● | ★ | ● | | | | | |
| 51 | ★ | ● | | ● | ● | ● | ★ | | | ● | | |

Abbreviations : Chl. = chlorite, Ill. = illite, comp. = compounds, St. = stainless, Carbonac. = carbonaceous compounds, Silic. = siliceous, ox. = oxide, Diam.= diamond, Endog.= endogenous. Scale : ● < 3%, 3% < ● < 10%, 10% < ★ < 20%, 20% < ● < 50%, ★ > 50%.

● : possible presence of small amounts of silicon carbide.

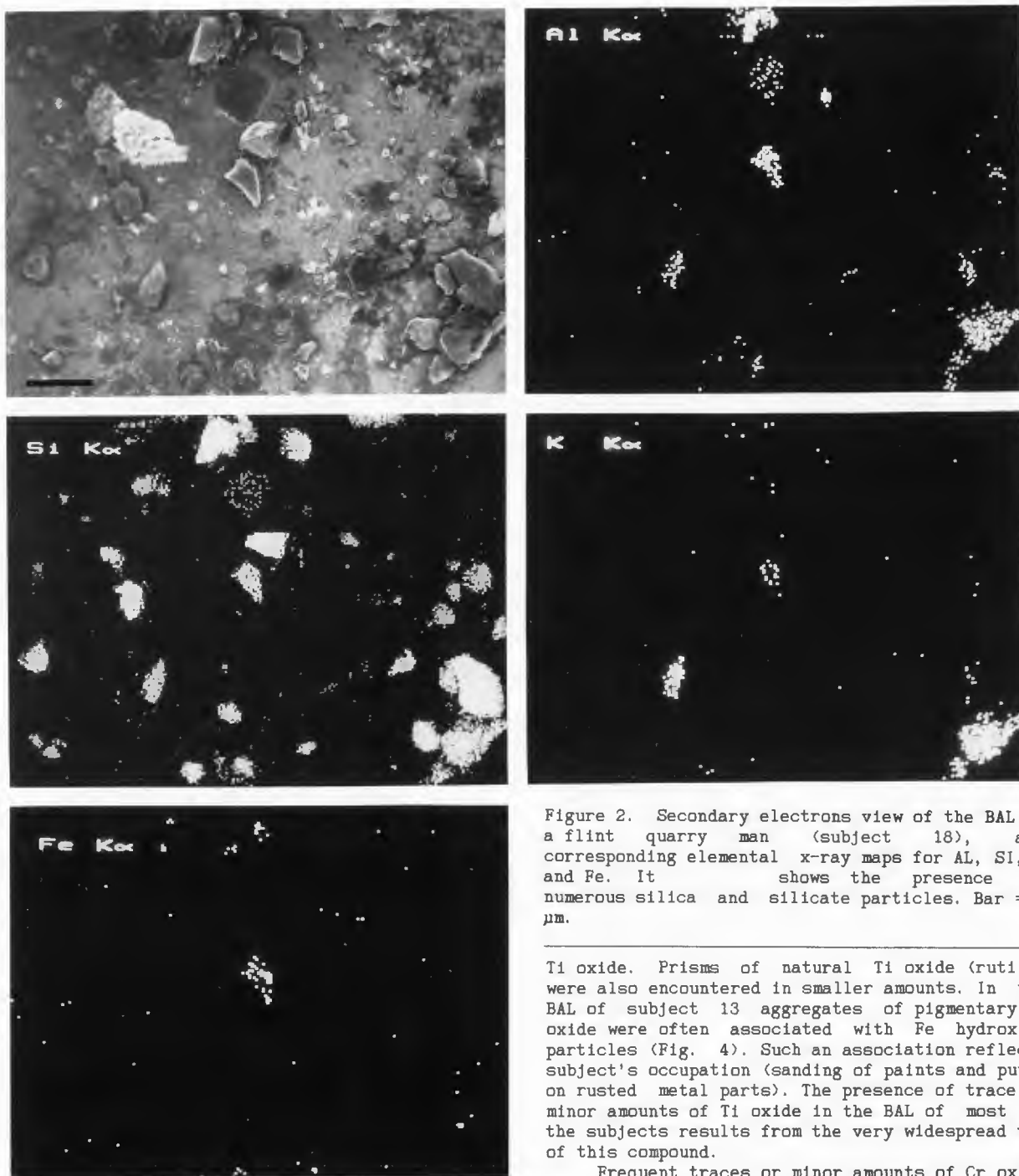


Figure 2. Secondary electrons view of the BAL of a flint quarry man (subject 18), and corresponding elemental x-ray maps for AL, SI, K and Fe. It shows the presence of numerous silica and silicate particles. Bar = 1 μ m.

Al compounds were found in concentrations higher than 10% in subjects 2 and 9 with confirmed exposure, in 26 with a probable exposure to abrasives and in 21 for whom both medical questioning and occupational inquiry failed to detect any exposure.

Ti oxide particles encountered in BAL show two distinct morphological habits. The most abundant are small (0.1 to 0.3 μ m) rounded grains, isolated or aggregated to form larger particles, which are characteristic of pigmentary

Ti oxide. Prisms of natural Ti oxide (rutile) were also encountered in smaller amounts. In the BAL of subject 13 aggregates of pigmentary Ti oxide were often associated with Fe hydroxide particles (Fig. 4). Such an association reflects subject's occupation (sanding of paints and putty on rusted metal parts). The presence of trace or minor amounts of Ti oxide in the BAL of most of the subjects results from the very widespread use of this compound.

Frequent traces or minor amounts of Cr oxide and chromite, traces of illmenite and rare occurrences of Zr oxide were also encountered. The presence of Cr oxide and chromite correlates well with proved or suspected exposure to abrasives.

Except for stainless steel, the most abundant alloys of Fe are FeCr, and to a lesser extent, FeNi. Alloys containing Fe associated with one or several of the following elements, Al, Ti, Cr, Mn, Ni, Zn, Mo, were rarely observed. Stainless steel and/or Fe alloys were present in concentrations higher than 5% in subjects 5, 7,

Non-fibrous Particles in BAL Fluids

Table 3. Relative concentration of the most frequent non-fibrous inorganic particles in BAL of 45 subjects*

| Particle type | Number of positive BALs | Concentration in positive BALs | | |
|--------------------------|-------------------------|--------------------------------|-----------------|--------|
| | | range (%) | mean \pm SD | median |
| All silicates | 45 | 5 - 92 | 50.8 \pm 23.7 | 47.6 |
| Silica | 45 | 2 - 65 | 21.4 \pm 13.8 | 21.0 |
| Feldspar | 38 | 1 - 40 | 5.5 \pm 6.4 | 4.1 |
| Talc + chlorite | 23 | 1 - 12 | 3.3 \pm 3.0 | 2.0 |
| Kaolinite | 40 | 1 - 24 | 8.4 \pm 5.7 | 6.5 |
| Illite + mica | 40 | 1 - 31 | 10.7 \pm 7.5 | 9.5 |
| Flyash + mullite | 22 | 1 - 17 | 4.9 \pm 5.4 | 3.0 |
| Fe compounds | 45 | 1 - 88 | 20.8 \pm 17.7 | 16.0 |
| Al compounds | 26 | 1 - 56 | 6.7 \pm 12.3 | 2.0 |
| Ti oxide | 38 | 1 - 31 | 6.0 \pm 7.6 | 3.0 |
| Stainless steel & alloys | 24 | 1 - 65 | 7.7 \pm 13.1 | 3.0 |
| W carbide | 7 | 1 - 58 | 24.5 \pm 23.1 | 19.0 |
| Carbonaceous compounds | 23 | 1 - 51 | 6.5 \pm 10.4 | 3.8 |

* Talc millers not included (see text)

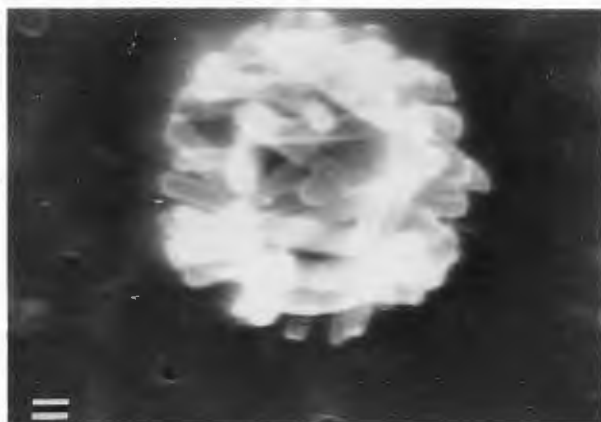


Figure 3. Spherical skeleton of mullite crystals from a flyash sphere (see text). Bar = 0.1 μ m.

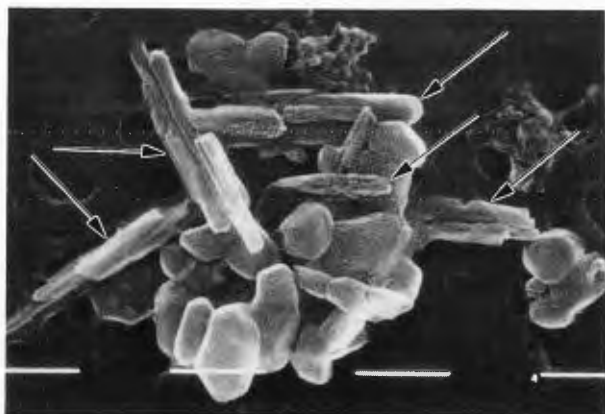


Figure 4. Aggregate of prismatic Fe hydroxide particles (arrows) and rounded particles of Ti oxide pigments from the BAL of subject 13 (see text). Bar = 0.3 μ m.

28, 37, 38, and 51 who all had definite or probable exposures to metallic particles and in subjects 11 and 33 for whom exposure would not be suspected from the available data.

Other metals or metal bearing compounds encountered in this study include Co, Ni, NiAl, Cu, Sn, Ce, CeLa, Ag, AgCl, Ta, W, Au, Pb and PbSn. Possible correlation of the presence of these particles in BAL with specific occupational exposures exists for Co, Ni, Cu, Sn, Au and Pb compounds.

Concerning Co, a high concentration of Co bearing particles was encountered in the two diamond polishers (subjects 15 and 16). These particles contain Co or Fe and Co in variable concentrations. They do not appear to be metallic Co but rather Co mixed with an amorphous carbonaceous substrate. Rare particles of W carbide containing Co were also observed in two hard metal workers (subjects 22 and 24).

W carbide appears either pure or in association with various amounts of Ta and Ti. Carbides of these two metals were also observed.

Small amounts of Ba and Ca sulfates were encountered in some samples but they do not correspond to specific exposures.

Concentrations of coal particles around 20% were recorded in subjects 14 and 49 who were coal miners. Coal particles were still present in the BAL of subject 49 although he had been working as W miner for 13 years at the time the BAL was performed.

Trace or minor amounts of endogenous particles were observed in several samples. For subject 5 there was a Ca phosphate coating on 73% of the particles, indicating a reaction of the lung with these particles.

Comparison of the relative concentrations recorded in repeated BALs are reported in Table 4. Despite some natural variability, there is good agreement between the results of the different BALs for each subject. There are no major changes in the relative concentrations of the detected compounds.

Table 4. Comparison of the main non-fibrous particle relative concentrations (%) recorded in repeated BAL.

| BAL sample Delay (months) | Subject 9 | | | | Subject 33 | |
|-------------------------------|-----------|----|-----|----|------------|----|
| | I | II | III | IV | I | II |
| | - | 15 | 17 | 29 | - | 42 |
| <u>Particle type</u> | | | | | | |
| Silica | 11 | 9 | 19 | 10 | 24 | 18 |
| Feldspar | 1 | 1 | 3 | 5 | 4 | 4 |
| Talc + Chlorite | - | - | - | 2 | 1 | 3 |
| Kaolinite | 1 | 3 | 5 | 3 | 7 | 5 |
| Illite + Mica | 8 | 5 | 6 | 2 | - | - |
| Fe compounds | 17 | 17 | 29 | 39 | 26 | 29 |
| Al compounds | 44 | 39 | 25 | 30 | 4 | 10 |
| Ti oxide | - | 2 | 3 | 3 | 2 | 5 |
| Stainless steel and alloys | 4 | - | 2 | - | 10 | 8 |
| Carbonaceous comp. | 6 | 1 | 1 | 3 | - | - |
| Chromite | - | 1 | - | - | 6 | 3 |
| Zircon | - | - | - | 1 | 8 | 5 |

Table 5. Non-fibrous particle concentrations and size distributions in 12 BAL fluids.

| Subject # | Particle concentration (x10 ⁶ /ml) | Diameter mean ± SD (µm) | % in size range | | |
|-----------|---|-------------------------|-----------------|--------|-------|
| | | | <1 µm | 1-2 µm | >2 µm |
| 3 | 1.34 | 0.8 ± 0.7 | 68 | 27 | 5 |
| 4 | 0.10 | 0.8 ± 1.2 | 77 | 20 | 3 |
| 7 | 1.11 | 0.5 ± 0.4 | 84 | 15 | 1 |
| 18 | 0.29 | 0.8 ± 0.8 | 82 | 11 | 7 |
| 19 | 0.59 | 0.6 ± 0.5 | 90 | 6 | 4 |
| 21 | 0.38 | 1.0 ± 0.6 | 48 | 47 | 5 |
| 22 | 0.21 | 1.2 ± 1.2 | 45 | 48 | 7 |
| 25 | 0.46 | 0.7 ± 0.6 | 71 | 26 | 3 |
| 27 | 0.77 | 0.8 ± 0.7 | 64 | 30 | 6 |
| 47 | 0.35 | 0.9 ± 0.9 | 67 | 28 | 5 |
| 49 | 9.90 | 0.6 ± 0.4 | 82 | 18 | 0 |
| 50 | 5.14 | 0.8 ± 0.7 | 74 | 19 | 7 |

Quantitative data about non-fibrous particles burden and size distribution obtained on 12 BALs are presented in Table 5. Non-fibrous inorganic particle absolute concentrations range from 0.1 to 9.9 x 10⁶ particles/ml BAL fluid. The lowest concentration corresponds to subject 4, who has no specific occupational particle exposure, while subjects 49 and 50 who have the highest concentrations were exposed during underground mining. Size distribution and mean diameter for each sample are highly variable. Mean diameter ranges from 0.5 to 1.2 µm. Particles sizes are log-normally distributed.

Discussion

The inhaled particles retained in the lung are best investigated by microscopic techniques.

Although light microscopy must not be neglected, electron microscopy (scanning or transmission) and the associated analytical techniques (EDS, electron diffraction) are the most efficient way to identify and size those particles, either *in situ* or on digested lung samples.

We have used these methods to study the non-fibrous inorganic particles contained in BAL fluid samples of 51 subjects with various occupational exposures. A precise identification of the particles is obtained in most cases. However, problems due to the inaccuracy of our EDS system to detect light elements (Z < 11) were sometimes encountered in differentiating metallic compounds from oxides or carbides.

The present investigation demonstrates that semi-quantitative particle analysis on BAL fluid samples is useful in confirming or clarifying known or disclosing unknown occupational dust exposures. As for asbestos fibers [17], it often appears to be more precise than questioning in monitoring non-fibrous particles exposures. At this step BAL results can be used to reconsider or redirect a professional inquiry. It also reflects the complexity of particles exposure. This complexity can result from the complexity of the aerosols to which the subjects were exposed during their occupation, or from changes in the type or the intensity of exposure during their career. The number of compounds recorded in each sample is a good indication of this complexity.

Even if detailed data are available about airborne particle types and levels at workplace, which is rarely the case, it is impossible to take into account all the parameters determining the particulate burden of the lung. The best way to document insoluble particle exposures is thus to investigate the retained dose in lung tissue or BAL samples. BAL can reflect exposure long after its cessation, up to 21 years for one subject of this study. It has also proved its efficiency to detect mixed exposures even 15 years after cessation of the first exposure. This indicates that even if most of the particles are readily cleared or transferred to interstitial tissue [22], a fraction remains trapped for a long time in the macrophages on the alveolar surfaces.

Reproducibility of the technique tested by analysis of repeated BAL samples is good although some variability in the relative concentrations is recorded. At least three reasons may be invoked to explain this variability. The first one is the reduced number of particles (approximately 100) analysed for each sample. Analysis of a higher number of particles would probably tend to reduce the differences. However, this would become too tedious and time consuming, unless an automatic image analyser is available. A capacity of two hundred analysed particles per hour was reported by Stettler et al [29] with such an analyser. A second reason can be variations between sampling sites, and a third can be modifications in the composition of the lung particulate burden with time. Effectively, what is observed while analysing BAL, or lung tissue samples, results from complex mechanisms involving particle deposition and clearance. Environmental and occupational airborne particles

both contribute to a variable extent to the particle burden of those samples. After cessation of the exposure particle clearance in conjunction with on going environmental particles deposition would tend to lower the traces of the occupational exposure. If the occupational particles dose is high or exposure is long, these mechanisms would have little influence on the semi-quantitative particle concentrations. However in the inverse case, there would be a progressive modification of those concentrations to theoretically reach a step where only the background environmental exposure could be detected. Factors, like smoking or air pollution which would affect clearance rates, must also be taken in consideration. Thus, the particle dose retained at the alveolar level must be considered as slowly changing with time under the effect of several interacting mechanisms.

It was suggested that smoking may be a non-neglectable source of kaolinite [4] particles in the lungs of smokers. However this hypothesis is in contradiction with the results obtained by Chung and Wiggs [7] and Paoletti et al. [27] on the particulate content of lung tissue of urban non-occupationally exposed smokers and non-smokers. They report an increase of the particle burden in smokers but no significant modifications between the particles species relative concentrations between the two groups of subjects.

Guidelines concerning the significance of particle concentrations in BAL with respect to occupational exposure may be established from our results. The presence of silica or Fe compounds must be considered as specific of an occupational exposure to these compounds if they represent more than 50% of the particulate burden. Talc, feldspars, Ti oxide and coal are specific if higher than 20%, Al compounds if higher than 10% and Cr oxide, stainless steel and alloys if higher than 5%. Specificity of kaolinite, illite and mica is difficult to appreciate. Since those minerals appear to be ubiquitous natural airborne contaminants, several tens of percent are probably necessary to reflect an occupational exposure. Specificity of the other compounds encountered in BAL samples must be considered on an individual basis.

Naturally, it must always be kept in mind that a positive BAL is only a proof of exposure, but not a proof of disease. Besides BAL particle analysis which is an attempt to identify possible pathogenic agents, other data such as clinical signs, x-ray films, lung function and eventually histology or immunological tests have to be considered and interpreted for the diagnosis of the disease and of its causes.

It must also be remembered that little is known about the correlations between particles present in BAL and in interstitial lung tissue. The only data available concern asbestos bodies for which a good correlation exists between lung tissue and BAL burdens. Grossly, it can be considered that asbestos body concentrations per gram dry lung tissue are one thousand times higher than those encountered per ml BAL fluid [16, 28]. There is actually no comparable data for the non-fibrous-particles. Mean particle

concentration reported for lung tissue samples of 10 urban subjects living in the Rome area and non-occupationally exposed to airborne particles (smokers and non-smokers) is 0.18×10^9 particles per gram dry lung tissue [27], and for smokers subjects from the Vancouver area, it is 0.47×10^9 particles per gram. Concentration ranges reported by Craighead et al. [8] ranged from 0.1 to 1.8×10^9 particles per gram for the general population and for 0.2 to 1.6×10^{11} particles per gram for subjects with silicosis. Particle concentrations recorded in BAL of the most comparable subjects (subject 4 can be considered as an urban non-occupationally exposed subject and subjects 49 and 50 as heavily occupationally exposed ones) are several thousand to several tens of thousand times lower. Particle concentrations in pairs of BAL and tissue samples need to be compared to evaluate the correlation that exists for the non-fibrous particle concentrations between these two types of samples.

The variability encountered in the mean particle size and in the particle size distribution is probably due to differences in the size distribution of the airborne particles subjects were exposed to.

Finally, concerning the limiting factors of non-fibrous particle analysis in BAL samples, in addition to the BAL sampling problems that can be encountered in severely diseased subjects and the impossibility to detect light elements ($Z < 11$), we must consider the inaccuracy of the preparation and analytical techniques used in the present work to detect soluble compounds or particles that are easily eliminated from the alveolar spaces. It is obvious that besides *in vivo* dissolution or modification of some particles, all digestion or ashing techniques used to make BAL or tissue samples compatible with further analysis by light or electron microscopy potentially induce dissolution or chemical modification. Little is known about their extent, but it seems that they have no appreciable effect on most of the compounds encountered. The problem of soluble compounds can partly be solved by the use of more appropriate elemental analysis techniques like x-ray fluorescence [23], atomic absorption [10] or proton induced x-ray emission [2]. *In situ* particle analysis in tissue sections [1, 18] or on macrophages collected from BAL is another alternative.

Acknowledgments

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References

1. Abraham JL, Burnett BR. (1983). Quantitative analysis of inorganic particulate burden *in situ* in tissue sections. Scanning Electron Microsc 1983;II: 681-696.

2. Bartsch P, Collignon A, Weber G, Robaye G, Delbrouck JM, Roelandts I, Yujie J. (1982). Distribution of metals in human lung: analysis by particle induced x-ray emission. *Arch Environ Health* 37: 111-117.
3. Berry JP, Henoc P, Galle P, Pariente R. (1976). Pulmonary mineral dust. *Am J Pathol* 83: 427-456.
4. Brody AR, Craighead JE. (1975). Cytoplasmic inclusion in pulmonary macrophages of cigarette smokers. *Lab Invest* 32: 125-133.
5. Churg A, Wiggs B. (1985). Mineral particles, mineral fibers, and lung cancer. *Environ Research* 37: 364-372.
6. Churg A, Wright JL, Wiggs B, Paré PD, Lazar N. (1985). Small airways disease and mineral dust exposure. *Am Rev Respir Dis* 131: 139-143.
7. Churg A, Wiggs B. (1987). Types, numbers, sizes, and distribution of mineral particles in the lungs of urban male cigarette smokers. *Environ Research* 42: 121-129.
8. Craighead JE, Kleinerman J, Abraham JL, Gibbs AR, Green FHY, Harley RA, Ruettner JR, Vallyathan NV, Juliano EB. (1988). Diseases Associated with exposure to silica and non-fibrous silicate minerals. *Arch Pathol Lab Med* 112: 673-720.
9. Daniele RP, Elias JA, Epstein PE, Rossman MD. (1985). Bronchoalveolar lavage: Role in the pathogenesis, diagnosis, and management of interstitial lung disease. *Ann Intern Med* 102: 93-108.
10. Demets M, Gheysens B, Nagels J, Verbeke E, Lauweryns J, van den Beekhout A, Lahaye D, Gyselen A. (1984). Cobalt lung in diamond polishers. *Am Rev Respir Dis* 130: 130-135.
11. De Vuyst P, Mairesse M, Gaudichet A, Dumortier P, Jedwab J, Yernault JC. (1983). Mineralogical analysis of bronchoalveolar lavage fluid as an aid to diagnosis of "imported pleural asbestosis". *Thorax* 38: 628-629.
12. De Vuyst P, Vande Weyer R, De Coster A, Marchandise FX, Dumortier P, Ketelbant P, Jedwab J, Yernault JC. (1986). Dental technician's pneumoconiosis: a report of two cases. *Am Rev Respir Dis* 133: 316-320.
13. De Vuyst P, Dumortier P, Léophonte P, Vande Weyer R, Yernault JC. (1987). Mineralogical analysis of bronchoalveolar lavage in talc pneumoconiosis. *Eur J Respir Dis* 70: 150-156.
14. De Vuyst P, Dumortier P, Moulin E, Yourassowsky N, Yernault JC. (1987). Diagnostic value of asbestos bodies in bronchoalveolar lavage fluid. *Am Rev Respir Dis* 136: 1219-1224.
15. De Vuyst P, Dumortier P, Schandené L, Estenne M, Verhest A, Yernault JC. (1987). Sarcoid-like lung granulomatosis induced by aluminium dusts. *Am Rev Respir Dis* 135: 493-497.
16. De Vuyst P, Dumortier P, Moulin E, Yourassowsky N, Rocmans P, de Francquen P, Yernault JC. (1988). Asbestos bodies in bronchoalveolar lavage reflect lung asbestos body concentration. *Eur Respir J* 1: 362-367.
17. Di Menza I, Hirsch A, Sébastien P, Gaudichet A, Bignon J. (1980). Assessment of past asbestos exposure in patients: occupational questionnaire versus monitoring in bronchoalveolar lavage. In *Biological Effects of Mineral Fibers*, JC Wagner (Ed). Iarc monographs 30: 609-614.
18. Gaudichet A, Pairon JC, Malandain O, Couste B, Brochard P, Bignon J. (1987). Etude mineralogique des particules non fibreuses du liquide de lavage broncho alveolaire (in french). *Rev Mal Resp* 4: 237-243.
19. Golden EB, Warnock ML, Hulet Jr LD, Churg AM. (1982). Fly ash lung: a new pneumoconiosis? *Am Rev Respir Dis* 125: 108-112.
20. Johnson NF, Halsam PL, Dewar A, Newman-Taylor AJ, Turner-Warwick M. (1986). Identification of inorganic dust particles in bronchoalveolar lavage macrophages by energy dispersive X-ray microanalysis. *Arch Environ Health* 41: 133-144.
21. Keyser T, Natusch DFS, Evans Jr CA, Linton RW. (1978). Characterizing the surface of environmental particles. *Environ Sc Technol* 12: 768-773.
22. Lippmann M, Yeates DB, Albert RE. (1980). Deposition, retention, and clearance of inhaled particles. *Br J Ind Med* 37: 337-362.
23. Maier EA, Dietermann-Molard A, Rastegar F, Heimburger R, Ruch G, Maier A, Roegel E, Leroy MJF. (1987). Simultaneous determination of trace elements in lavage fluids from human bronchial alveoli by energy dispersive x-ray fluorescence: 3. routine analysis. *Clinical Chemistry* 33: 2234-2239.
24. Miller RL, Churg AM, Hutcheon M, Lam S. (1984). Pulmonary alveolar proteinosis and aluminium dust exposure. *Am Rev Respir Dis* 130: 312-315.
25. Natusch DFS, Wallace JR, Evans CA. (1974). Toxic trace elements: Preferential concentration in respirable particles. *Science* 183: 202-204.
26. Parkes WR. (1982). Occupational lung disorders. 2nd Ed. Butterworths, London U.K. 529 p.
27. Paoletti L, Batisti D, Caiazza S, Petrelli MG, Taggi F, De Zorzi L, Dina MA, Donelli G. (1987). Mineral particles in the lungs of subjects resident in the Rome area and not occupationally exposed to mineral dust. *Environ Research* 44: 18-28.
28. Sébastien P, Armstrong B, Monchau G, Bignon J. (1988). Asbestos bodies in bronchoalveolar lavage fluid and in lung parenchyma. *Am Rev Respir Dis* 137: 75-78.
29. Stettler LE, Groth DH, Platek SF. (1983). Automated characterization of particles extracted from human lungs: three cases of tungsten carbide exposure. *Scanning Electron Microsc.* 1983;1: 439-448.
30. Tosi P, Franzinelli A, Miracco C, Leoncini L, Minacci C, Baldelli C, Gotti G, Governa M. (1986). Silicotic lymph node lesions in non-occupationally exposed lung carcinoma patients. *Eur J Respir Dis* 68: 362-369.

Non-fibrous Particles in BAL Fluids

Discussion with Reviewers

L. E. Stettler : Have multiple preparations from the same BAL sample been analyzed? If so, what types of variation were seen in absolute and in relative particle concentrations?

Authors : No, such preparations were not attempted.

L. E. Stettler : The authors data for subject 42 reflecting an exposure 21 years prior to the BAL is very interesting. Do you have any data concerning the absolute particle concentration for this subject? If so, how does this data compare with the non-occupationally exposed urban subject (subject 4)? Have you performed additional BAL's for this subject as was done for subjects 9 and 33? If so, how have the absolute particle concentrations changed?

Authors : There were no other data available for subject 42 than those reported in Table 2. Particularly, the absolute particle concentrations were not determined for the talc workers because the platy particles (talc, chlorite) were too abundant. Plates were often lying one on the other making it impossible to obtain a reliable particle count (Fig. 5). We estimate that the absolute particle concentration for subject 42 is 10 to 100 times higher than for subject 4.

L. E. Stettler : Are the particles sizes seen in the BAL samples log-normally distributed?

Authors : χ^2 test showed that particles sizes in BAL samples are log-normally distributed ($P < 0.025$)

V. L. Roggli : Do the authors have any data regarding variability of the results with respect to sampling site (e.g., right middle lobe vs. left upper lobe, etc.)? Is there any significant variation with respect to which fraction of BALF is examined (e.g., 1st vs. 2nd vs. 3rd fraction)? How much variation in particle counts is observed from one grid square to another by TEM?

Authors : We have presently no data about the variations of non-fibrous particle counts and proportions with respect to BAL sampling site or fraction. Although we do not know if non-fibrous particles have the same behaviour as asbestos bodies (ABs) towards BAL sampling, data existing about ABs allow us to draw some trends. Performing bilateral BAL on the lingula and right middle lobe of two subjects with unilateral asbestos related disease showed that AB recovery is influenced by the presence of disease. It was much higher in the diseased (compressed) lung than in the "healthy" one (unpublished results). This can be explained by a lesser efficiency of BAL in the diseased lung. Moreover, BAL fluid and cells recovery is 20 % higher in the middle lobe or in the lingula than in the lower lobes [31].

The first fraction of a BAL must be discarded since AB counts are roughly ten times lower in this fraction than in the following ones. From the second fraction counts remain in the same range, the maximum being obtained on the third one [32].

Concerning the variability of the non-fibrous particle counts from one grid square to

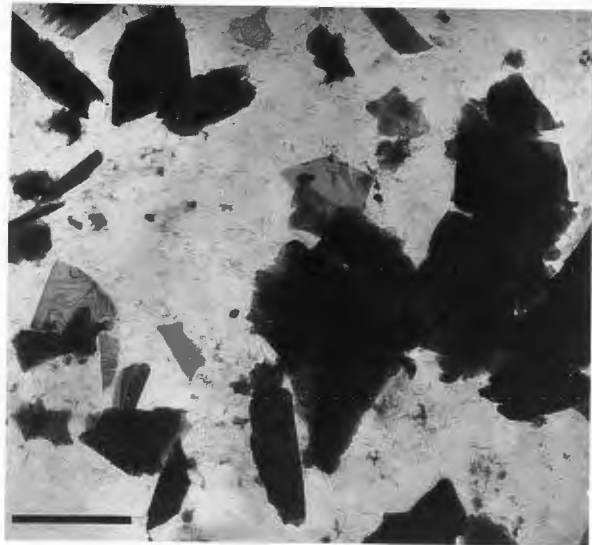


Figure 5. TEM view of the particles recovered from the BAL of a talc worker (subject 42) showing the difficulty to obtain a reliable particle count for samples containing a high concentration of platy particles. Bar = 5 μ m.

another, the maximum observed differences were less than 15 % of the mean count.

V. L. Roggli : Materials and Methods section indicates that the authors employed analytical TEM equipped with a STEM attachment, and portions of cellulose ester membrane filters were transferred to copper grids and the filter removed by a modified Jaffe wick technique. However, the illustrations (Figs 2-4) show what appears to be intact polycarbonate filters examined by secondary electron imaging. Please comment.

Authors : Our STEM attachment is equipped to perform secondary, backscattered as well as transmitted electrons imaging. Although routine sample observation and particle analysis involve conventional TEM (Fig. 5), presented illustrations are secondary electron images because they give a better idea of the particle morphology. When viewed in secondary electrons, holes in the carbon film, traces of bubbles or imperfect fusion of the cellulose ester filter during mounting on glass slide for light microscopy may be confused with the holes of a polycarbonate filter. A closer look at these artefacts show that they have variable sizes (Fig. 4) or are too small (Fig. 3) to be compatible with holes in usual polycarbonate filters.

V. L. Roggli and J. L. Abraham : Did you prepare blank samples, and if so, were the blank results subtracted from the cases for which quantitative data were reported? Could particles in blanks account in part for the ubiquitous nature of silica, iron, kaolinite, illite and mica in BALF samples?

Authors : Blanks were obtained by sampling 20 ml of fresh saline simultaneously with some BAL samples and running the whole preparation procedure in parallel. The maximum particle count obtained on a blank was $1.3 \cdot 10^4$ particles per ml. Considering that this is an order of magnitude lower than the lowest particle count in BAL (subject 4, table 5) and that blanks were not systematically prepared, we chose to neglect blank values. Blank particle content has little influence on ubiquitous particle levels in BAL samples.

J. L. Abraham : Please discuss the problem of assessing whether the BAL sample of particulates represents alveolar or interstitial dust burden. Also, please discuss your experimental approach, if any, to investigate this problem.

Authors : By definition, the only informations collected by BAL sampling relate to the alveolar dust burden. Except for asbestos bodies [16,28], correlation with interstitial dust burden is presently unknown. There is also little information available about transfert and clearance rates for particles in the alveolar and interstitial domains. Clearance rates may vary from one type of particles to another (e. g. chrysotile fibers have a much higher clearance rate than amphibole ones). The possible experimental approaches to investigate this problem are either to compare particle burdens of digested BAL and lung samples or to analyse *in situ* on tissue sections the particle content of alveolar macrophages and of the interstitium. In both approaches it would be necessary to divide the proportion of each type of particle by the proportion of an ubiquitous particle (e. g. silica) to compare the alveolar and interstitial dust burdens.

J. L. Abraham : Please discuss the problems in quantitation of BAL samples. There may be variable contact of the fluid with various airspaces. How does one know if the fluid is sampling small airways versus alveoli, etc.? Is there some sort of experimental tracer technique which might be used to provide some sort of internal standard for the BAL sampling?

Authors : These problems are reviewed in a recent report giving technical recommendations and guidelines for BAL [33]. Numerous parameters like the volume, the temperature and the pH of the instilled fluid, the time it remains in contact with the lung, the location of the sampling site and the presence of obstructive disorders might play a role in the efficiency of BAL fluid and cells recovery. Expressing quantitative results as a number of particles per ml BAL fluid is rather simple and easy to use. Although it would be advisable to get some sort of internal standard taking a possible dilution effect into account, there is presently no general agreement about the nature of this standard. Attempts to use macrophages or albumin concentrations to standardize results are untrustworthy as these parameters may drastically be modified in response to an inflammatory reaction of the lung [34].

J. L. Abraham : What is the significance of the percentages reported as guidelines in the Discussion? Quantitation is needed. For example, expressing a result as a percentage where 50% of the burden is silica at 1 billion particles per milliliter has intuitively not the same significance as 50% of the particles being silica at a concentration of 1 million particles per milliliter. Please see also Mason et al. (1982) [35].

Authors : We agree with this comment, however our paper is focused on the help of particle analysis in BAL to disclose occupational exposures and not on the relationship that may exist between particle burden and disease. A percentage of one type of particle higher than the given value has to be considered as the probable signature of an occupational exposure, and this irrespectively to quantitation.

Additional references

31. Pingelton AK, Harrison GF, Stechschulte DJ, Wesselius LJ, Kerby GR, Ruth WE. (1983). Effect of location, pH and temperature of instillate in bronchoalveolar lavage in normal volunteers. *Am Rev Respir Dis* 128: 1035-1037.
32. Sébastien P. (1982). Possibilités actuelles de la biométrie des poussières sur échantillon de liquide de lavage broncho-alvéolaire. *Ann Biol Clin* 40: 279-293.
33. Costabel U, Danel C, Haslam P, Higgenbottam T, Klecht H, Pohl W, Rennard S, Rossi G, Rust M, Semenzato G. (1989). Technical recommendations and guidelines for broncho-alveolar lavage (BAL). *Eur Respir J* 2: 561-585.
34. Stockley RA. (1984). Measurement of soluble proteins in lung secretions. *Thorax* 39: 241-247.
35. Mason GR, Abraham JL, Hoffman L, Cole S, Lippman M, Wasserman K. (1982). Treatment of mixed-dust pneumoconiosis with whole lung lavage. *Am Rev Respir Dis* 126: 1102-1107.