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Evaluation by Electron Microscopy Techniques of Asbestos Contamination in Industrial, Cosmetic, and Pharmaceutical Talcs

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Talc powders from national and international markets were analyzed in order to assess their fiber contents and the proportion of asbestos in the fibrous material. Samples of talc powders used as excipients in pharmaceutical and cosmetic preparations demonstrated fiber contents up to 30% of total particles. About a half of the talc powders revealed the presence of asbestos: in five samples chrysotile (a serpentine asbestos) was present, in the other ones tremolite and anthophyllite (an amphibole asbestos). The amounts of asbestos vary up to 90% in the different samples of the fibrous fraction. About 75% of observed asbestos fibers were thinner than 0.4 μm, i.e., below the resolving power of light microscopy which until now was the most utilized technique for evaluating the environmental pollution due to asbestos.

INTRODUCTION

Talc is a Mg silicate with a particular lamellar (sheet) structure in which each lamella consists of one sheet of Mg $(OH)_2$ between two layers of SiO_4 (1).

In natural deposits Mg is often substituted in talc crystals by other cations such as Fe, Ni, Cr, Mn, etc. Deposits are not generally monomineralic, but, since they result from geologic processes which caused the formation of several different mineral phases, they are heterogeneous as far as the kind and relative amount of the minerals (2). Thus, it is not surprising that materials known as talc powders may contain less than 50% of talc (3).

The most common minerals that may be found mixed with talc in mineral deposits are listed in Table 1. Among them, two fibrous kinds of amphibole, tremolite and anthophyllite, and a fibrous kind of serpentine mineral, chrysotile, constitute some of the best known varieties of asbestos (2, 4, 5).

Talc powders are widely employed in a number of industrial processes and in commonly used products (i.e., in the manufacturing of pottery articles and insulating materials, in paper manufacturing, as additives in asphalts, in pesticides, in cosmetic products, and as excipients in pharmaceutical preparations).

It is well known that professional exposure to talc may cause the kind of fibrosis known as talcosis. Moreover, a large number of epidemiological data point out the

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TABLE 1

MINERALS COMMONLY ASSOCIATED WITH TALC IN NATURAL DEPOSITS

Carbonates	
	Calcite, dolomite, magnesite
Amphiboles	
	Tremolite, anthophyllite
Serpentines	
	Chrysotile, antigorite, lizardite
Others	
	Quartz, mica, chlorite, rutile, pyrophyllite

risk of cancer connected with the fibrous components of talc powders, i.e., talc fibers and fibrous impurities.

"Asbestos bodies" in the lung tissue of workers exposed to talc have been reported several times in the literature (7–11).

An epidemiological study on workers in talc deposits has demonstrated a three- to fourfold increase in cancer risk as compared with the risk of the population in general (12). Moreover, the same histological lung alterations are shown by workers exposed to talc powders and by workers exposed to asbestos (13). Recently, a connection between ovarian cancer and the use of asbestos containing talcs has also been suggested (14, 15). However, there is a considerable lack of analytical data about the fibrous components of talc.

In 22 talc powders examined, values ranging from 8 to 30% of fibrous particles were reported in 1968 by U. S. authors (16). A similar study carried out by NBS investigators (17) evidenced percentages from 2 to 30% of fibrous particles in all the analyzed samples. In both cases it was not specified what fraction of such percentages was constituted by asbestos.

Studies carried out in the United Kingdom on talc powders used for different purposes revealed that 3 out of 24 specimens contained tremolite (18). More complete data concerning 20 talcs for cosmetic use and 1 for pharmaceutical use purchased in the New York City area from 1971 to 1975 have been published (19): 10 among the cosmetic talcs examined contained, from 1 to 14% (w/w), tremolite and anthophyllite, and two of them also contained traces of chrysotile.

Taking also into account the lack of data on the Italian situation, a systematic analysis of talc powders employed or at least marketed in Italy was carried out in our laboratory to determine the percentage of fibrous particles and the amount of asbestos in such powders. Moreover we analyzed 14 talc powders provided by the European Pharmacopoeia from various geographical areas and to be utilized for different purposes.

In this study we utilized electron microscopy (EM) and associated analytical techniques such as electron diffraction and X-ray microanalysis which allow the morphological and structural characterization as well as the elemental analysis of particles at high levels of resolution.

MATERIALS AND METHODS

A small amount of talc obtained from the original package without mixing and homogenizing it, in order to avoid any fragmentation of particles, was weighed and

suspended in a solution of 0.5 to 0.8% Formvar in dichloroethane at a concentration of 0.02 to 0.05 g/ml talc.

After air insufflation by a Pasteur pipet to avoid sedimentation, two or three drops of the suspension were put on a just-cleaved mica sheet and rubbed with another mica sheet.

After solvent evaporation, the film including talc particles was separated from the mica sheet on the surface of twice distilled H_2O and collected on 200-mesh copper grids. A thin carbon film was then evaporated on the grids.

The samples were observed by a TEM Siemens Elmiskop 102 at 10,000 magnification and 100 kV HT. To obtain information on the crystalline structure of mineral particles, selected-area electron diffraction (SAED) was utilized. Chemical elements present in the mineral particles were detected by X-ray microanalysis, equipped with a wave dispersion spectrometer.

Statistical Evaluation of Data

We checked the statistical compatibility of the results of replicate determinations of fibrous fractions for the same talc specimen. The χ^2 test was utilized to check the hypothesis that the differences in the values of the fibrous fractions were due to chance (21). For each kind of talc the average value of the percentages of fibers and of nonfibrous particles were calculated. The numbers of fibers and of nonfibrous particles measured in each determination have been utilized as elements of a contingency table and χ^2 was calculated comparing the numeric values obtained and the ones expected (i.e., the corresponding average percentages).

At a significance level of $\alpha = 0.05$, the values obtained for χ^2 were not generally significant enough [$\chi^2_{exp} < \chi^2(0.95)$] to reject the initial hypothesis.

However, in some cases we obtained comparatively high χ^2 values [$\chi^2_{exp} > \chi^2(0.95)$].

Careful reexamination of the specimen characteristics and the counting methods evidenced that the less reliable results concerned counts performed on fields where large clusters of particles were observed or where, due to their number, many particles were in contact.

We realized also that the wide range of the counting results could be related to the lack of a defined threshold for the minimum size required for a particle to be counted.

After stating new and more strict counting criteria (particle clusters and particles smaller than 0.2 μm were not included in the counts), we reexamined the same talc specimens and obtained a very good statistical compatibility with the initial hypothesis.

In Table 2 the values of χ^2 we obtained and the ones corresponding to a 95% probability at the same degree of freedom are listed.

To check whether the method of sample preparation could cause an increase or a decrease in the fibre percentage, for each kind of talc powder we analyzed the correlation between the total number of particles per unit area of the film (the area of one mesh of the grid) and the percentage of fibrous particles on such a surface. This correlation was studied both on different Formvar films and on different areas of the same film.

In all cases the correlation parameter r^2 (21) between the particle number and the fibrous fraction in the unit area resulted in values very close to zero, as shown in Table 3.

TABLE 2

χ^2 TEST TO CHECK THE REPRODUCIBILITY IN THE EVALUATION OF THE FIBROUS FRACTION IN TALC POWDERS

Sample	χ^2 Observed	χ^2 (0.95)
A	9.9	16.9
B	1.61	5.99
C	10.6	11.1
D	7.58	9.49
E	3.65	7.81
F	2.95	7.81
G	0.37	3.84
H	2.34	5.99
I	7.58	9.49
J	3.65	7.81
K	2.95	7.81
L	4.32	5.99
M	1.39	9.49

Statistical Criteria for Evaluating the Fiber:Particle Ratio in Talc Samples

A binomial distribution is expected for the values of the fibrous fraction of a given number of particles and for the percentage of asbestos fibers in a given number of fibers. This allows calculation of the number of particles to be observed in order to obtain the required accuracy of measurement.

For a binomial distribution the relative standard deviation (as percentage of the mean value μ) calculated by means of the a priori probability P of observing a fiber and by means of the number N of observed particles is

$$\sigma/\mu = [(1 - P)/(N - P)]^{1/2}$$

The total number N of particles to be observed in order to evaluate the fiber:particle ratio may be calculated by means of this equation according to the required accuracy of measurement.

Table 4, in which the standard deviation is reported as a function of P and N, shows that, in order to evaluate the fiber concentration with a relative standard deviation of less than 10–15%, N must be about 10^3, even for concentrations between 5 and 30%, and must increase by at least of a factor 10 for lower concentrations.

TABLE 3

CORRELATION BETWEEN PARTICLE NUMBER PER UNIT AREA AND FIBROUS PARTICLE PERCENTAGE

Sample	Correlation parameter, r^2
A	0.22
B	0.13
C	0.03
D	0.13
E	0.002
F	0.41
G	0.06

TABLE 4

RELATIVE STANDARD DEVIATION FOR A BINOMIAL DISTRIBUTION

P	$N = 100$	$N = 1000$	$N = 10{,}000$
0.01	99%	31%	10%
0.05	43%	14%	4.3%
0.10	30%	9%	3%
0.15	24%	7%	2.4%
0.20	20%	6%	2%
0.25	17%	5%	1.7%
0.35	15%	5%	1.5%

N = total number of particles to be observed. P = probability of observing one fiber.

Since some talc specimens may contain very few asbestos fibers, and therefore are barely detectable, it is necessary to define an upper limit for the pollution level, in case that no asbestos fibers should be detected in a given specimen. Even in such a case we cannot infer that the talc specimen is completely asbestos free.

We can simply calculate the probability P of finding at least one asbestos fiber among a number N of fibrous particles as a function of the ratio v between the number of asbestos fibers and total number of fibers [$P = 1 - (1 - v)^N$].

From Table 5, in which the probability is reported as a function of N and v, we infer, for instance, that for very small concentrations ($v \ll 10^{-4}$), the probability of observing one asbestos fiber is very close to zero for samples containing 10^2–10^3 fibrous particles.

To fix an upper limit for the estimated asbestos concentration in the sample we chose a value of the probability P of singling out at least one fiber of asbestos in the sample of fibers observed: in order to obtain the results we are looking for, we set a value of $P = 0.9$ (90% of probability). Then we calculated the concentration that, for the number of fibers observed, provided such a probability ($P = 0.9$). This value

TABLE 5

PROBABILITY OF OBSERVING AT LEAST ONE ASBESTOS FIBER AMONG N FIBROUS PARTICLES AS A FUNCTION OF THE RATIO v BETWEEN ASBESTOS FIBERS AND TOTAL FIBERS

	N					
v	50	100	200	500	1000	2000
10^{-4}	0.005	0.01	0.02	0.05	0.10	0.18
5×10^{-4}	0.025	0.05	0.10	0.22	0.39	0.63
10^{-3}	0.05	0.10	0.18	0.39	0.63	0.86
2×10^{-3}	0.10	0.18	0.33	0.63	0.86	0.98
3×10^{-3}	0.14	0.26	0.45	0.78	0.95	1.00
5×10^{-3}	0.22	0.39	0.63	0.92	0.99	1.00
7×10^{-3}	0.30	0.50	0.75	0.97	1.00	1.00
10^{-2}	0.39	0.63	0.87	0.99	1.00	1.00
2×10^{-2}	0.64	0.87	0.98	1.00	1.00	1.00
5×10^{-2}	0.92	1.00	1.00	1.00	1.00	1.00
10^{-1}	1.00	1.00	1.00	1.00	1.00	1.00

of asbestos concentration in the sample has been considered as the upper limit since a higher concentration would provide a probability higher than 90%, that is, practically the certainty, of observing one asbestos fiber.

RESULTS

Twenty-nine different samples of talc for industrial, cosmetic, and pharmaceutical uses have been analyzed: 15 from the Italian market and 14 provided by the European Pharmacopoeia from the international market and from various geographic areas.

The powder samples prepared by the Formvar film method showed to be suitable for studies by electron diffraction and X-ray microanalysis and for further observation by light microscopy. Moreover the samples showed a considerable degree of stability, even after repeated observations.

The statistical analyses showed the reliability and reproducibility of the results obtained for powder samples prepared by the above-described method.

According to the criteria accepted by the Council of European Communities (32), particles have been considered as fibrous when having a length:width ratio greater or equal to 3 and a width less than 3 μm.

Fibers respectively greater and less than 5 μm in length have been considered separately, according to the above-mentioned criteria, which consider as more hazardous, because of their biological effects, the fibers longer than 5 μm. The counts for the evaluation of fibrous particle percentages have been performed on a total number of particles ranging from 2×10^3 to 10×10^3 for each kind of talc, so in general the resulting error in the percentages (standard deviation calculated by the expression given in the statistical criteria) was not greater than 10%.

To evaluate the pollution due to asbestos in the studied talcs, the fibrous kinds of amphiboles, tremolite and anthophyllite, and the fibrous kind of serpentine, chrysotile, have been investigated (2–4).

The electron diffraction patterns of the various mineral phases in the samples are not straightforward (27); nevertheless the patterns show peculiarities which allow determination, with certainty, the presence of serpentine asbestos (Fig. 1) or amphibole asbestos (Figs. 2 and 3) (23, 28, 29) among the particles which constitute the talc powders.

The identification of the kind of mineral in amphibole asbestos may be confirmed through the identification of the characteristic chemical elements by means of the electron microprobe.

Table 6 reports the concentrations of such elements in the most common kinds of asbestos. The identification of the kind of amphibole present in the talcs may be performed by means of the evaluation of the Ca (tremolite) and Fe (anthophyllite) concentrations in the mineral.

For each kind of talc powder, a sample of 100 random fibrous particles has been considered. We studied the electron diffraction pattern of each sample and its Ca and Fe contents, by means of X-ray microanalysis. As reported in Tables 7–10 the fibrous particle percentages ranged from 2 to 30% in all of the talc powders analyzed. As reported in Tables 7–9, in 8 out of 15 Italian talcs the presence of asbestos fibers has been revealed, in 7 samples there were fibers of tremolite, and in 1 sample there were fibers of chrysotile.

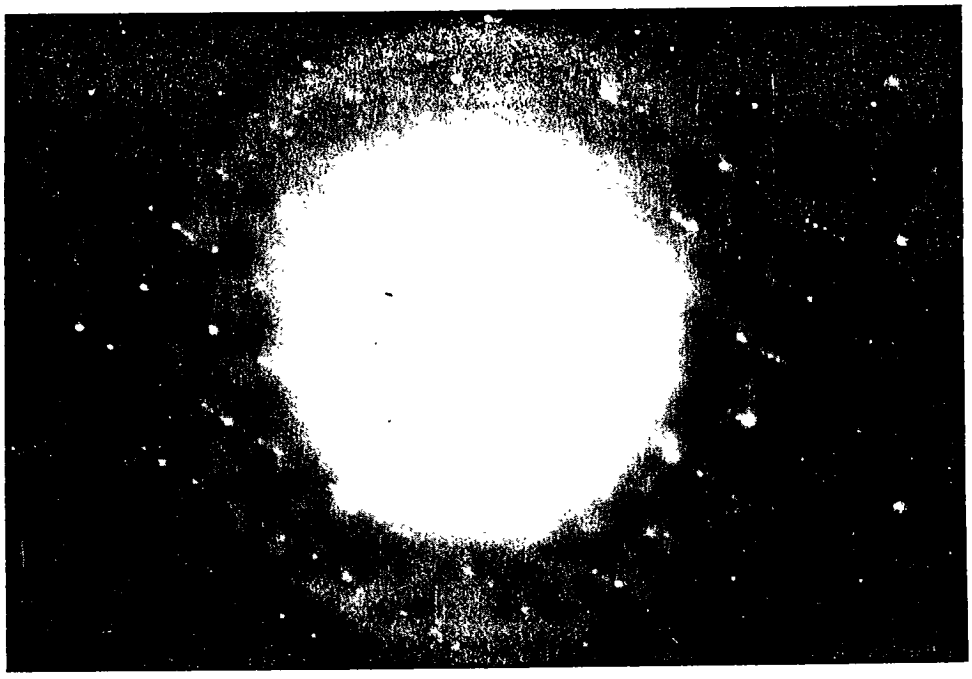

FIG. 3. Electron diffraction pattern of a tremolite fiber.

The presence of asbestos fibers was revealed in 6 out of 14 talcs from the European Pharmacopoeia: in 3 samples there was chrysotile, in 2 samples the amphiboles tremolite and anthophyllite were present, and in 1 sample there was tremolite and chrysotile. In the 2 talc samples containing amphibole asbestos, tremolite and anthophyllite were present in great percentages, reaching in both cases about 20% of the entire particulate. Figures 4 and 5 show a few typical fibers of such mineral varieties observed in some of the samples examined.

Interestingly, about three-fourths of the asbestos fibers observed in each sample had a diameter smaller than about 0.4 μm, that is, below the resolving power of

TABLE 6

CHEMICAL COMPOSITION IN OXIDE PERCENTAGE OF THE MOST COMMON VARIETIES OF ASBESTOS

	Chrysotile	Crocidolite	Amosite	Anthophyllite	Tremolite
SiO_2	41.8–42	49–53	49–53	56–58	55–60
MgO	41.8–42.8	0–3	1–7	28–34	21–26
FeO	0.1–1.6	13–20	34–44	3–12	0–4
Fe_2O_3	0.2–1.3	17–20	—	—	0–0.5
Al_2O_3	0.1–0.5	0–0.2	—	0.5–1.5	0–2.5
CaO	0–0.1	0.3–2.7	—	—	11–13
K_2O	0–0.1	0–0.4	0–0.4	—	0–0.6
Na_2O	—	4–8.5	—	—	0–1.5
H_2O	13.6–14	2.5–4.5	2.5–4.5	1–6	0.5–2.5

TABLE 7

PERCENTAGE OF FIBROUS PARTICLES AND OF ASBESTOS FIBERS IN SOME INDUSTRIAL TALCS

	% Fibers in the particulate	% Fibers > 5 μm in the particulate	% Asbestos fibers versus total fibers	% Asbestos fibers in the particulate	Variety of asbestos
A	26.5 ± 1.7	2.8 ± 0.6	<2	<0.5	—
B	5.7 ± 0.3	0.8 ± 0.1	8 ± 2.7	0.5 ± 0.2	Tremolite
C	4.7 ± 0.7	0.5 ± 0.2	4 ± 1.9	0.2 ± 0.1	Chrysotile
D	2.6 ± 0.3	0.6 ± 0.1	<2	<0.05	—

phase-contrast microscopy, until now the most widely used technique for the observation of particles and mineral fibers.

For the talc samples in which the presence of asbestos fibers was not detected, the upper limits chosen for the asbestos content were those evaluated by the above-mentioned criteria (90% probability criteria). Such upper limits are listed in Tables 7–10.

It must be pointed out that in all talc powders analyzed, quite frequently, fibrous talc particles have been observed morphologically to be very similar to amphibole fibers (Fig. 6) but easily recognizable by their typical electron diffraction patterns (Fig. 7) (19–23).

In the examined samples, particles of minerals usually found together with talc (Table 1), in particular Ca and Mg carbonates, have been frequently observed; as already mentioned, until now no attempts have been made to evaluate quantitatively their content in talc samples.

DISCUSSION

It is well known that occupational exposure to talc is associated with a diffuse interstitial lung-scarring talcosis. Moreover, many experimental and epidemiological data show that the fibrous fraction of talc powders is more hazardous than the platy one because of presence of asbestos fibers which contaminate natural talc deposits.

The aim of this study is the evaluation of the percentage of fibers and, in particular, of asbestos fibers present in talc powders.

TABLE 8

PERCENTAGE OF FIBROUS PARTICLES AND OF ASBESTOS FIBERS IN SOME PHARMACEUTICAL TALCS

	% Fibers in the particulate	% Fibers > 5 μm in the particulate	% Asbestos fibers versus total fibers	% Asbestos fibers in the particulate	Variety of asbestos
A	5.4 ± 0.8	1.1 ± 0.4	8 ± 2.7	0.4 ± 0.2	Tremolite
B	8.7 ± 1.0	3.1 ± 0.6	<2	<0.2	—
C	4.5 ± 0.6	0.4 ± 0.2	14 ± 3.4	0.6 ± 0.2	Tremolite
D	3.1 ± 0.4	0.9 ± 0.2	21 ± 4	0.7 ± 0.2	Tremolite
E	3.7 ± 0.4	1.0 ± 0.2	17 ± 3.7	0.6 ± 0.2	Tremolite

TABLE 9

PERCENTAGE OF FIBROUS PARTICLES AND OF ASBESTOS FIBERS IN SOME COSMETIC TALCS

	% Fibers in the particulate	% Fibers > 5 μm in the particulate	% Asbestos fibers versus total fibers	% Asbestos fibers in the particulate	Variety of asbestos
A	6.1 ± 0.9	1.6 ± 0.5	<2	<0.1	—
B	21.6 ± 1.6	5.0 ± 0.9	<2	<0.4	—
C	11.1 ± 1.1	3.2 ± 0.6	<2	<0.2	—
D	4.9 ± 0.5	0.7 ± 0.2	32 ± 4.7	1.6 ± 0.3	Tremolite
E	10.3 ± 0.7	3.2 ± 0.4	<2	<0.2	—
F	5.1 ± 0.6	1.8 ± 0.4	10 ± 3	0.5 ± 0.2	Tremolite

By means of the method described above, it is not possible to obtain a good estimate of the percentage by weight of other minerals present in talcs.

In some samples, a very high level of asbestos contamination was revealed, possibly related to risk for people in contact in various ways with talc powders. This underlines the need of a deeper knowledge of the characteristics of talc currently in use and of suitable regulations, should the results of this study be confirmed on a vast scale, taking also into account the lack of national and international legislation about talc powder characteristics. The sole regulations concerning the amount of fibrous amphiboles in talc were issued in 1976 by the CTFA: It was stated that talc powders utilized in cosmetics and in toiletries should contain at least 90% talc and no revealable asbestos fibers.

TABLE 10

PERCENTAGE OF FIBROUS PARTICLES AND OF ASBESTOS FIBERS IN TALCS PROVIDED BY THE EUROPEAN PHARMACOPOEIA

	% Fibers in the particulate	% Fibers > 5 μm in the particulate	% Asbestos fibers versus total fibers	% Asbestos fibers in the particulate	Variety of asbestos
A	4.1 ± 0.3	2.1 ± 0.2	<1	<0.04	—
B	25.01 ± 1.1	6.39 ± 0.6	87 ± 1.2	21.7 ± 1.0	Tremolite 88% Anthophyllite 12%
C	3.0 ± 0.24	0.83 ± 0.13	<1	<0.03	Traces of chrysotile
D	2.86 ± 0.35	1.43 ± 0.25	<2	<0.06	—
E	3.55 ± 0.37	1.9 ± 0.2	<2	<0.07	—
F	6.8 ± 0.48	2.8 ± 0.3	<1	<0.07	—
G	2.3 ± 0.25	0.56 ± 0.13	<2	<0.05	—
H	1.5 ± 0.23	0.32 ± 0.1	<2	<0.03	—
I	20.3 ± 0.87	7.7 ± 0.57	92 ± 1.3	18.7 ± 0.8	Tremolite 95% Anthophyllite 5%
J	3.3 ± 0.38	2.0 ± 0.3	<2	<0.07	—
K	4.4 ± 0.43	1.8 ± 0.27	3 ± 1.2	0.13 ± 0.05	Tremolite 50% Chrysotile 50%
L	3.7 ± 0.37	1.8 ± 0.26	4 ± 1.4	0.15 ± 0.05	Chrysotile
M	3.9 ± 0.41	1.5 ± 0.26	<2	<0.08	—
N	5.0 ± 0.47	2.3 ± 0.32	3 ± 1.4	0.15 ± 0.07	Chrysotile

As far as talc used as excipients in various pharmaceutical preparations, both Italian and European Pharmacopoeia have so far not stated regulations about analytical control of asbestos contamination. This problem has been recently pointed out and it is probable that regulations in this connection may be enacted soon.

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