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CONSULTING • ULTRAMICROANALYSIS • MICROSCOPY • SMALL PARTICLE PROBLEMS • SOLID-STATE CHEMISTRY

3 December 1973

Hearing Clerk
Food and Drug Administration
Room 6-86
5600 Fisheries Lane
Rockville, Maryland 20852

Gentlemen:

We are writing to comment on the Commissioner's proposal covering "Asbestos Particles in Food and Drugs" as published in the Federal Register, Vol. 38 No. 188, pages 27076-27081, 28 September 1973. We are interested in helping the FDA choose a practical analytical procedure for the detection and identification of asbestos.

Our laboratory has analyzed many hundreds of asbestos and asbestos-containing samples by most of the various analytical procedures: polarized light microscopy, x-ray diffraction, scanning electron microscopy with energy-dispersive x-ray analysis, transmission electron microscopy with selected area electron diffraction, electron microprobe analysis and electron microscope microprobe analysis. We would like to begin by giving you our evaluation of each of these methods.

The methods to consider are:

1. Morphology by light and electron microscopy
2. Optical properties by light microscopy
3. Diffraction by x-ray or electron diffraction
4. Elemental analysis by electron or ion microprobe

The various instruments available are capable of a variety of combinations of these methods (See Table 1 attached to this letter). The advantages and disadvantages of each area are discussed individually.

The polarizing light microscope (LM) requires great skill and background obtained only with long practice. A microscopist trained in optical crystallography and familiar with the serpentines and amphiboles still requires hours of time per sample to find and identify each of the possible asbestos types. The problem is complicated by the presence of interfering fibers (3:1 aspect ratio!), e.g., paper, talc on edge, diatoms, quartz, mineral wool, plant hairs and fibers etc. Finally, all of the asbestos types show considerable variation in all of their properties and these variations must be familiar to the microscopist.

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Dispersion staining (DS) greatly speeds up the detection and identification of asbestos fibers. It is the only practical way to apply optical crystallography to samples containing small percentages of asbestos. The technique can be learned much more rapidly than optical crystallography yet it is securely based on optical crystallographic properties.

X-ray diffraction (XRD), also based on crystal properties, is somewhat less sensitive than dispersion staining but requires no more background and skill. The sensitivity can be increased from 0.5-1.0% to 0.1-0.5% by step-scanning. One advantage over LM and DS is smaller size limit — about 50 nm compared with about 5,000 nm (5 μ m).

Scanning electron microscopy with an energy dispersive x-ray analyzer is an overrated tool. Many substances besides asbestos are fibrous and it is nearly impossible to obtain a good chemical analysis of submicrometer particles in a particulate matrix with EDXRA in the SEM; there is too much interference. Fitting the SEM with a wavelength dispersive x-ray analyzer greatly improves its usefulness.

The transmission electron microscope (TEM) with selected area electron diffraction is an ideal tool when the asbestos fiber size is $\leq 0.2 \mu$ m. The shape of fibers down to 0.005 μ m is immediately apparent on scanning a sample and the diffraction pattern is immediately discernible.

The electron microprobe analyzer (EMA) with its wavelength dispersive x-ray analyzer is better than the SEM fitted with EDXRA but is surpassed by an SEM fitted with wavelength dispersive x-ray analyzer. The better morphological image of the SEM tips the balance.

EMMA, the combination transmission electron microscope-electron microprobe analyzer is, by far, the most useful of the analytical tools for asbestos. It may not be needed if the fiber size is large enough (about 0.5 μ m in smallest dimension), but smaller particles can be characterized by morphology, diffraction and chemical analysis in this one instrument. It is ideal for water samples containing suspended asbestos fibers.

The ion microprobe, although a highly sensitive trace analytical tool, is not usually used for asbestos fiber analysis. It does, however, detect fine fibers and identifies them by qualitative chemical analysis (quantitative when compared with standards).

Summarizing then — if the size of the asbestos fibers is very small (as it nearly always is for water samples) LM is useless and a diffraction method must be used. If the size is large enough, say 1 x 3 μ m, and the percentage high enough, say $\leq 0.01\%$, then LM can do the job. If high sensitivity is not needed, say about 0.2% or greater, then XRD is the best method. Very fine particles require the TEM with selected area diffraction.

It should not be necessary to emphasize that no method is better than the analyst who makes the analysis. Few laboratories have all of the above instruments and highly skilled analysts for each. Instead, each tends to use the tools available and the methods with which their personnel are more or less familiar. In a situation as important as this, the choice should not be based on availability of tools and skills. Fortunately, the tools for detection and identification of asbestos are neither the most expensive nor the most sophisticated.

Our procedure (in brief), based on the analysis of many hundreds of samples, is:

1. Screen the sample by DS using Cargille high dispersion liquid 1.550.
2. If positive: a) identify the asbestos type by DS or XRD, b) determine amount by counting using criteria suggested in the FDA method of 28 September 1973, or by XRD. (We might add that a 3:1 length to width ratio is not exactly a fiber. The Particle Atlas specifies 10:1; quartz flakes are often >3:1).
3. If negative: a) conclude that less than 0.01% of chrysotile or amphiboles are present, b) use TEM or EMMA with EDXRA and SAED to be sure submicroscopic asbestos particles are not present.

This is a complete procedure which can usually be shortened considerably in practice. For example, a mineral sample or a sample containing a mineral fraction will generally not have been subjected to a procedure that eliminates larger fibers, e.g., ultrasoneration or sedimentation. Hence TEM is not necessary and DS can be used.

Before describing in more detail precisely how we proceed, let me comment on the proposed FDA procedure.

1. To do optical crystallography on talc the particles must be well dispersed. One mg under an 18 mm cover slip is far too crowded if one hopes to find individual fine asbestos fibers; one-tenth that concentration is much better.
2. It is true that all refractive indices of chrysotile are less than 1.574, but so are the indices of numerous other elongated substances including ubiquitous paper and glass fibers. In other words, 3:1 aspect ratio particles having all indices <1.574 are very probably not asbestos. To be certain they are chrysotile requires:
 - a) remounting another aliquot in, say, 1.560 to see if $n_{\parallel}$ is about that value and if the other two n 's are about 1.550. Worse still, one has to find those now nearly invisible (without DS) fibers among millions of other particles; or
 - b) scan the sample at room temperature in a hot stage so that a suspected fiber in 1.574 could be shown to disappear with sodium light on heating at about $25 + [(1.574 - 1.560)/0.00045]$ or 56°C for $n_{\parallel}$ and about $25 + [(1.574 - 1.550)/0.00045]$ or 78°C for $n_{\perp}$. One would at least have the same particle in view throughout the test but it would still take hours, or even days, to check 100 particles by this procedure.
3. Talc, often on edge, has one index less than 1.574 and it takes time to check the other index to be sure they are not chrysotile.
4. It is likewise true that all amphiboles have all indices higher than 1.590 but the same difficulties hold true here as are given above for chrysotile. The amphiboles are even worse, however, because there are more of them, each one varies considerably in optical properties and there are many possible interfering substances.
5. Counting the number of fibers is not a good measure of % asbestos. We find the size of individual fibers varies 10^6 -fold, at least.

6. It requires a highly skilled microscopist to identify the amphiboles; especially in small amounts, in confusing mixtures and as small fibers. Of all the microscopists in the world fewer than 1% would be so qualified. Nearly all of these qualified microscopists are mineralogists and a few chemical microscopists. Very, very few industrial laboratories, especially in food and drugs, have such personnel nor are they available on the job market in the number that would be necessary if this procedure is adopted as written.

Now, what would we suggest as modifications that might improve the proposed method? We recognize that the proposed method is basically good in that it is based on crystallographic data, the classical approach to the problem. We would keep this base but shift to diffraction rather than crystal optics. The diffraction method is easier to teach, easier to learn, just as dependable, works for all fibers (large or small) and is more rapid. It is better than crystal optics in assessing the type of amphibole especially in complex mineral series like tremolite — actinolite — ferroactinolite or the cummingtonite — grunerite series. It is, however, somewhat less sensitive than light microscopy. With step scanning, a time-consuming but potentially automatic procedure, 0.2–0.5% of a given asbestos might be detected; by ordinary slow continuous scanning 0.5–1.0%.

Criteria for the certain identification of each asbestos type can be found in the literature. We use the following criteria for chrysotile and tremolite.

Chrysotile — An x-ray powder pattern showing both the 002 (7.3 ± 0.1 A; $I/I_0 = 1.00$) and 004 (3.65 ± 0.05 A; $I/I_0 = 90 \pm 20$) reflections and showing no line in the 14.6 A chlorite region is necessary evidence for the presence of chrysotile. A line at 14.6 A in addition to, and of the proper intensity relative to, the 7.3 A and 3.65 A lines identifies chlorite rather than chrysotile.

Tremolite — Necessary evidence for the presence of tremolite is an x-ray powder diffraction pattern with lines at 8.4 ± 0.1 A, 3.27 ± 0.05 A, 3.03 ± 0.05 A and 2.92 ± 0.05 A. (Note: zinc and magnesium stearate may interfere at 8.4 A and calcite at 3.03 A.)

Similar criteria have been set for the other amphiboles. If greater sensitivity is required we recommend step-scanning. This must be done through the regions of the three strongest lines for each of the asbestos types.

If still greater sensitivity is desired or if, for any reason, light microscopy is the preferred method, we then suggest dispersion staining. The overriding reason for DS rather than the classical methods of optical crystallography is the following. One cannot by the latter methods mount a sample in the diagnostic refractive index mounting liquid for, say, chrysotile because chrysotile thereby becomes nearly invisible and, especially in trace amounts, small needles would not be detected. For this reason your proposed method requires mounting in 1.574 so that chrysotile fibers can be seen and their index relative to 1.574 determined. However, many other substances also have indices lower than 1.574 and, although birefringence, extinction, sign of elongation and contrast may eliminate some, this takes time to check. DS, on the other hand, allows the analyst to mount the sample in 1.550, the liquid most likely to make chrysotile disappear when classical methods are used. Now, however, with DS chrysotile fibers show two brilliant and distinctive colors, one for $n_{\parallel}$ and one for $n_{\perp}$.

This uniquely identifies chrysotile fibers almost at a glance; glass fibers, paper fibers, talc on edge, quartz, splinters etc all show, at least, different colors and an experienced microscopist can quickly identify them.

Similarly for the amphiboles, we mount the sample in 1.605 rather than 1.590 and for a corresponding reason. In 1.605, tremolite is easily identified by either yellow and blue colors on crystals with oblique extinction or yellow and golden magenta on crystals with parallel extinction ($n_{\parallel}$ and $n_{\perp}$, respectively). Furthermore, actinolite and anthophyllite have distinctive and different colors in this same liquid. One is left only with amosite (positive elongation) and crocidolite (blue fibers with negative elongation). All five of these amphiboles can thus be detected and identified in this one liquid.

In conclusion, if forced to analyze a sample by the proposed method, we would require several (2-8) hours of a skilled crystallographer's time; this would average \$250 per sample. The time can be cut to 0.5-1 hour and the cost to about \$50 when dispersion staining is used. It is also practical to use less highly trained microscopists for the dispersion staining procedure. Although we're not sure, reading the proposed method, whether we are excluded from using dispersion staining, it is obvious the writer of the procedure did not visualize doing so.

We have just completed, for example, an intensive 2-day course in these methods for EPA personnel and have scheduled a similar course for food and drug industry personnel 25-26 February 1974. It is feasible to train a microscopist with little previous knowledge of crystallography how to detect and identify asbestos by dispersion staining in two days. Not so by the classical method; here, we would need 10 days and even then less than half of the students could dependably analyze any and all samples for serpentine and amphiboles.

We have also enclosed copies of two papers from our laboratory on asbestos. One is, unfortunately, still in press and without the cited Figures. We hope, in any case, that some of this material based on our own experience may be helpful to you. If we can help in any further way, please be sure of our desire to cooperate.

Sincerely,



Walter C. McCrone
Scientific Adviser

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