Chapter

Analytic Analyses of Human Tissues for the Presence of Asbestos and Talc

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Abstract

This chapter discusses the historic and current criteria for the analysis of cosmetic talcum powder and the finding the components of the talcum powder in human tissues. It describes how technicians and scientists have looked in the past for these components and how they should be looked at properly today. Within the chapter it has been shown that it can be complicated, especially when the tools and the methods used are not adequate or sensitive enough. It also goes on to describe methods for analysis that are sensitive enough in both mineral analyses and in human tissue. It also defines the terms that are necessary to use for inclusion of structures based on the scientific knowledge we have today not confused with what either industry or their defenders are trying to use to confuse or defend their positions.

Keywords: electron microscopy, human tissue talc components

1. Introduction

One of the best and concise reviews of what has been defined as asbestos is in a report by the U.S. Department of the Interior: U.S. Geological Survey by Virta [1]. Briefly, asbestos and talc are minerals that are mined from the earth. The asbestos is defined as having six different types of magnesium (Mg) silicates (Si). An important feature of many of the types is that they may or may not contain iron when they are removed during mining. They can have other ions as well and those include sodium (Na); calcium (Ca) or manganese (Mn) along with the Mg and Si. These minerals are defined by the presence of these elements and their ratio one to another. There are numerous publications defining the mineralogic nature of these minerals throughout the literature besides what is stated in Virta [1]. They are also defined initially by their color when in the ground and raw, by their size, shape, how they were formed and their crystalline structure by light and electron microscopy. The types are divided in two groups, serpentine and amphiboles. The serpentines principally are chrysotile and the amphiboles consist of five different types based on the ratio of the Mg to Si and other elements that are integrated into the molecular structure. The amphiboles consist of crocidolite, amosite, anthophyllite, tremolite and actinolite. They are mined in both open and closed type mines. The elemental composition of these six types of asbestos is seen in **Table 1**. The talc is a basic $H_2Mg_3Si_4O_{10}(OH)_2$.

These mined minerals, both asbestos and talc, have in the past been used in many products and have been shown to have detrimental effects in humans and

animals when they enter the body of these organisms [2]. The effects range from tumors to fibrosis. The asbestos has been classified as a carcinogen and has been known to cause lung cancers, mesotheliomas, gastrointestinal cancers and more recently has been implicated in causing ovarian cancers and others. In the case of talc and talcum powder products, it has been implicated as causing these tumors either indirectly because it is contaminated with asbestos or the talc is a carcinogen or co-carcinogen even without the contaminating asbestos. Further, asbestos, generally in high doses is a well-known cause of interstitial fibrosis, asbestosis, and pleural plaques in lungs. Talc in relatively high doses is also known to cause fibrotic lesions, specifically in the lung. This type of fibrosis is referred to as granulomas.

The mechanisms of causation of these diseases have been shown to be either direct or indirect. What I mean by direct is the interaction of the asbestos fiber or talc fiber or particle with DNA in the cell eliciting mutations. The indirect methods of causing these same mutations is the release of oxidants either within the cells or from macrophages that have either completely engulfed the fibers or particles or partially engulfed them because they are just too large to be contained within the cells. These oxidants cause DNA mutation, which can cause the cells to convert to cancer cells. In addition, when these fibers and particles get into the cell, the cells are known to release cytokines and chemokines that can result in the recruitment of inflammatory cells and the in the development of the fibrotic lesions.

The relationship between asbestos fibers and amounts, size, dimensions, and type has been correlated with the development of diseases. It has been determined that the greater amount of asbestos present in the peripheral lungs or other tissues of known tumorigenesis, the greater the risk of developing that tumor or fibrotic change. The longer and thinner the fiber, the greater the risk. Also, amphiboles bare a greater risk than chrysotile unless the chrysotile is relatively long fiber type and numerous and even than it must require a greater latency between exposure and the development of tumors specifically. Crocidolite by far is considered the most carcinogenic with amosite not far behind and then anthophyllite. Tremolite and actinolite tend to parallel chrysotile because they are generally shorter and less numerous because they are contaminates with the chrysotile or talc.

An important link and correlation between environmental exposure and causation of the diseases describes above is the finding of these particles in human tissue. The remainder of this chapter is dedicated to the specific criteria and methodologies for defining and identifying these fibers and particles in human tissues which can be very different and much more difficult to identify in from those evaluated from the same minerals that come directly from mining. This chapter addresses many of these issues in defining these fibers after they have been subjected to tissue modification after entering the human body.

NAME	COMPOSITION
CHRYSOTILE	$(Mg_6O_4(OH)_8)^{-4}(Si_4O_{10})^{-4}$
RIEBECKITE (CROCIDOLITE)	$Na_2(Fe^{+2},Mg)_3Fe^{+3}Si_8O_{22}(OH)_2$
GRUNERITE (AMOSITE)	$(Fe^{+2})_2(Fe^{+2},Mg)_5Si_8O_{22}(OH)_2$
ANTHOPHYLLITE	$Mg_7Si_8O_{22}(OH)_2$
TREMOLITE	$Ca_2(Mg_5Si_8O_{22}(OH)_2$
ACTINOLITE	$Ca_2(Mg,Fe^{+2})Si_8O_{22}(OH)_2$

Table 1.
This table illustrates the chemical composition of the various asbestos fibers.

Analysis of human tissues for the presence of asbestos and talc is nothing new [3, 4]. However, what makes this type of analysis unique and now very much at the forefront is that these components have been identified in and possibly attributed to the development of tumors not considered in the past [3].This author has looked at numerous types of tissues and tumor tissue from same and in a variety of other tissues and organs [5–7]. What is emerging is the question of protocols and methods for detecting these particles and fibers, identifying them and attributing them to the disease processes. The history behind asbestos exposure and disease is well documented with causing lung tumors, mesotheliomas in pleura and abdomen and asbestosis in lung. Only some of the effects of talc have been documented, most of which are associated with the development of granulomas in the lung and in the pleural spaces when the talc is injected into the space to avoid the accumulation of fluid, talc pleurodesis.

More recently, within the last 10 years there has been attribution of cosmetic talcum powders causing mesotheliomas and possibly other lung tumors [4]. However, the attribution has been directed to the contaminating asbestos in the product [4]. Companies that currently sell and those that sold this product in the past are claiming that their products are free of asbestos. They base this on tests that have been done in a number of laboratories using a variety of testing protocols. However, further testing using more sensitive methodologies have shown these products to still contain asbestos.

It is the specific intent of this chapter to address all the issues with regard to the methodology of testing of cosmetic talcum powders for the presence of asbestos and to be able to document the presence and type of asbestos in human tissue studies in persons that have used these products with no history of exposure to asbestos from other sources and differentiate the particle type.

2. Historic testing of cosmetic talcum powders

The testing of talcum powder goes back to 1968, Cralley et al. [8] tested 22 different samples of talcum powder off the store shelf for fibrous and mineral content. They found that all 22 containers had a significant amount of fibrous components by light microscopy and phase contrast (PCM). The type of fibers were not identified by PCM or by XRD and assumed to be fibrous talc with smaller contaminates of tremolite, anthophyllite, chrysotile and pyrophyllite. Without identifying the fiber types, they identified fibers that could not be seen by light microscopy and concluded that it was these fibers that could be the source for ferruginous bodies seen in humans.

In the 1970s, numerous investigators analyzed talcum powders. Walter C. McCrone Associates, Inc. looked at talcum powders for a variety of different companies and groups including NIOSH. They used polarized light microscopy (PLM), XRD and Transmission Electron Microscopy (TEM) and reported finding asbestos fibers in many of the samples [9–12]. In 1972, at New York University Chemistry Department tested a sample of a specific talcum powder called 1615 [11]. XRD indicated that the fibers were suspect for asbestos and then the talc was subjected to a more critical testing where they identified both tremolite and chrysotile [13].

In 1974, Rohl and Langer [14] tested a number of talcum powder specimens using both light microscopic techniques, XRD and analytic electron microscopy (ATEM) with selected area electron diffraction (SAED) and electron microprobe and indicated they were able to detect only a very small amount of the fibrous asbestos particles by PLM or XRD mainly because of the size of the particles and

recommended that it was essential to do analytic TEM to analyze talcum powder for the presence of asbestos. In another article in 1974, Rohl [15] indicated that the asbestos in the talcum powder was directly from its mining. However, in both studies they concluded that a negative finding of asbestos in these products by just XRD could mean there were possibly billions of fibers in just a half gram of the talc if tested by a more sensitive technique, i.e. TEM.

In 1976, Rohl and Langer [16] reported on 20 off the shelf talc or talcum powder products of which they were able to detect asbestiform fibers in 10 of the 20. They used a combination of XRD, PLM, scanning electron microscopy (SEM) and TEM. They used EDS and SAED with the TEM samples to identify the asbestos fibers. They concluded that the great majority of the talc asbestos fibers tested by XRD and PLM would go undetected as compared to SEM and TEM specimens.

In 1990, Kremer and Millette [17] published on the same powder used by the McCrone Laboratory in 1985 and employed a different methodology of suspending the material in a solution of methylcellulose to view the fibers by TEM and found a variety of different minerals, including asbestos.

3. Historic methods for observing talc components

What is of great interest is that there are two methods promoted by the cosmetic industry, CTFA-J4-1 [18] and USP-Talc [19] which only employ XRD and light microscopic techniques. They also state that using TEM with SAED is much more sensitive technique but they do not recommend using that methodology. The unfortunate part of all this is that the industry relies on this method of testing knowing full well that they will not find the great majority of contaminating asbestos fibers by these techniques.

However, there have been many techniques published that use a combination of both XRD, light microscopy (PLM or PCM) and which state that if there is a negative finding by these techniques it is important to look by TEM or use SEM as a screening technique. Some of these techniques include the EPA 1993 bulk method [20] as one such method. Most of the techniques not only require that TEM be used, but both SAED and EDS be performed to be able to determine the identity of the type of fiber one is seeing. These include the AHERA methodology which employs the Yamate et al. [21] method. Other methods that are frequently used are those from ASTM D6281 [22], D5755 [23], D5756 [24], and D6480 [25] all of which require TEM. There are two others called ISO10312 [26] and ISO13794 [27] which are very much the same as the ASTM methods. The techniques for verification of asbestos fiber types require SAED confirmation. However, in some cases where there may potentially be a question or a problem of confirmation zone-axis maybe required and is described in both the ASTM D6281 [28] technique and in Yamate et al. [29]. However, this is only if there is a question, since in most instances it does not give further support to routine SAED. When combining the newer more sensitive EDS equipment with SAED, zone-axis analysis will not add anything. The most important point here is that there is no specific defined method for identifying asbestos in talcum powder products. However, the use of the most sensitive techniques available is imperative.

4. Differentiating asbestos fibers

There are six types of asbestos that have been described and identified as detrimental. These are categorized into two types, serpentine, chrysotile, or the

amphiboles, crocidolite; amosite; anthophyllite, tremolite and actinolite. As seen in Chart 1, the six asbestos types and talc show their chemical composition and how they are both very similar and or different based purely on their chemical composition. In differentiating asbestos fibers there are two approaches to the problem that can/are seen differently depending on who is looking at the fiber(s). This includes a definition of what asbestiform means and based on who is looking will determine which definition may be applied. When a mineralogist is looking at fibers their criteria requires a population of fibers that have to meet the 3:1 ratio, equal to or longer than 5 μm with parallel sides that has grown in an asbestiform mineral habit. On the other hand, when viewed by someone looking at single fibers the only distinctions that can be made are based on the observed morphology of that fiber. It is not possible to relate it to the environment from which it was formed. The criteria under the latter situation is the one that all government agencies adhere to and require for it to be an asbestos fiber and that is that the fiber should be greater than 0.5 μm in length, have at least a 3:1 ratio of length to width and have parallel sides. That is what qualifies it to be an asbestos fiber.

In 1990, Wylie [30] published some suggested criteria which were primarily based on light microscopic criteria and not electron microscopy. Wylie et al. [31] suggested that it had to have a 20:1 or greater and had to be very thin fibers or fibrils, less than 0.4 μm in width and two other criteria which included parallel fibers in bundles, splayed ends of fiber bundles, fibers in the form of thin needles, matted masses of individual fibers and finally fibers showing curvature to be considered as asbestos. In the EPA R-93 [32] this was repeated in the glossary. However, it is possible to see that these light microscopic criteria are useless when viewing a single fiber or fibril by transmission electron microscopy. It has been determined that if one were to use this criteria, approximately 80% of the asbestos fibers would be misclassified. EPA R-93 method [32] suggest the use of 10:1, ratio based to some degree, on a 1985 Wylie publication [31] indicating that if 20:1 were used with an amosite population, as much as 50% of asbestiform asbestos fibers would not be counted. Even the bureau of Mines Circular [32] indicates that a 5:1 ratio is the most realistic. The 5:1 ratio is in fact used by AHERA, ASTM methods D6281, D5755, D5756 and D6480 and ISO 10312 and 13794. The width of the fiber as described by Harper et al. [33] seems to be the best discriminator. In a publication by Kelse and Thompson [34] from RT Vanderbilt further supports the concept that any fibers equal to or greater than 5 μm in length and less than 0.25 μm in diameter are asbestos fibers and almost all less than 0.5 μm in width are fibers and not cleavage fragments. However, these are purely mineralogy distinctions and have virtually no application to biologic systems since the cells that are activated by these fibers in human body do not make these distinctions. The cells only are effected by the shape, size dimensions and surface charge on these fibers which can cause a form of oxidant injury or mechanical alteration of the cellular DNA in the mesothelial or ovarian epithelial cells that take them up and in macrophages and inflammatory cells that engulf them causing the release of cytokines, chemokines and molecules associated with oxidant injury which can indirectly effect mesothelium and ovarian epithelium to become tumors. Of course this excludes the concept that the same molecules can also cause the development of fibrosis or asbestosis. Therefore, this entire argument rose by a very few mineralogists that cleavage fragments not be considered as harmful, is just wrong.

The other issue that arises from a similar argument is talc itself. Talc can also be present in the form of fibers that can mimic, but can be differentiated analytically from asbestos and can cause fibrotic lesions in some mammals and in human lungs [35, 36]. Therefore, it is realistic to consider talc, especially in the fibrous form, a potential causative factor in the development of mesotheliomas and ovarian

cancers. There is, has been, and is currently significant research ongoing to prove that the talc can be considered a carcinogen, alone, as a co-carcinogen with the asbestos or as a promoter with the asbestos, just based on its ability to produce an inflammatory response.

Zone indexing of asbestos fibers and talc fibers for the purpose of differentiating them has been described and shown to be relatively unnecessary procedure [37]. EDS spectra can be indistinguishable between anthophyllite and talc [37]. However, when anthophyllite is compared to talc fibers by SAED talc fibers no matter how they are turned or tilted show the typical hexagonal pattern. On the other hand, anthophyllite can only show a pseudohexagonal pattern if tilted to a specific angle. Therefore, the only issue would be that one would see less anthophyllite if tilted in that specific angle as compared to talc, but talc would never be confused with anthophyllite if SAED is performed in only a single angle.

5. Analysis of human tissues

There have now been many reports, possibly hundreds that describe the protocols for identifying asbestos and talc in human tissues. However, when one looks at these protocols it is possible to break them down to three similar, but yet different means of looking for these particles in these human tissue preparations. As fully described below, the remaining material after tissue digestion can be prepared by the filtering method a portion of the filter is put directly on an SEM stub and then analyzed. The Alternatively small portions of the filter can be placed onto TEM grids and then observed by either SEM or TEM. Lastly, the material can be placed directly on a formvar support film on a TEM grid and then directly analyzed by TEM.

6. Analysis of asbestos by SEM

There are at least two investigators that look at human tissue preparations; one of which has been doing these analyses for years by SEM and that is Roggli [38]. Based on all government criteria SEM analysis is not an acceptable criteria. All government agencies that describe doing electron microscopy observation and identification of asbestos require TEM with at the least SAED, but EDS is always listed as a criteria. SEM analysis does not allow the technician, examiner or scientist to evaluate the crystalline structure of the fiber or particle of interest. SAED is what is considered the gold standard for identifying asbestos fibers and other particles such as talc. As will be shown as this explanation unfolds, the identification of asbestos fibers and specific types of asbestos fibers in human tissues is far more difficult than that of the mineralogist identifying them from ground up rocks or mined minerals. This is the case mainly because the longer these fibers are present in a biologic environment with cells, tissues, animals or humans, the fibers are modified and frequently can only be distinguished using SAED. When SEM is used there is a significant potential for error. The error is most likely to occur when distinguishing fibers between anthophyllite, chrysotile, tremolite and non-asbestos talc fibers. Pure morphology by SEM on single fibers is very similar in appearance. EDS analysis of the same fibers are more difficult to get the optimal elemental composition because the electron beam energy is significantly lower, generally never more than about 40 KV whereas in a TEM it is generally 75–200 KV. It has been long known that the higher the KV the greater the penetration of the beam into the fiber. Lower energy levels will only affect the

very surface of the crystalline structure or fiber. As stated before, fibers removed from biologic systems are modified as their surfaces by the interacting environment. Biologic interactions results in the removal of molecular components from the surface referred to as leaching. The leaching is mostly associated with removal of magnesium, which can lead to the change in the Mg to silica, Si ratio which can put fibers into different categories or types based purely on elemental analysis. The most effected fibers or particles are chrysotile type asbestos fibers and talc fibers and particles. These are most susceptible to leaching and ultimate relatively rapid breakdown of the structure. Examples will be given below when discussing changes in TEM. The alternative to leaching is that elements in the form of molecules can become adherent to the fiber or particles. The most common element and ion that adhere is iron, Fe. When the fibers or the particles are present in tissue for long periods, years, the iron, in combination with protein molecules can produce ferruginous bodies or asbestos bodies on asbestos fibers. When there are substantial amounts of iron and protein to form bodies they are easy to identify even by light microscopy. However, there can be lighter coatings not forming the pearl like structures on the fiber or covering the particles and then it is just seen as increased iron which could lead to an inaccurate identification by EDS analysis which has already been argued in letters to the editor following a publication [2] where one laboratory wanted to identify an anthophyllite fiber as an amosite fiber. In addition, other elements such as sodium, Na, aluminum, Al and calcium, Ca, can adhere to the fiber surface also leading to a misidentification when looking at fibers with the SEM by morphology and EDS alone. This will be discussed later with examples in the TEM section.

7. Methods for SEM or ATEM preparation

7.1 Methods for filtering

The filtering methodology has been published many times and is used by laboratories that evaluate air, water, bulk and human tissue samples [29]. With human tissue samples the material must first be digested and cleaned with distilled water to remove any biologic material. This is performed by a variety of techniques which have previously been employed. When the tissue is received in formalin, the tissue is either dried or completely and weighed or is just blotted dry. In the former the results will be expressed as dry weight and the later wet weight. Either way they are approximately comparable by approximately a factor of 10. Either way the tissue is then treated with either hypochlorous acid, (Clorox) or 5% potassium hydroxide, KOH, which acts to digest away any biologic material or it becomes soluble in either solution. The inorganic material is then separated by centrifugation and repeated, ×5, sequential washes in distilled water. The remaining inorganic and metal materials are then put into a final suspension of distilled water and filtered onto either polycarbonate or missed ester type filters. After drying the filters are cut into small pieces and placed on formvar coated copper or nickel locator grids or directly onto a SEM stub. The filters are lightly coated with evaporated carbon to help prevent transposition or release of the fibers and particles during the collapse protocols. The filters are collapsed with either acetone or ether depending on filter type. Some investigators use low temperature ashing to remove any residual biologic material, however that is rarely done today. The ashing was most often used for filters that were prepared from water and air sampling which where the material present on the filters is not predigested with Chlorox or KOH. The grids or stubs are then ready for observation.

7.2 Methods for drop method

An alternative method that this author has used for over 45 years was first defined by Langer et al. [39] where the digested material is resuspended in a known amount of distilled water and then 10 μl drops are placed directly on formvar coated grids and dried. The grids are then ready to view by scanning or transmission electron microscopy evaluation.

8. Methods for asbestos fiber and talc particle analysis

The problems associated with SEM have been defined above and will not be discussed here.

When we look at the prepared grids, whether the grids are viewed and evaluated by three criteria, morphology, EDS and SAED, the grids are first scanned to make sure that they have less than 5% broken openings. Dependent upon the criteria used in the laboratory, the grids are critically evaluated at magnifications between 10 and 20 K, one grid at a time, for the presence of asbestos fibers or whatever is being evaluated.

To determine if a fiber is asbestos is based on well-established criteria. If a fiber has parallel sides and has a 3:1 or 5:1 aspect ratio it has the morphological criteria for a fiber. If the fiber demonstrates individual smaller components within the larger fiber, referred to as fibrils, each of which is a fiber if seen alone is than a better criteria for the classification as asbestiform by mineralogy criteria. When pathologically evaluating the morphology of an asbestos fiber a mineralogists criteria of being asbestiform or grown in an asbestiform habit is not at all considered. However, when the asbestos fibers are seen as a bundle, would be considered an asbestos fiber by either a pathologist or mineralogist. The chemistry of the fiber has to contain specific elements which include sodium, magnesium, silica, calcium, manganese and iron. They also have been found in approximate ratios using silica as a reference. Each type of asbestos type has a specific ratio when in its natural form. Crocidolite, amosite and tremolite are generally easily recognized by EDS alone. Chrysotile and anthophyllite can look very similar. Also one must exclude fibrous talc when making these determinations since it too looks very much like chrysotile and anthophyllite by EDS alone. The third criterion is selected area electron diffraction which determines crystalline structure of the fiber or material. This technique produces patterns that identify the crystal very much the way fingerprints identify people. When a fiber cannot be identified by morphology and EDS, SAED is the determining technique. SAED can only be performed with a transmission electron microscope. It is possible to screen for fibers and particles by XRD, PLM, PCM and SEM, however, for definitive identification, TEM using morphology and SAED or ATEM using both EDS and SAED are absolutely required. Even then it may be difficult to identify the fiber type because of all the issues described above as interference in the ability to specifically identify a fiber. Amosite and crocidolite are generally the easiest to identify. Chrysotile, anthophyllite and fibrous talc can easily be misidentified. Tremolite/actinolite can also be determined but with difficulty and the use of SAED to differentiate it from chrysotile, anthophyllite or fibrous talc. So when evaluating human tissue isolation of fibers and particles, there are many elements present in tissue that can ionically or covalently adhere to the outer most part of the fiber or particles. A few of these elements include sodium (Na), aluminum (Al), calcium (Ca) and iron (Fe). When it is not possible to identify any features by morphology, most laboratories first focus on the EDS which will give us the element composition and

the ratio of one element to another. One has to consider that the fibers and particles once in a cell are attached by acids, and enzymes that can modify the surfaces by eroding the fiber, usually by leaching the Mg. However, many elements can be added to the surface. When Na is added and Mg is partially leached amosite can appear to be crocidolite (**Figure 1**). When Mg is leached from anthophyllite and Fe is added it can appear to be amosite (**Figure 2**). When chrysotile has Mg leached and Fe added in appears to be anthophyllite (**Figure 3**). It is very difficult to sort between tremolite and actinolite because Fe can be added. Fibrous talc can look like chrysotile and anthophyllite by EDS only (**Figure 4**). If there is a lot of Calcium phosphate as background and interference with some added iron, it may not be possible to confirm tremolite or actinolite by EDS (**Figure 5**). One more exhibits anthophyllite with leached magnesium and some added iron; however, it could be easily be confused with being tremolite or actinolite (**Figure 6**). There are many cases like this that end up being defined by the SAED and not EDS and morphology alone.

The series of fiber TEM micrographs and their corresponding EDS show how difficult it could be with only morphology and EDS to define asbestos fiber type removed from humans. It then becomes critical to perform SAED on these fibers to determine the crystalline structure based on the dispersion patterns. However, this technique can also be problematic in identifying fiber types. It is possible if the anthophyllite is tilted just right it can look like talc in the SAED pattern and if the D-space measurement can also be the same [29, 37]. However, the opposite is not true. Talc never looks like anthophyllite by SAED pattern or d-spacing measurements. As a result the only effect this could have is to reduce the amount of anthophyllite if present.

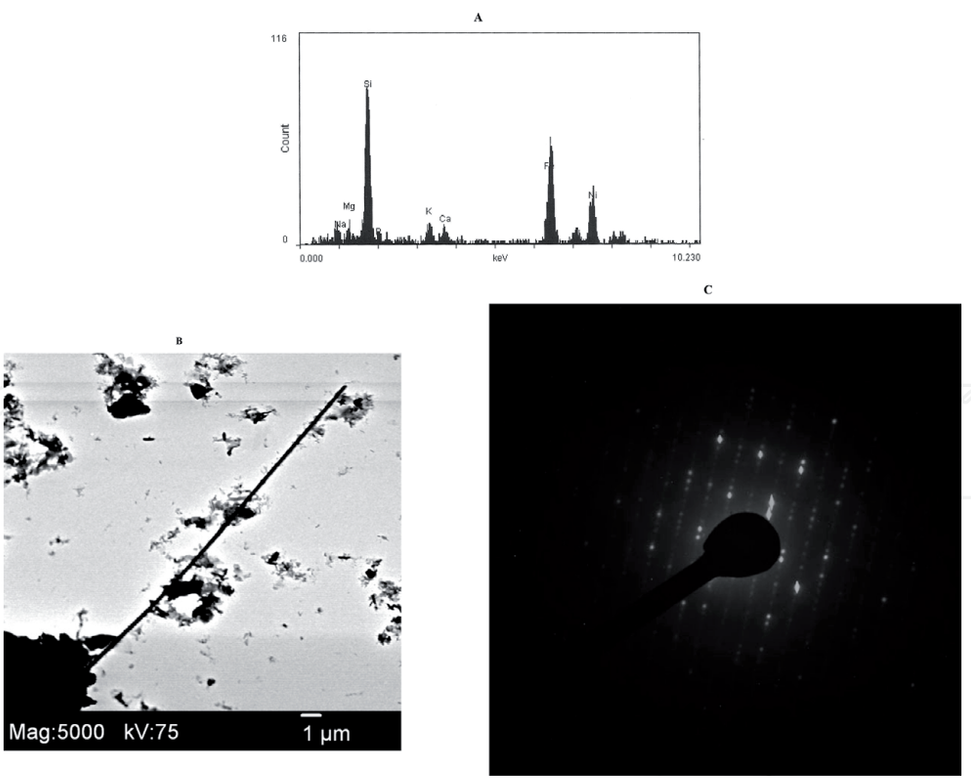

Figure 1.
This EDS spectrum (A) represents the asbestos fiber seen in (B). At first look this long narrow fiber would correlate with the spectra of a crocidolite fiber having approximate ratios of 1:1:10:6 Na:Mg:Si:Fe. The potassium, K, is from adherent from the tissue digestion and the calcium, Ca, is surrounding interference material with the phosphate, P. However, when SAED was performed, the diffraction pattern was that for amosite and not crocidolite indicating that the sodium, Na, was either interference from the surrounding area or adherent to the fiber itself. The SAED in 1C confirms that it is amosite.

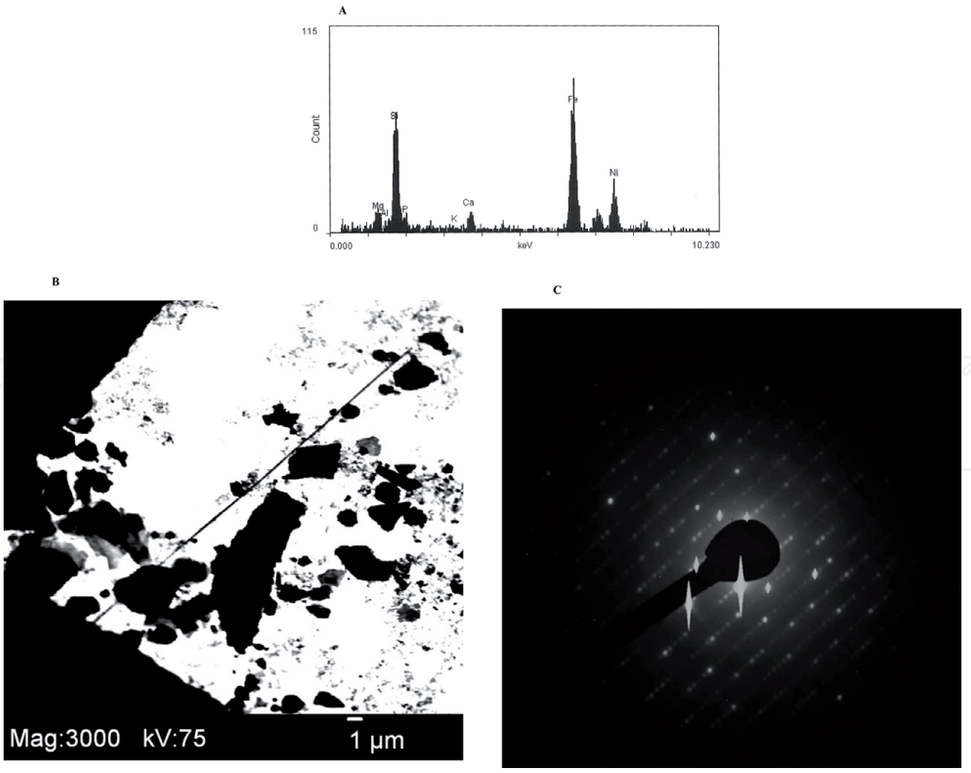

Figure 2.
This EDS spectrum (A) represents the asbestos fiber seen in (B). Other than the calcium phosphate, $CaPO_4$, it would be consistent with it identifying an amosite asbestos fiber. However, there is more Fe than would be expected and a little less Mg. When SAED was performed, this fiber turned out to be anthophyllite with significant Fe more than likely coming from interference iron particles surrounding the fiber and some leached Mg. The SAED in 2C confirms that it is amosite.

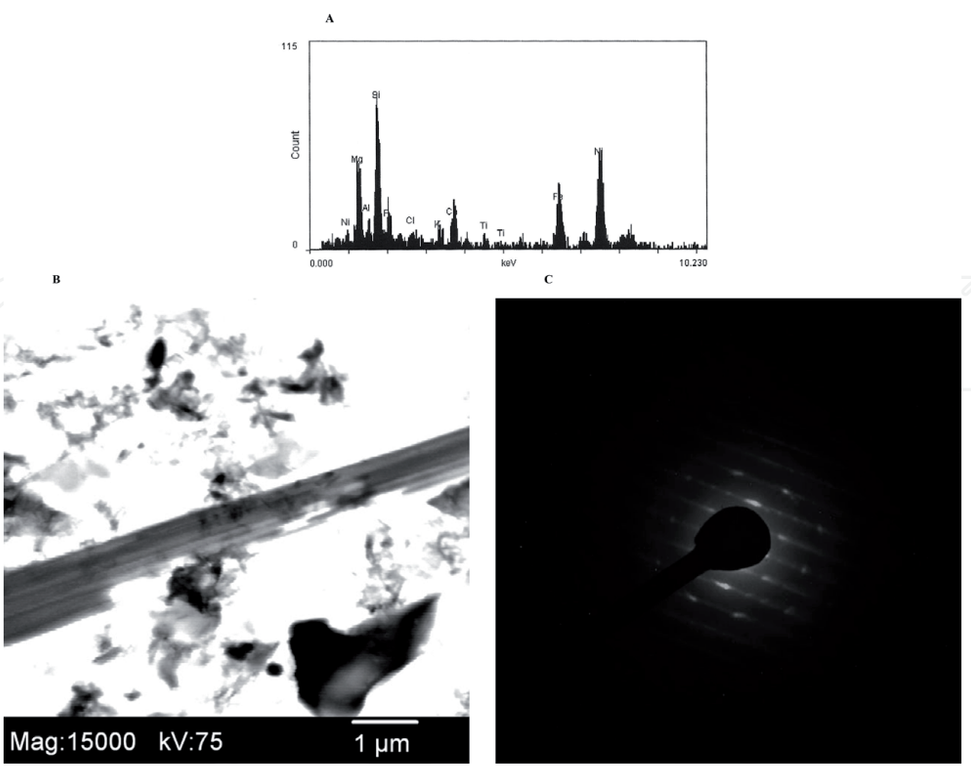

Figure 3.
This EDS spectrum (A) represents the asbestos fiber seen in (B). This EDS would best fit anthophyllite type asbestos with some increased Fe. There may be a slight too much Mg for anthophyllite. SAED of this fiber proved to be chrysotile with increased Fe and Mg leaching. The other elements identified, calcium phosphate, potassium chlorine and a little aluminum are from the surrounding interference. The SAED in 3C confirms that it is chrysotile.

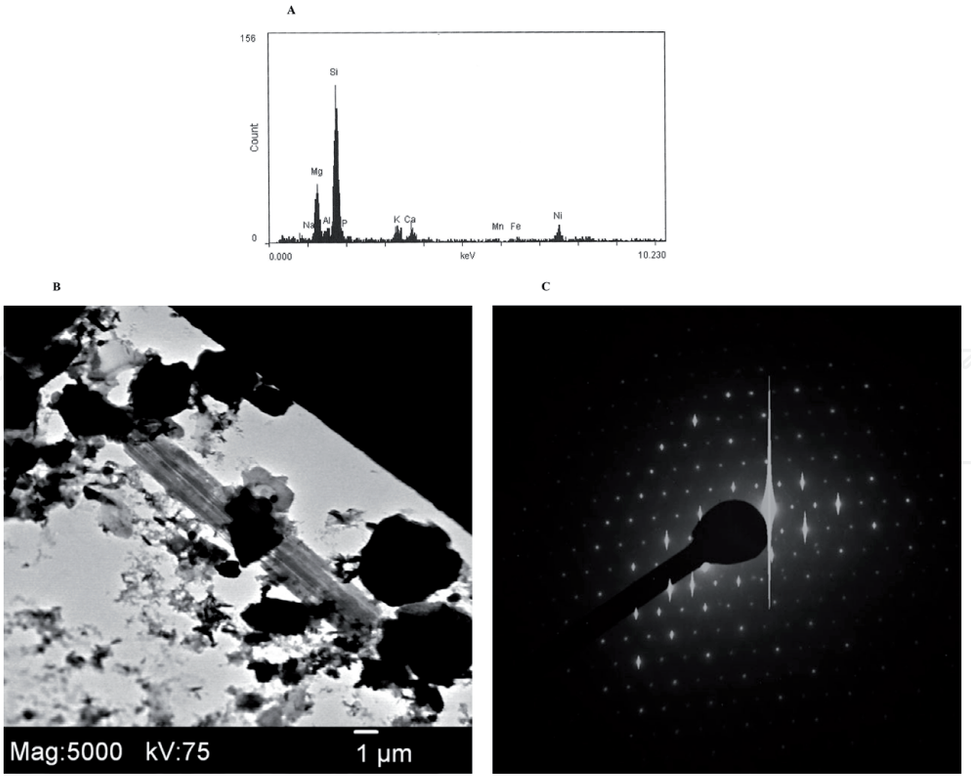

Figure 4.
This EDS spectrum (A) represents the asbestos fiber seen in (B). This EDS could represent a chrysotile asbestos fiber with leached Mg or an anthophyllite type fiber with no Fe. However, SAED exhibited the classic hexagonal pattern of a talc fiber. The SAED in 4C confirms that it is talc.

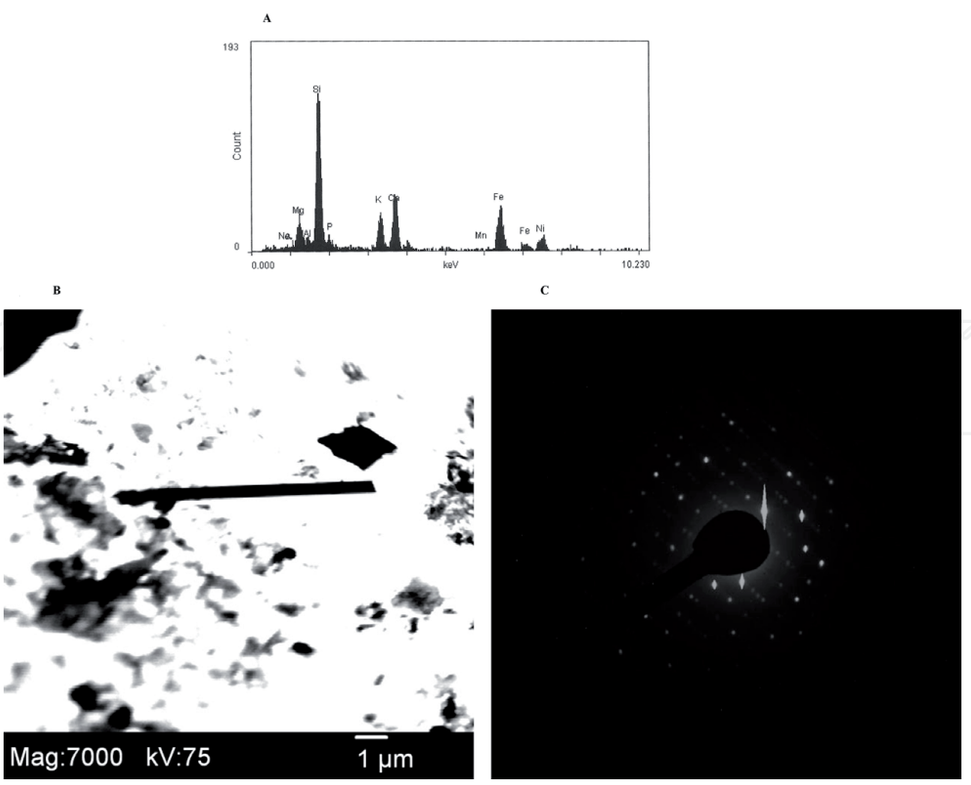

Figure 5.
This EDS spectrum (A) represents the asbestos fiber seen in (B). This appears to be anthophyllite asbestos with some leached Mg and slightly more Fe. The K is from the digestion and there is some calcium phosphate. SAED exhibits the typical pattern for tremolite/actinolite type asbestos. This further demonstrates that the much of the calcium was from the fiber and not the surrounding calcium phosphate. The SAED in 5C confirms that it is tremolite.

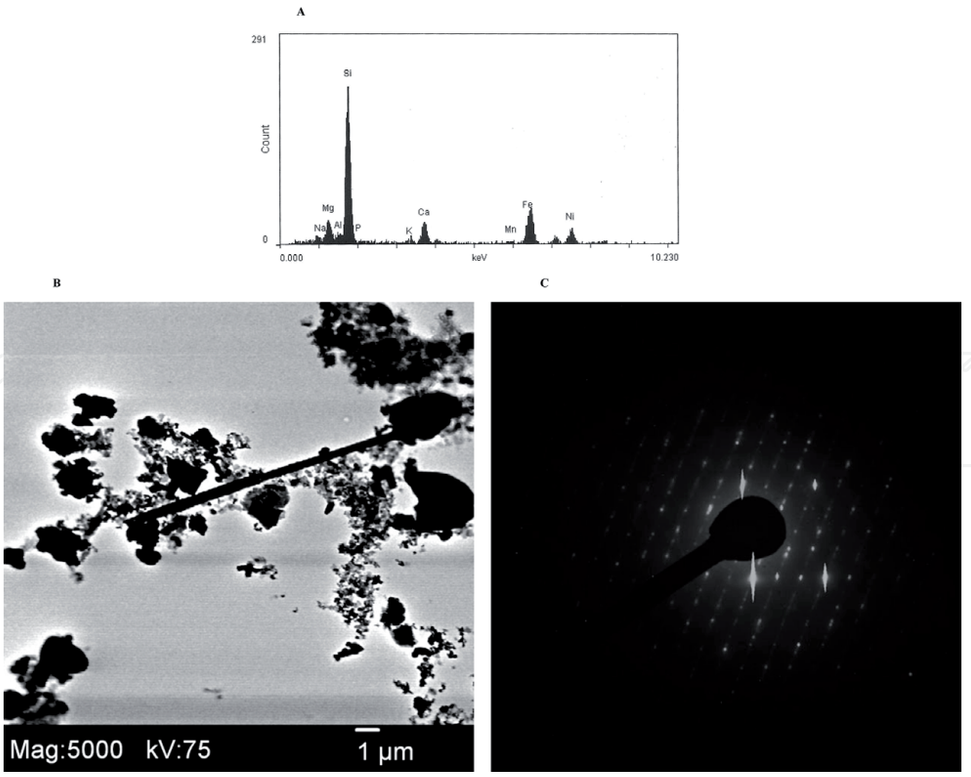

Figure 6.
This EDS spectrum (A) represents the asbestos fiber seen in (B). This EDS appears to represent a tremolite/actinolite fiber. There is a little added Fe. However this is an anthophyllite asbestos fiber based on the SAED. There is interfering Ca particles, in this case it was not associated with phosphate. The SAED in 6C confirms that it is anthophyllite.

One other issue that must be addressed is that of cleavage fragments in human tissue. The entire concept of determining if a fiber is asbestos or a cleavage fragments by mineralogists is defined by whether it is asbestiform or not. By their definition asbestiform relates to the way that the manner in which the crystals initial formed. They would refer to it as asbestos only if it was formed in an asbestiform habit, meaning that all the fibers were completely linear and just seen together as a bundle very much like a thick telephone wire. Any other type of arrangement would be considered a cleavage fragment as it may separate from the larger mass. If that larger mass was not asbestiform and it was possible to see a structure that resembled a fiber with parallel sides, the mineralogist would call it a cleavage fragment based on knowing that none of the particles in the larger population were linear fiber types, as they refer to them as asbestiform. It has been shown that the great majority of fibers that may be considered to be cleavage fragments are generally very short with very small aspect ratios. They most often look like chunks rather than fibers and are also generally thicker than fibers seen as asbestiform asbestos fibers. There have been papers published that indicate that if the fiber has a 20:1 ratio then it is asbestos [30]. There are also papers that indicate that a ratio of 8:1, they are asbestos [33]. Possibly the best criteria is when a fiber has a minimum aspect ratio of 5:1 and the width is 0.25 µm or less it is definitively asbestos [34]. The definition of asbestiform for a pathologist or appropriate testing laboratory or someone looking for asbestos fibers in tissue is purely based on the criteria of having a length to width ratio of 3:1 or 5:1 with parallel sides. In the absence of a population of fibers and the mineralogical identifiable non-asbestiform mineral there are no reliable criteria at the light or electron microscopic level to call it an asbestos fiber or a cleavage fragment other than those relating to size and shape described above.

There are two basic reasons that the above criteria for fibers are not considered cleavage fragments, but asbestos fibers, if from human tissue. The first of which is when such a fiber is analyzed from a human tissues preparation and there are only a few fibers it is impossible to identify it as from an asbestiform habit of growth. Therefore, all governmental organizations only refer to the criteria of aspect ratio and parallel sides. Further, it has been determined that the fibers identified as asbestos, the size, shape and type of fiber is critical in attributing it to causation. The longer and thinner fibers have been most commonly attributed to tumor and asbestosis development. Another criteria is that the charge distribution on the surface of fibers, asbestos by mineralogy definition or cleavage fragments are not significantly different from asbestiform fibers defined by mineralogist's criteria and will have the same oxidative effects which indirectly cause genetic or DNA mutations or elicit chemokines or cytokines resulting fibrosis, asbestosis. Lastly, fibers identified as asbestos found in human tissue analyses have been attributed to and correlated with the history of exposure and the above diseases in tens of thousands of cases over the last 60 years. This is without determining if it is a cleavage fragment or not.

9. Background controls

Background controls are imperative when performing asbestos fiber burden analyses on human tissues. It is extremely important because without them there is no criteria for comparison to assess whether what a scientist or technician is finding has any relevance with regard to exposure history and what remains in the tissue depending on latency or how long it has been since the patients' exposure(s).

To attribute a patient as a background exposure, it is imperative that a complete patient history must be taken by a skilled doctor or industrial hygienist so that it can be determined that the patient had absolutely no exposure to asbestos. That means that the patient did not mine or mill asbestos, did not work with a product containing asbestos or did not use a product that may have been contaminated with asbestos. In many of the "background controls" used by other investigators that perform asbestos fiber burden analyses, the history taken usually only states that the patient did not work with an asbestos product. In one case it was documented that the patients did not work with the products but came from an area of the country where there was significant asbestos product manufacturing. That alone should have excluded that population. So when one explores the literature and finds that there is a group of patients exposed to asbestos products and developing disease are being compared to a population where only occupation is the only excluding factor, that is not adequate criteria for calling it a background control. This is referred to as a cohort comparison. One last criterion is that if a patient or the patient tissues are analyzed as background controls and they exhibit either crocidolite or amosite, they should be immediately eliminated as background controls. The reason for them not being considered as background controls is that these fiber types are commercial forms of asbestos that are not found in this country. Therefore, it has to be assumed that the asbestos was from a product containing that type of asbestos and was exposed.

In this author's laboratory, the patients, or the tissues were very critically screened for potential exposure history by very skilled pulmonologists that were trained and worked in coordination with our Environmental Sciences Department. Over the last 35 years this authors laboratory has analyzed tissues from lungs of

207 patients used as background controls. It was only in the initial 25 patients that 3 exposed patients actually slipped through. However, based on finding 1 patient with one amosite fiber, 1 patient with one crocidolite fiber and 1 patient with high concentrations of long chrysotile fibers were the only ones that ultimately proved after extensive further questioning of the family, it determined that these three patients were in fact exposed.

Another criterion to be considered is the timing of background controls, when they were taken compared to the patient that is being analyzed. It has become very apparent that the numbers of asbestos fibers that are being found in patients both exposed and those of background controls have been declining over the years. The phenomenon is the result of the outlawing of most uses of asbestos. Therefore, workers are no longer exposed to asbestos and asbestos products and only those that had been in the past will present with asbestos in their tissues. Another criterion to consider is that over time even the commercial amphiboles will be decreased due to dissolution in the body and removal from the primary site of entrance, presumably the lung. It is a well-known and documented fact that chrysotile has a relatively short half-life in human tissue as compared to amphiboles and therefore, even high exposures of chrysotile, may not be detected in an asbestos fiber burden analysis many years later. It should, however, be noted that chrysotile fibers are not totally removed from the lungs in weeks or months making them relatively non-toxic. Only very long thick fibers are removed from the lung in this period of time. Chrysotile fibers as long as a few hundred micrometers in length can reach the periphery of the lung and once there can be present for years before they are broken down and transported out of the lung or to other tissues. One of the most common hallmarks of a chrysotile exposure is the residual tremolite that one finds in an analysis. Tremolite is a known contaminate of chrysotile that is an amphibole and therefore is more resistant to rapid breakdown and removal. Tremolite tends to be shorter in length and is frequently taken up by macrophages and moves with the smaller broken down chrysotile as compared to the commercial amosite and crocidolite type asbestos. These factors all apply to the background population. Over the 35 plus years of

Current levels of asbestos fiber burden observed in digests of lung tissue from our autopsy and surgical population with no history of asbestos exposure. All fibers regardless of size are counted.
[#]Chrysotile type asbestos: Range 0–30,000 fibers/gram wet weight lung
Mean 857 fibers/gram wet weight lung
[*]Amphiboles type asbestos: Range 0–345 fibers/gram wet weight lung
Mean 10 fibers/gram wet weight lung
[+,#]Chrysotile & Amphibole: Range 0–690 fibers/gram wet weight lung
Mean 20 fibers/gram wet weight lung
[**]Asbestos bodies: Range 0–1 bodies/gram wet weight lung
Mean <1 body per gram wet weight lung.

[*]*Amphiboles include: tremolite.*

[**]*Asbestos bodies counted by light microscopy of cytocentrifuge preparations. Levels are too low to be detected by electron microscopy.*

[+]*The combination of chrysotile and amphibole fiber burdens represent only cases from the 35 case pool studied where both types of fibers were seen together.*

[#]*100% of the fibers counted were less than 5 μm in length and 100% of those fibers were less than 1 μm in length.*
[@]*All amphiboles fibers were tremolite.*

Table 2.
This table illustrates the range, means and types of asbestos found in the lungs of patients that have had absolutely no exposure to asbestos except for the air they breathe in the New York metropolitan area.

looking at tissue analyses and background controls, it is clear that the amount of background seen is also decreasing. It was once believed that individuals just breathing the air in New York City or for any other city in the world, people would have millions of asbestos fibers in their lungs. This author does not believe that it is true any longer. Based on the most current study group of background controls, it has been determined that no matter how sensitive the testing is done, the great majority of individuals do not exhibited any asbestos in their lungs. The few that have been shown to have asbestos, is restricted to finding very short, less than 1 μm in length, chrysotile fibrils and similarly sized tremolite and nothing else. The results of the analyses of 35 patients meeting all the criteria mentioned above as background controls are shown in **Tables 2–4** for the tissues commonly analyzed in the laboratory.

From a techniques point of view, it is imperative that the analyses of the patient are done with the same degree of sensitivity as the background controls.

Current levels of asbestos fiber burden observed in digests of paratracheal and parabronchial lymph node tissue from our autopsy and surgical population with no history of asbestos exposure.
[#]Chrysotile type asbestos: Range 0–690 fibers/gram wet weight lymph node.
Mean fibers/gram wet weight lymph node.
[*,@]Amphiboles type asbestos: Range 0–690 fibers/gram wet weight lung
Mean 20 fibers/gram wet weight lymph node.
[+,#,@]Chrysotile & Amphibole: Range 0–1380 fibers/gram wet weight lung
Mean 39 fibers/gram wet weight lymph node.
[**]Asbestos bodies: Range 0–1 bodies/gram wet weight lymph node
Mean <1 body per gram wet weight lymph node.

[*]*Amphiboles include: tremolite*

[**]*Asbestos bodies counted by light microscopy of cytocentrifuge preparations. Levels are too low to be detected by electron microscopy.*

[+]*The combination of chrysotile and amphibole fiber burdens represent only cases from the 35 case pool studied where both types of fibers were seen together.*

[#]*100% of the fibers counted were less than 5 μm in length and 100% of those fibers were less than 1 μm in length.*
[@]*All amphiboles fibers were tremolite.*

Table 3.
This table illustrates the range, means and types of asbestos found in the paratracheal and parabronchial lymph nodes of patients that have had absolutely no exposure to asbestos except for the air they breathe in the New York metropolitan area.

Current levels, 2009–present, of asbestos fiber burden observed in digests of 15 abdominal organs and tissues from our autopsy and surgical population with no history of asbestos exposure. All fibers regardless of size are counted.
Chrysotile type asbestos: Range 0 fibers/gram wet weight abdominal organs and tissues
Mean 0 fibers/gram wet weight abdominal organs and tissues.
[*,@]Amphiboles type asbestos: Range 0 fibers/gram wet weight abdominal organs and tissues
Mean 0 fibers/gram wet weight abdominal organs and tissues.
Chrysotile & Amphibole: Range 0 fibers/gram wet weight lung
Mean 0 fibers/gram wet weight abdominal organs and tissues.
[**]Asbestos bodies: Range 0 bodies/gram wet weight abdominal organs and tissues.
Mean <1 body per gram wet weight abdominal organs and tissues.

[*]*Amphiboles could include: tremolite or anthophyllite.*
[**]*Asbestos bodies counted by light microscopy of cytocentrifuge preparations. Levels are too low to be detected by electron microscopy.*

Table 4.
This table shows that in patients with no history to asbestos or talc exposure there was no evidence of asbestos in the abdominal organs including any gynecological organs as the ovaries, uterus, fallopian tubes and cervix.

10. Summary and conclusions

Based on what has been presented above shows that it is clear that there are many possible methods for looking at talcum powders for contaminating asbestos and human tissue for the presence of asbestos, talc and talc contaminants such as aluminum silicates and silica. The difference between these techniques and methods are their sensitivity. The ability to identify these structures go from the least sensitive light microscopic methods using XRD, PLM or PCM to SEM with EDS and then to the most sensitive using a TEM and employing all the analytic methods of EDS and SAED. Sensitivity based on this equipment is based solely on the ability for the instruments to resolve the structures. In most, if not all these methods of looking at the material, sensitivity relies on how one prepares the specimen and how much of the specimen one examines. Therefore, when looking for small fibers or particles that contaminate the talcum powder or the human tissue it is a must, especially when not seen by less sensitive techniques as light microscopy, that the samples have to be examined with an analytic TEM, ATEM and an adequate amount has to be viewed to insure that if the contamination is low or very low, it can still be detected. A perfect comparison is the testing for drugs in blood. If one employs the least sensitive instrument and looking at a relatively tiny sample of blood, small amounts of drugs will not be detected and patients or the addict will not be considered positive when in fact they had taken drugs. Therefore, to identify contaminates in cosmetic talcum powder that will cause disease in humans, one must not only employ the proper instrumentation but also analyze an adequate amount of the talcum powder or human tissue preparation.

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