

Mesothelioma in an Individual Following Exposure to Crocidolite-containing Gaskets as a Teenager

RONALD FRANKLIN DODSON, SAMUEL P. HAMMAR, LEE W. POYE

Mesothelioma is considered a signal tumor for asbestos exposure and typically occurs decades after first exposure to asbestos. Tissue analysis often indicates past exposure to mixed types of asbestos. This report describes the case of a 58-year-old man who developed mesothelioma after reported exposure to crocidolite from asbestos-containing gaskets beginning at age 16 during three summers during high school and for approximately four hours per day during the last semester of his senior year. He had no further known exposure to asbestos. Analytical transmission electron microscopy analysis of digested tissue samples revealed elevated levels of crocidolite asbestos fibers and the presence of crocidolite cored ferruginous bodies. This case is unique in that it establishes that relatively short and/or intense exposures to crocidolite asbestos traumatically released from a previously classified Category 1 nonfriable asbestos containing material (NESHAP) was confirmed via tissue burden analysis years following the historically defined exposures. *Key words:* mesothelioma; asbestos; crocidolite asbestos; analytical transmission electron microscopy (ATEM)

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INTRODUCTION

Asbestos has been used in over 3,000 individual products primarily due to its unique properties such as increasing tensile strength and fire resistance; resistance to strong chemicals was used in applications including insulating products and as components of

friction products.¹⁻³ For clarification the term “asbestos” as used in this publication refers to the six elongated mineral structures conforming to definitions used in regulatory guidance documents by OSHA⁴ and AHERA.⁵

According to Wagner and Pooley³ “the world production of asbestos in 1976 was 5,000 million kg., of which 97% was chrysotile and the remainder was crocidolite and amosite.” Furthermore, Wagner and Pooley indicated that the unique properties of crocidolite included “its resistance to acids and sea water.”³

Despite its useful properties, asbestos is a dangerous substance in that it induces fibrotic conditions in the lung and pleura and two major cancers; namely, mesothelioma and lung cancer. A recent review concluded specifically that there was evidence following exposure to asbestos “to infer causal relationship for laryngeal cancer; to be suggestive for pharyngeal, stomach and colorectal cancers; and to be inadequate for esophageal cancer.”⁶ Additionally, the International Agency for Research on Cancer (IARC) Monograph Working Group comprised of 27 scientists from eight countries determined that “there is sufficient evidence in humans” to indicate asbestos is also a causal agent for laryngeal and ovarian cancer.⁷

All asbestos-related diseases are considered to be dose-response related with increased risk and increased incidence of these diseases occurring in relationship to increasing asbestos exposure. It should be appreciated, however, that the risk for developing an asbestos-related disease is also based in part on individual levels of susceptibility for the given levels of past exposure.

Latencies for various asbestos-related diseases vary considerably and there is no single latency period for any specific asbestos-induced disease. For mesothelioma, there is a wide range of latencies and, as reported by Lanphear and Buncher,⁸ mesothelioma is considered to have one of the longest latency periods of the asbestos-induced diseases.

The medical world’s recognition of the association between asbestos and mesothelioma was based primarily on the report by Wagner, Sleggs, and Marchand in 1960,⁹ where they reported 33 cases of mesothelioma in

Received from: The patient whose case is reported here was referred to the authors by his attorneys, who paid for services rendered. Send correspondence to Ronald Dodson; email:<ron@eri-consulting.com>.

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the Northwestern Cape Providence of South Africa. This was an area in which crocidolite asbestos was mined and milled, and it was thought that 32 of the 33 cases that developed mesothelioma had been exposed to crocidolite asbestos. This report demonstrated that not only did the miners and persons directly handling asbestos develop mesothelioma, but also other individuals who were in the vicinity of the asbestos. The report by Wagner, Sleggs, and Marchand⁹ was also significant for citing two cases of mesothelioma reported in Quebec miners in 1952, and an additional 11 cases of mesothelioma reported in 1958, by McCaughey et al.¹⁰

Epidemiologic (statistical) evidence that asbestos causes mesothelioma was reported in the early 1960s by Selikoff and colleagues.^{11,12} Selikoff et al.¹¹ studied a group of insulators who were members of the Heat and Frost Insulation Union and showed that about 10% of these individuals exposed to asbestos died from mesothelioma. The exposure of these individuals was primarily to chrysotile and amosite asbestos, although crocidolite was also one of the types of asbestos to which they were exposed.

Over time, it has become apparent that all types of asbestos cause mesothelioma.¹³ There is evidence based on some characteristics that the amphiboles are more tumorigenic on a fiber-for-fiber basis (given equal dimensions) in causing mesothelioma than is chrysotile asbestos. However, there is significant evidence that chrysotile causes mesothelioma.¹⁴ Nevertheless, there are those who consider chrysotile limited in potential for inducing mesothelioma—with some going as far as to offer the opinion that it is “safe”¹⁵ and, thus, of little or no concern as a causative agent for mesothelioma. For example, in a recent review of the epidemiology of malignant mesothelioma, McDonald¹⁶ indicated that “a survey in Canada in 1980–1999 and other surveys demonstrated that crocidolite, amosite, and tremolite could explain almost all cases of mesothelioma.” The concentration of asbestos necessary to cause mesothelioma is unknown for any individual person. There are many examples of low-level exposure to asbestos and the development of mesothelioma.^{17–20} As stated by Peto and associates in 1999: “The great majorities of mesotheliomas are caused by asbestos and that a country’s mesothelioma rate is therefore a quantitative indicator of its population’s past exposure—mainly occupational—to asbestos.”²¹

The following case of mesothelioma involves an occupationally defined exposure to material classified as Category 1 nonfriable asbestos-containing material (NESHAP-40 CFR Subpart M) that was traumatized during preparation of the gasket material (made friable) resulting in the release of respirable asbestos dust. The crocidolite type of asbestos, which was a component of some of the gasket material (with other gasket material being neoprene, cork, gum rubber and some potentially containing chrysotile asbestos), was

being prepared by the individual. Experts agree that crocidolite is a highly carcinogenic form of asbestos.

CASE HISTORY

A 58-year-old male nonsmoker presented for medical evaluation because of shortness of breath. The individual was a college graduate and had worked in computer support and information technology following his graduation. The only contact he had with asbestos-containing materials consisted of work he had done starting at the age of 16 in an industrial-specialty company for three summers and for a period from January to June in his senior year of high school when he worked from 1:00 to 5:00 PM within the same facility. An appreciable amount of the prepared gaskets sold by the specialty company were to petrochemical industries.

The individual’s role in the facility (stated as 90% of the time) was to cut gaskets from sheet rolls using a “jigsaw-like hand tool” with shear-like blades. He would often cut the gaskets while on his hands and knees and described appreciable dust being generated in the process of cutting the material. When necessary, he would put the gaskets on a die and “press out” the suitable pattern. He stated 60% of his time was spent cutting dark gray gaskets, which were identified as crocidolite-containing gaskets. He also cut gaskets described as those made of neoprene, cork, and gum rubber. He recalled working with sheet gaskets that were crocidolite-containing and possibly some gaskets that contained chrysotile asbestos. He did occasional cleanup work; including sweeping the facility and cleaning scrap bins. He stated that orders placed with the company would result in him cutting several hundred gaskets per day.

The clinical evaluation by a pulmonologist in response to the patient’s complaint of shortness of breath included an evaluation of a chest radiograph. The chest radiograph revealed a right pleural effusion involving 2/3 of the right chest cavity. Thoracentesis was performed and the pleural fluid contained benign mesothelial cells. Approximately two weeks later, thoracoscopy was performed and the pleural tissue was biopsied and showed a malignant epithelial mesothelioma based on histologic and immunohistochemical evaluation. Seven days later a CT scan revealed calcified parietal pleural plaque in the right lower hemithorax. The individual was referred to a specialized surgical center where a right extrapleural pneumonectomy was performed. Tissue samples confirmed a right pleural epithelial mesothelioma, which was diagnosed 40+ years from first exposure to asbestos.

Tissue blocks were prepared for pathological evaluation and subsequently referred to RFD with a request to determine the presence and numbers of ferruginous bodies and uncoated asbestos fibers. Previous evaluation of tissue sections by several pathologists had not

identified ferruginous bodies in sections of lung tissue. Thus, a pathological definition of “asbestosis”¹ often considered as reflective of a heavier exposure related disease could not be made from evaluation of tissue sections.

METHODS AND MATERIALS

The materials submitted to the laboratory of RFD consisted of five blocks and 21 slides. The material was sent by an attorney representing the individual discussed in this case report and his family. The request was for an analysis to be conducted for determination of the numbers of ferruginous bodies and the numbers and types of asbestos fibers in the tissue. The clinical data received with the authorization indicated the individual suffered from mesothelioma but there was no indication as to the potential sources or types of asbestos exposure(s). Approximately one-half of the more normal-appearing lung parenchyma from three blocks was removed. As much paraffin as possible was cut from the edge of the block face adjacent to the tissue after which the tissue underwent deparaffinization. The procedure for deparaffinization included melting the remaining paraffin followed by putting the lung tissue through six changes of xylene and six changes of ethanol. The deparaffinized weight of the lung tissue samples was 0.6398 gm deparaffinized wet weight. The tissue samples were then subjected to a modified bleach digestion procedure.²²

The digestate was collected on either 0.22 μ m pored mixed cellulose ester (MCE) filters for the analysis by light microscopy for the presence of ferruginous bodies or on 0.2 μ m polycarbonate filters and prepared for analysis as to the presence of uncoated asbestos fibers and ferruginous bodies by ATEM. The wedges of the MCE filters were mounted on a glass slide, cleared by acetone vapor and scanned by light microscopy at 200–400x magnification. A structure that conformed to the appearance of an asbestos-cored ferruginous body at the light microscopy level was noted as a classical ferruginous body.

Strips of the carbon-coated polycarbonate filters were mounted on 100-mesh copper grids and the filter matrix was dissolved using a modified Jaffe wick procedure using chloroform. This resulted in a carbon extraction replica that contained the entrapped fibers, ferruginous bodies, and other particulates. The analysis of the grids was conducted in a JEOL 1200EX transmission electron microscope. The fibers or cores of ferruginous bodies were analyzed as to crystalline characterization by selected area diffraction (as necessary for establishing an amphibole, serpentine, or other pattern). The elemental composition of the structures was determined by an IXRF Systems: Kevex pulse processor model 4461. Scans in the ATEM were made of 30 random grid squares on three grids at

15,000 \times with counts and analysis including all fibers greater than or equal to 0.5 μ m long and with an aspect ratio of greater than 5:1. The detection limit at 15,000 \times was calculated as 4,165 fibers/gm deparaffinized wet weight of tissue. A scan at lower magnification (2,000 \times) was made of an additional 60 grid squares in an attempt to find ferruginous bodies. The cores of ferruginous bodies found in the low magnification scan were evaluated as were any uncoated fibers (> 3 μ m in length).

Laboratory background evaluations were conducted for comparative assessment as quality controls within the laboratories confirming the “cleanliness” of the filters and solutions used in the process thus verifying that no asbestos was contributed from these sources. At the time the tissue samples were submitted to RFD for quantitative analysis of tissue burden, there was no information provided as to the history of either the source of the presumed exposure or data regarding potential extent of exposure to a specific asbestos containing product.

The request for permission to submit this unusual case for publication was granted by the attorney representing the patient and his family. Permission to publish was also granted directly to one of the authors (RFD) by the individual who is the subject of this report.

RESULTS

Ferruginous bodies (FB) were found in the scan of the prepared MCE filter by light microscopy. These structures were consistent with the appearance at the light microscopy level of asbestos-cored ferruginous bodies in that they were formed on an elongated core, the core was clear, and the surface consisted of deposits of beaded rust-colored material (iron). The 46 ferruginous bodies found in the area scanned were equivalent to 934 ferruginous bodies per gram of deparaffinized wet weight of tissue.

The ATEM analysis conducted at 15,000x identified 16 crocidolite asbestos fibers. These fibers were equivalent to 66,644 fibers per gram of deparaffinized wet weight of tissue. The crocidolite fibers ranged from 2.5 to 16 μ m in length (average length 7.1 μ m). No other types of asbestos bodies or fibers were detected in the areas scanned at 15,000 \times . The additional scanned areas reviewed in the lower magnification scan (2,000 \times) identified one crocidolite-cored ferruginous body (10 μ m in length) and four additional uncoated crocidolite fibers (ranging from 6 μ m to 21 μ m in length).

DISCUSSION

The lack of identification of ferruginous bodies in tissue sections by several pathologists reflects the lim-

ited sensitivity for finding ferruginous bodies in tissue sections.²³ The more sensitive method for assessing tissue content for the presence of ferruginous bodies and uncoated asbestos fibers is by tissue digestion as described in this report.²⁴

The numbers of ferruginous bodies per gram as determined by light microscopy from digested tissue in this case are appreciably higher than the number used in two of our laboratories (RFD, SPH) for defining levels found in lung tissue from the general population (≤ 20 FB/gm wet weight of tissue).²⁴ Most ferruginous bodies (with the exception of some unusual exposures to longer serpentine fibers) are formed on amphibole cores of asbestos and generally on one of the commercial types (amosite or crocidolite) of asbestos.^{25,26} In this case, the commercial amphibole- crocidolite was confirmed by ATEM as the core material of a ferruginous body.

The material from which the individual indicated his asbestos exposure occurred was to sheet gasket material, which was described by color, application (petrochemical), and specification sheets as crocidolite-containing. Crocidolite is recognized for its resistance to strong chemicals, including acids.³ The confirmation as to the composition of some types of gasket materials was made by a further clarification from the specialty company for which he had worked and from the company that supplied the gaskets in question. The possibility the individual had cut chrysotile-containing gaskets existed but, within the limit of detection of the analysis, no chrysotile was found. The absence of chrysotile within the limits of detection used in the study may be attributable to: the limited amount of exposure to this type of asbestos; or explained by the opinion that chrysotile is inhaled on average as a shorter-length fiber than amphiboles and can, thus, be more readily cleared from, or dissolves rapidly, in lung tissue over time and has, therefore, been "eliminated" by the time the tissue was sampled.²⁴ However it should be noted that a counter to the argument in the second point is that chrysotile (including short fibers of chrysotile) have been found in lung tissue years after last occupational exposure.²⁵ Detection of short/long thin chrysotile and thinner amphiboles does require higher magnification evaluation by ATEM as per the requirement of the use of this instrument for certain clearance projects under AHERA Regulations. An extensive discussion of the detection limits for light microscopy, scanning electron microscopy, and analytical transmission electron microscopy, is provided in the HEI-Asbestos in Public Buildings Document.²⁷ The limits for detection of fibrous particulates (including asbestos) as inherent in the light microscope and scanning electron microscope are based on the lower limits for detection of such particulates based on diameter not length. Thus, lower magnification by even ATEM would not be expected to detect short-thin and/or

long- thin fibrous structures if such fibers are very thin such as those often found as the majority component in tissue burden. The present tissue evaluation was conducted at higher magnification by ATEM and included shorter fibrous structures in the count scheme thus any chrysotile within the evaluated samples would have had to have been at levels below the detection limit used in the assessment.

Gasket materials are categorized under National Emission Standards Hazardous Materials (EPA-40 CFR 61 Subpart M) as Category 1 nonfriable asbestos-containing materials (ACM). This document speaks to activities associated with demolition but has application to nondisturbed as well as disturbed asbestos-containing materials in general. This category includes "asbestos-containing packings, gaskets, resilient floor coverings, and asphalt roofing products containing more than 1 percent asbestos as determined using the method specified in appendix E, subpart E, 40 CFR part 763, section 1 Polarized Light Microscopy." This definition implies the material is presumed "encapsulated" and is assumed to be less likely to emit fibers than friable materials containing asbestos. However, not to be overlooked is the further clarification in NESHAP that "Regulated asbestos-containing material (RACM) means (c) Category 1 nonfriable ACM that will be or has been subjected to sanding, grinding, cutting, or abrading." Logically, this point recognizes that appreciable fibers can be released from a previously considered "nonfriable" asbestos-containing material when subjected to actions such as performed by the subject of this case report.

A position statement that all asbestos types are linked to all asbestos-related diseases was once again reiterated by an editorial that appeared in *Lancet*.²⁸ There still remain questions regarding the varying degrees of risk that different types of asbestos carry for producing various diseases. The mechanisms for inducing diseases include the simple physical factor that the material is a fibrous dust inhaled in various lengths to the more complex aspects involving cellular/biochemical reactions by which irreversible cellular damage can result in development of an array of pathological events, including development of tumors.^{29,30} Some measurements of cell damage may favor more likely changes being induced by one asbestos type in one form of testing while another evaluation using a different model may give differing levels of pathological endpoints between the forms.

Published information has led to the introduction by some of a causation theory of asbestos-related diseases designated as the "amphibole hypothesis."¹⁵ The advocates of the amphibole hypothesis have considered crocidolite to be the most toxic asbestos type in causing mesothelioma.¹⁵ The link between exposure to crocidolite and the risk of developing mesothelioma as described in this case report was well established in the

report in South Africa by Wagner, Sleggs, and Marchand⁹ and Wagner and Pooley.³

The lung tissue from the majority of mesothelioma cases evaluated in our laboratory (RFD) contained a mixture of asbestos types.^{25,26} The lung tissue analyzed in this patient contained (within the limits of detection) only crocidolite asbestos. Subsequent documents received from the patient's historical file indicated he was occupationally exposed to a traumatized crocidolite-containing material while a teenager that was corroborated by findings obtained from analysis of the patient's lung tissue by ATEM years after his exposure to crocidolite asbestos.

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