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Characterization of minerals in pleural plaques from lung tissue of non-human primates

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Abstract

To examine the hypothesis that secondary minerals precipitate in the lung after the inhalation of fibrous minerals, pleural plaques from 11 non-human primates (NHP) were examined using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), and powder X-ray diffraction (XRD). Two NHPs served as controls, and nine were exposed to either low (1 f/cc) or high (1000 f/cc) levels of chrysotile, asbestiform grunerite (amosite), asbestiform riebeckite (crocidolite), or glass fibers. XRD analysis revealed apatite in pleural plaques of six NHPs, and SEM-EDS analysis found small quantities of apatite in three additional NHPs. XRD analysis identified calcite in one control NHP and one exposed to chrysotile (with talc present) and in the NHPs exposed to high doses of glass fibers, chrysotile, and grunerite. XRD analysis detected 2:1 expandable clays in NHPs exposed to high levels of glass fibers, chrysotile, grunerite and riebeckite. XRD also detected amphiboles in NHPs exposed to high levels of grunerite and riebeckite. SEM-EDS analysis revealed Mg-rich silicates in a control NHP and one NHP exposed to low doses of chrysotile. Asbestos bodies coating fibers were detected in NHPs exposed to high levels of grunerite and chrysotile. Iron-rich silicate fibers without coatings were identified in the NHP exposed to high levels of riebeckite using SEM-EDS. SEM-EDS also revealed Na-rich silicates in one NHP exposed to low doses of chrysotile. Because minerals other than exposure minerals were detected in many of the NHPs, the hypothesis that secondary minerals may form in the lung after exposure to fibrous minerals should be considered when addressing the health effects of minerals.

Key words: asbestos; in vivo; SEM; powder X-ray diffraction; pleural plaques.

Introduction

Inhalation of asbestiform minerals in both the occupational and environmental settings may cause increased risk of respiratory diseases (Hodgson and Darnton, 2000; McDonald and McDonald, 1997; Ross and Nolan, 2003; Sullivan, 2007), and research is exploring the possibility of respiratory disease caused by environmental exposure to asbestiform amphiboles (Gunter et al., 2007, and references therein). The respiratory diseases that can occur from inhaling asbestiform minerals range from asbestosis to mesothelioma, a rare form of cancer first identified as a cluster among individuals exposed to asbestiform riebeckite in South Africa (Wagner et al., 1960). Another physiological result from asbestos inhalation is the formation of pleural plaques first described among talc workers (see Porro et al., 1942). Pleural plaques were not associated with asbestos until the 1950s (Jacob and Bohlig, 1955), and today their presence in the lung is considered a biomarker of asbestiform mineral exposure (Christen et al., 1997; Clarke et al., 2006).

Pleural plaques are dense collections of collagen fibers and are usually located on the parietal pleura, which is a thin layer of membrane that covers the interior of the thoracic wall (Clarke et al., 2006) and on the diaphragm (Christen et al., 1997). Their sizes range from a few millimeters to several centimeters in diameter (Christen et al., 1997). Sometimes these collagen fibers become calcified, and during the process of calcification (which is actually the precipitation of apatite, $Ca_5(PO_4)_3(OH)$), inhaled particles may become trapped in plaques. For this reason, pleural plaques from a previous inhalation study using NHPs exposed to fibrous materials were chosen for this study.

Identification and quantification of minerals in the lung are useful for understanding the in situ behavior of minerals and the association of mineral abundance or type with disease. Lung burden studies have quantified the presence of uncoated asbestiform minerals as well as coated asbestiform minerals (asbestos bodies) in the lung (e.g., Wagner et al., 1986; Stephens et al., 1987; Wagner et al., 1982; Gibbs et al., 1994). These studies focused on the fiber morphology, type, and abundance, and on the presence of asbestos bodies. To our knowledge, the mineral composition of pleural plaques has largely been ignored. Comprehensive mineralogical characterization of pleural plaques is important because geochemical modeling has shown that secondary minerals (including the apatite that forms through calcification of pleural plaques) may precipitate from primary minerals inhaled into the lung (Taunton et al., 2010; Wood et al., 2006). The goal of this study is to use scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDS), and powder X-ray diffraction (XRD) to characterize the types of minerals found in calcified pleural plaques in NHPs involved in an inhalation study. We aim to correlate the minerals in the lung with the inhaled material to which the NHPs were exposed as well as to the minerals predicted to form from the starting material.

Methods

The authors received pleural plaque samples from eleven NHPs that were previously used in an inhalation study of fibrous materials (Hiroshima et al., 1993). Table 1 lists the type of fiber, the dose, and the number of days exposed for each NHP. The exposure occurred in dust rooms of what was then called the NCOH (National Centre for Occupational Health), and now known as the National Institute for Occupational Health (NIOH), which is part of the National Health Laboratory Service (NHLS) in Johannesburg, South Africa. The NHPs were not bred in captivity, but were captured on farms in what was the Northern Transvaal, now part of Limpopo and Mpumalanga Provinces. Thus their pre-capture history of exposure to environmental

Table 1. Non-Human Primate sample number, mineral of exposure, dose and number of days exposed.

Sample	Exposure Mineral	Dose	Number of exposure days
RNO6-02	Control	-	1701
RNO6-11	Control	-	1245
RNO6-03	Chrysotile	Low: 1 f/cc	1383
RNO6-04	Chrysotile	Low: 1 f/cc	1078
RNO6-10	Chrysotile	Low: 1 f/cc	1383
RNO6-08	Chrysotile	High: 1000 f/cc	280
RNO6-05	Glass Fiber	High: 1000 f/cc	378
RNO6-01	Riebeckite	Low: 1 f/cc	1441
RNO6-06	Riebeckite	Low: 1 f/cc	1441
RNO6-09	Riebeckite	High: 1000 f/cc	459
RNO6-07	Grunerite	High: 1000 f/cc	1202

dusts and fibers is unknown.

Three NHPs were exposed to low levels (i.e., 1 f/cc) of chrysotile ($Mg_3Si_2O_5(OH)_4$), and one NHP was exposed to high levels (i.e., 1000 f/cc) of chrysotile. One NHP was exposed to high levels of asbestiform grunerite [$Fe_7(Si_8O_{22})(OH)_2$; commercially called amosite]. Two NHPs were exposed to low levels of asbestiform riebeckite [$Na_2(Fe, Mg)_3Fe_2Si_8O_{22}(OH)_2$; commercially called crocidolite], and one NHP was exposed to high levels of asbestiform riebeckite. One NHP was exposed to high levels of glass fibers, and two NHPs served as controls. Four NHPs died during the study: both the controls, RN-04 exposed to low levels of chrysotile, and RN-05 exposed to high levels of glass fibers. The deaths cannot be attributed to starting material, dose, or exposure time. Therefore, the authors submit that the deaths of these animals do not affect the results of the lung mineral characterization. The remaining NHPs were sacrificed at the end of the study.

Upon autopsy, pleural plaques from lung tissue samples were fixed with formalin then digested with potassium hydroxide. This procedure removes organic material, but does not affect the inorganic material in the lung. The particulates were centrifuged, washed, and re-suspended with deionized water in 35 ml centrifuge tubes.

In preparation for XRD analysis, the suspensions were shaken then ultrasonicated. Samples were filtered onto 20mm diameter silver membrane filters as in Norton and Gunter (1999), leaving approximately one to two milliliters of each sample in the centrifuge tube to use for scanning electron microscopy. Eight-hour scans were performed on each sample with a Siemens D5000 θ-θ diffractometer equipped with a solid-state detector using CuKα radiation operating at 40 kV and 30 mA with a step size of 0.02° 2θ from 2-40°. Count times were 25 seconds per step. Detection limits for this type of method are approximately 0.05%, or better (Sanchez and Gunter, 2006).

For SEM-EDS analysis, one drop of the residual material that remained in the centrifuge tube after XRD preparation was pipetted onto

carbon tape mounted on an aluminum stub. The samples were not coated. Sample imaging and EDS analysis were performed using an AMRAY 1830 scanning electron microscope at 15kV and a working distance of 24-26 mm.

Results and Discussion

Controls

XRD patterns of control NHP samples are shown in Figure 1a, b. XRD analysis detected apatite in both control NHPs. Geochemical modeling predicted the precipitation of hydroxylapatite out of a solution similar to that of lung fluid (Taunton et al., 2010; Wood et al., 2006). Moreover, pleural plaques are not unique to asbestiform mineral exposure even though they are used as an exposure biomarker (Clarke et al., 2006). Thus, it is reasonable to find apatite in lung tissue, even though the prevalence of pleural plaques in the general population (those that are not exposed to fibrous materials) ranges from 0.02% to 12.8% (Clarke et al., 2006).

One control lung sample contained calcite ($CaCO_3$; Figure 1a) as determined by XRD; however, no calcite was found using SEM-EDS. Results from Wood et al. (2006) predicted that dolomite [$CaMg(CO_3)_2$] should be saturated in the lung, but the literature reports that calcite and dolomite actually are rare in the body (Mansfield 1980; Gault et al., 1993; Osbourne et al., 1986). One reason for this could be that calcite is predicted to dissolve in less than 1.5 years when modeled with simulated lung fluid (Taunton et al., 2010). Even so, geochemical modeling does predict the formation of calcite in reactions with

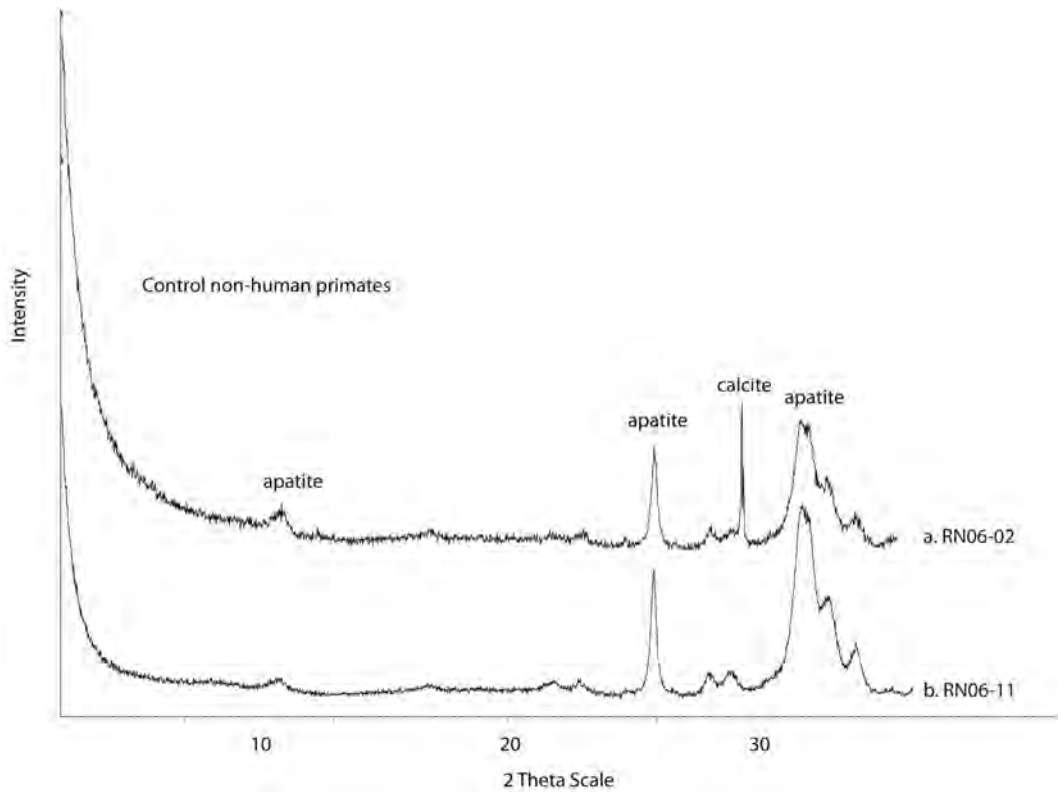

Figure 1. XRD patterns from control non-human primates keyed to sample names in Table 1.

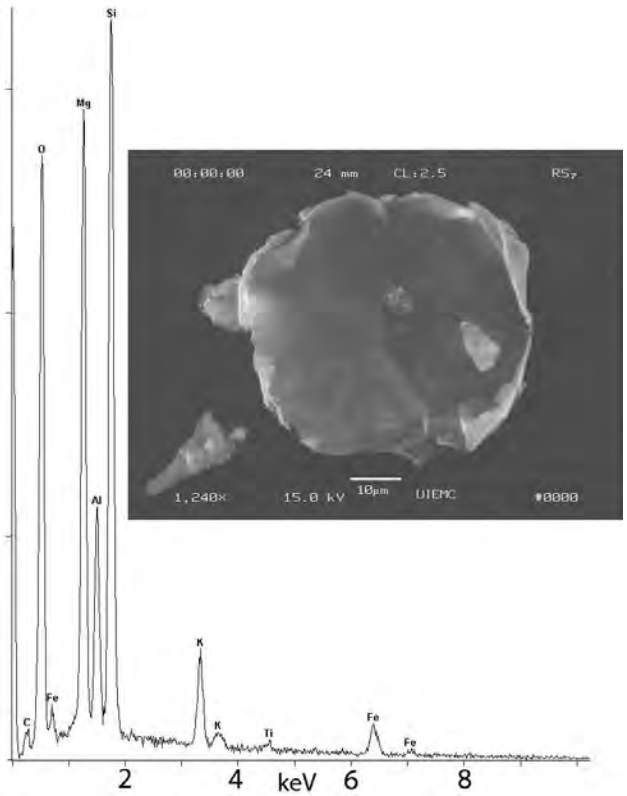

Figure 2. SEM image and EDS spectrum of Mg-rich sheet silicate in a control non-human primate.

high concentrations of chrysotile, anorthite, and talc (Taunton et al., 2010).

In the control that contained calcite, SEM-EDS detected Mg-rich sheet silicates, an example of which is shown in Figure 2. The large size of this particle dictates that it was not inhaled. Plumlee et al. (2006) reported that particles that are tens of microns in diameter are cleared through mechanisms such as coughing and increased mucus production. The mineral in Figure 2 has a diameter of approximately 60 μm, which suggests in situ mineral precipitation.

Chrysotile exposure

Figure 3a-d show XRD patterns for NHP exposed to low levels (Figure 3a-c) and high levels (Figure 3d) of chrysotile. Apatite was present in two of three lung samples of NHPs exposed to low levels of chrysotile and not present in the NHP exposed to high levels of chrysotile. Calcite was present in the lung of one low-level exposed NHP. As stated above, geochemical modeling predicted calcite formation during chrysotile dissolution (Taunton et al., 2010). Talc [$Mg_3Si_4O_{10}(OH)_2$] was identified in the NHP with high chrysotile exposure, and a 2:1 clay mineral was detected in both low and high exposure NHP. Results from Taunton et al. (2010) predicted that talc forms as a secondary phase during chrysotile dissolution

in simulated lung fluid.

SEM-EDS analysis found Mg-rich and Na-rich silicate minerals in NHPs exposed to low doses of chrysotile. These particles range from approximately 40 to 100 μm in length, which suggests that they formed inside the lung. Iron-rich particles surrounding fibers were imaged and analyzed in the sample from the high chrysotile exposure NHP (Figure 4). It is reasonable to identify these particles as asbestos bodies. Asbestos bodies are iron-protein-mucopolysaccharide coatings on asbestiform minerals. They are formed by macrophages in the lung during phagocytosis of a fiber (Suzuki and Churg, 1970). Asbestos bodies are not common with chrysotile exposure; however, they do occur (Hiroshima et al., 1993; Hiroshima and Suzuki, 1993; Pooley, 1972; Suzuki and Churg, 1970). Perhaps the absence of asbestos bodies around chrysotile relates to chrysotile's relatively fast dissolution rate (6 to 18 months for a 1 micron diameter chrysotile cylinder to dissolve; Hume and Rimstidt, 1992; Parry, 1985; Jurinski and Rimstidt, 2001) compared to amphibole mineral dissolution.

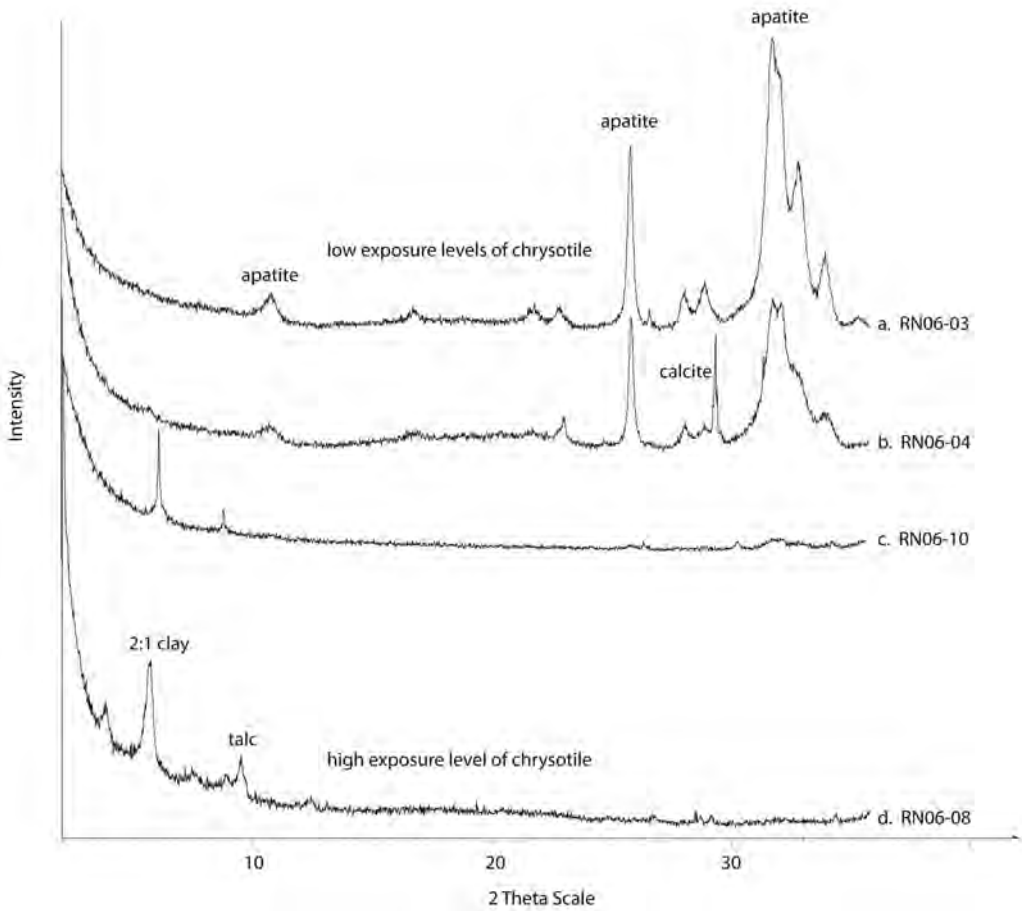

Figure 3. XRD patterns from non-human primates exposed to low and high levels of chrysotile keyed to sample names in Table 1.

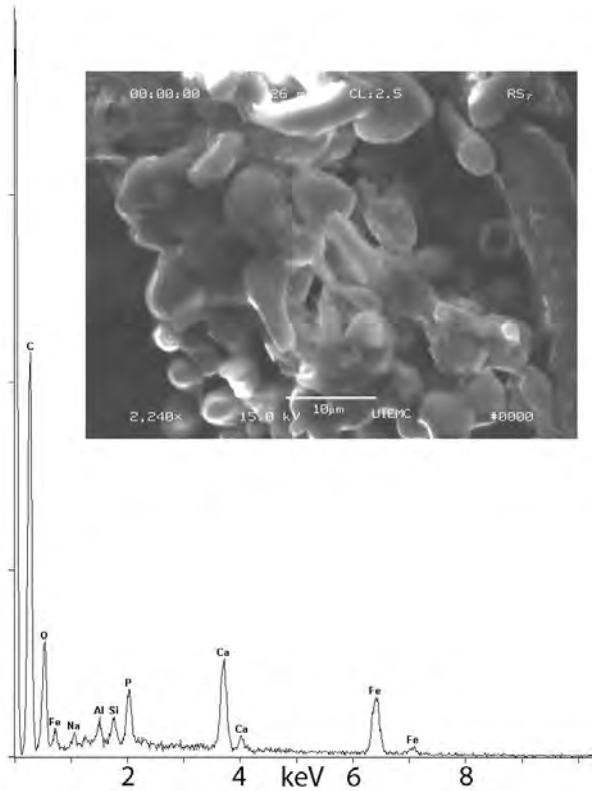

Figure 4. SEM image and EDS spectrum of Fe-rich particles surrounding fibers in a non-human primate exposed to high levels of chrysotile.

Glass fibers

Talc, and possibly rectorite [$(Na,Ca)Al_4(Si,Al)_8O_{20}(OH)_4 \cdot 2(H_2O)$], were identified by XRD in the NHP exposed to high levels of glass fibers (Figure 5). Another 2:1 clay mineral was also detected. No glass fibers or asbestos bodies were imaged, and SEM-EDS analysis was difficult due to the small amount of sample. One C-rich particle was imaged. Because the starting material composition for these fibers is unknown, it is difficult to compare these results to the literature or to the minerals in this study. Hesterberg and Hart (2000) compared synthetic vitreous fibers (man-made glass fibers) to riebeckite and grunerite dissolution and found that the rate constants for the synthetic fibers were one to three orders of magnitude faster than the amphibole fibers. Eastes and Hadley (1995) determined that 1.5 years after the last day of exposure, less than 5% of the original dose of synthetic fibers remained in the lungs of rats compared to 22% of riebeckite fibers. Both of these studies suggest that, in vivo, synthetic fibers dissolve more quickly than amphibole fibers. Also, Weir et al. (2007) could not detect volcanic glass in the sheep lungs harvested from sheep living in northern Idaho, while Norton and Gunter (1999) pointed out that volcanic glass is a major component of the respirable dust in the region. Based on these studies, one can interpret

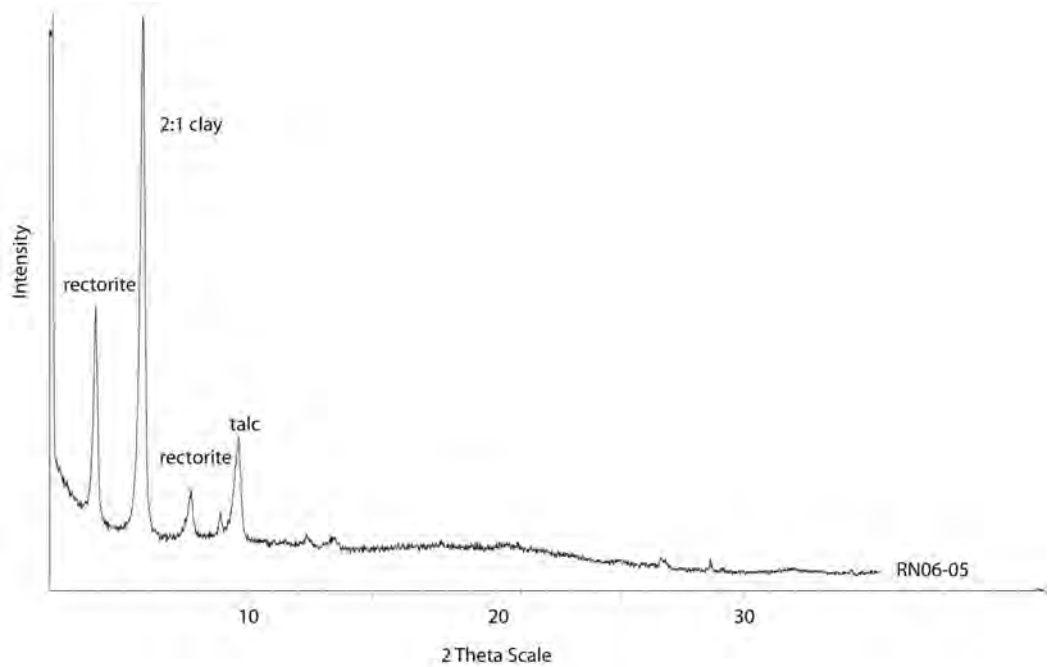

Figure 5. XRD pattern from a non-human primate exposed to high doses of glass fibers.

that the majority of glass fibers had dissolved by the time the NHP died.

Riebeckite

XRD and SEM-EDS analysis of the two NHPs exposed to low levels of asbestiform riebeckite revealed only apatite (see Figure 6a, b for XRD patterns). XRD analysis of the NHP exposed to high levels of riebeckite revealed quartz (SiO_2), an amphibole mineral (presumably riebeckite), and no apatite (Figure 6c). SEM-EDS analysis of the sample from the high riebeckite exposed NHP found Fe- and Na-rich fibers, presumably riebeckite (Figure 7a). Additionally, an Al-bearing oxide was also detected (Figure 7b).

Occupational exposure to asbestiform riebeckite gives one of the highest incidences of mesothelioma (Berry, 1999). Hodgson and Darnton (2000) report 17.8 % (5/28) mesothelioma deaths among Massachusetts workers making cigarette filters with asbestiform riebeckite. One possible explanation for this fact is that both riebeckite and grunerite contain iron. Through interaction with microorganisms and physiologic fluid in the body, the iron in these types of asbestos can form free radicals and reactive oxygen species, which ultimately cause tissue damage (Gold et al., 1997). In the present study, low doses of riebeckite only produced apatite; no riebeckite was detected using either XRD or SEM-EDS. This suggests that most of the riebeckite fibers had dissolved at the time of sacrifice and the only particles left in the lung were related to the calcification of pleural plaques (i.e., apatite), that riebeckite remained in the lung tissue and not the pleural plaques, or that the concentration of riebeckite was below the detection limits for XRD and SEM-EDS. No asbestos bodies were detected. In the high riebeckite dose sample, fibers were still present

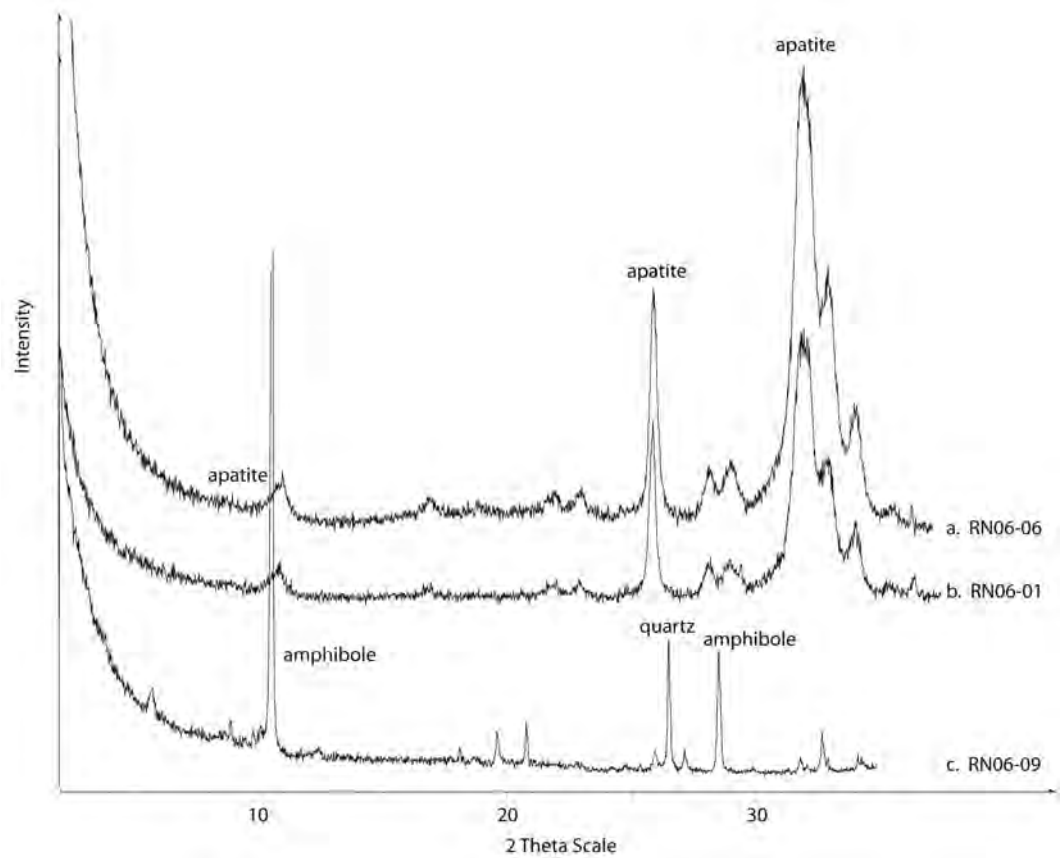

Figure 6. XRD patterns from non-human primates exposed to low and high levels of riebeckite keyed to sample names in Table 1.

and asbestos bodies were detected in the pleural plaque sample. Aluminum oxides were also present in the high dose sample, but not the low dose samples. This suggests that perhaps some of the starting material in the high dose sample had transformed into a secondary phase. Aluminosilicates have been found in the cores of senile lesions in the brain that, in part, define Alzheimer's disease (Candy et al., 1986). How these aluminosilicates form is not known, but the present study suggests they may form as secondary weathering products of foreign minerals in the lung. The cations released from mineral dissolution in the lung could be incorporated into the blood stream and transported to other places in the body where, depending on the concentrations of other ions in solution, they could precipitate into aluminosilicate or aluminum oxide minerals. Also, even though riebeckite and grunerite dissolution in the lung could not be modeled because thermodynamic and kinetic data for these minerals do not exist, results from Taunton et al. (2010) predicted the formation of aluminum oxides and aluminum silicates during feldspar dissolution in simulated lung conditions.

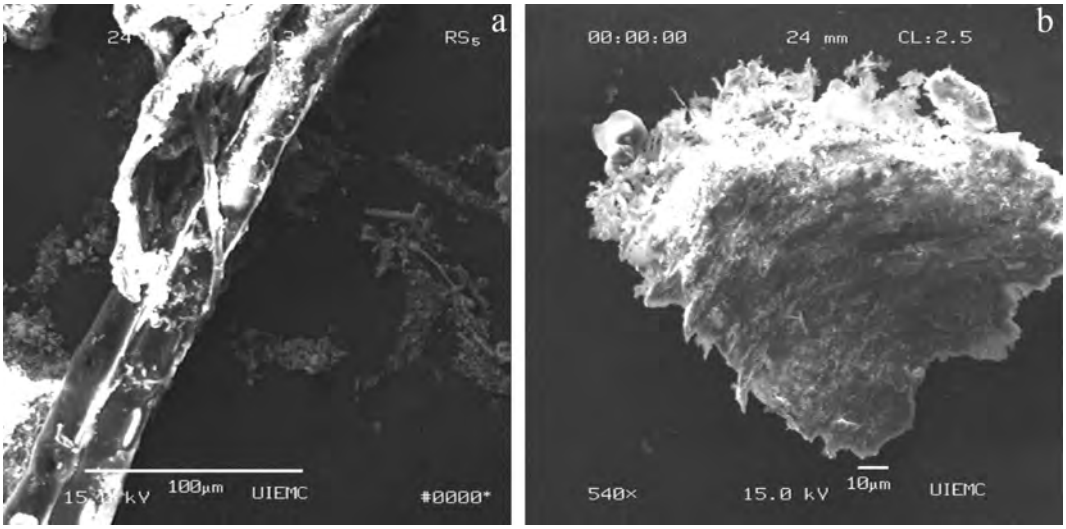

Figure 7. SEM images of particles in the lung of the non-human primate exposed to high levels of riebeckite. a: Fe-rich silicate fiber; b: Al-rich oxide.

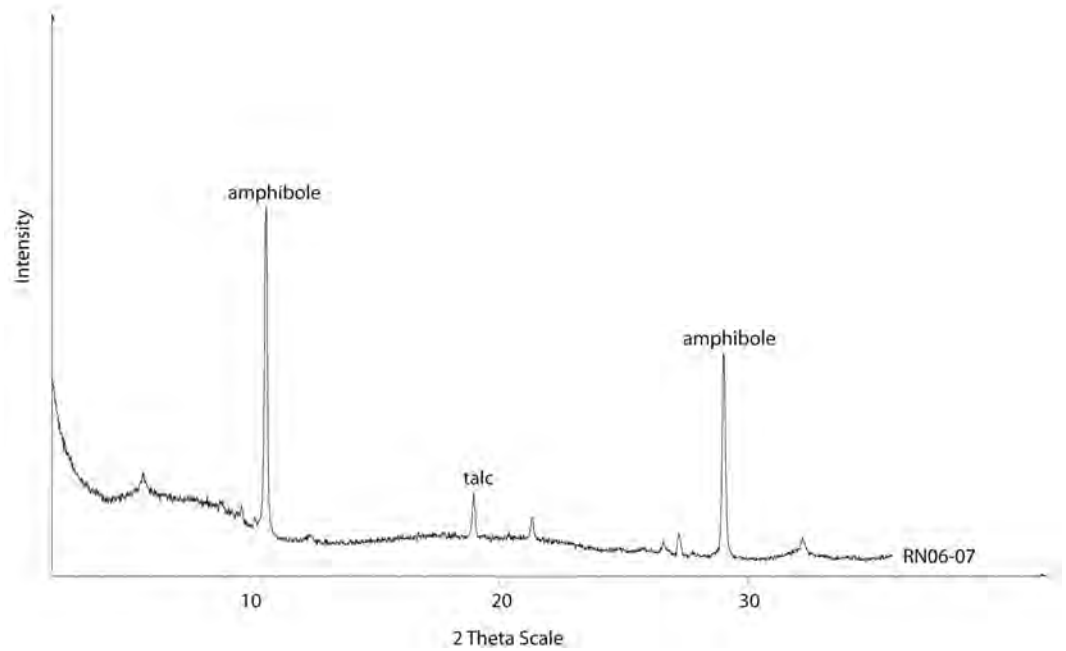

Figure 8. XRD pattern from a baboon exposed to high doses of grunerite.

Grunerite

Talc and an amphibole (presumably grunerite) were detected in the NHP that had high exposure to asbestiform grunerite (Figure 8). SEM-EDS analysis showed fibers covered by asbestos bodies (Figure 9a, b). This particular NHP had the greatest number of exposure days compared to the other NHPs with high exposures (an order of magnitude higher). Based on comparison of SEM-EDS data, this NHP also had the highest concentration of asbestos bodies.

Calcification of pleural plaques

It is interesting to note that XRD analysis did not detect apatite in any of the NHPs with high exposures to any of the minerals or in the one NHP with low exposure to chrysotile. With the exception of the NHP with high grunerite exposure, the number of exposure days for high dose NHPs was only about one third as long as those that had low dose exposures. Perhaps either the high concentrations of fibers or the secondary precipitates they induced altered the surrounding physiologic fluid enough to inhibit calcification. SEM-EDS analysis did detect small quantities of calcium and phosphorous in NHPs exposed to high doses of grunerite and chrysotile. This suggests that apatite could be present in all samples, but at concentrations below the detection limit for the instrument (at least 0.05 % for this method of analysis, Sanchez and Gunter, 2006). However, it is unclear why one NHP with a low chrysotile exposure contained apatite and another baboon with the same level and duration of exposure did not. The third low chrysotile exposure NHP died three years before the other two, and this NHP contained apatite in its lung sample.

Health effects of minerals

A wealth of information exists on the health effects of minerals (e.g., Guthrie and Mossman, 1993, and references therein; Berry, 1999; McDonald and McDonald, 1997). All of these studies focus on primary mineral inhalation as the cause of lung disease. What the present research shows is that secondary precipitation of minerals in the lungs could also have a role in lung pathologies. We argue that previous lung burden studies may have ignored a small but important suite of secondary minerals that in some way catalyze or inhibit the disease process in the lung.

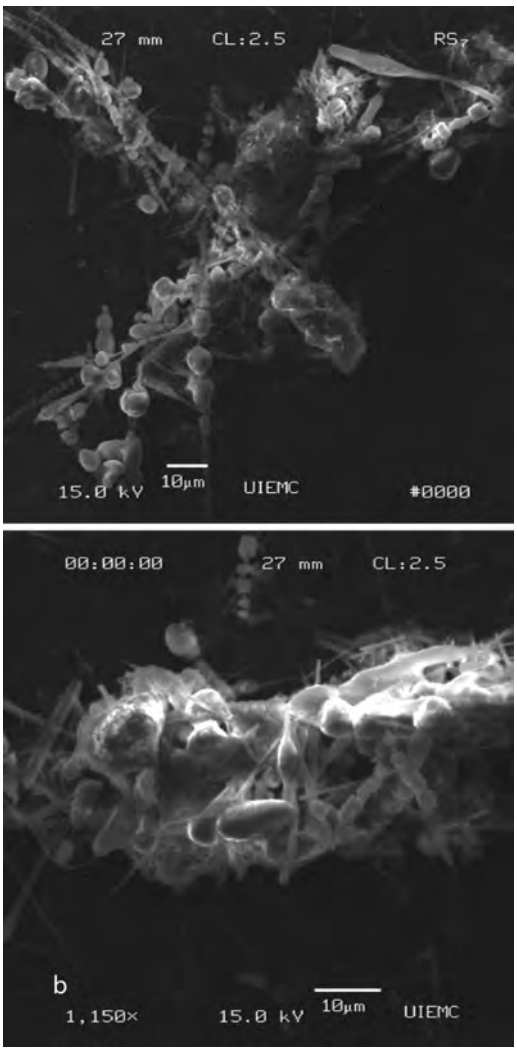

Figure 9. SEM images from a baboon exposed to high doses of grunerite. Note the ferruginous bodies surrounding the fibers.

More extensive characterization of all minerals in pleural plaques and lung tissue, not just those to which the subject was exposed, is needed to test the hypothesis of secondary mineral involvement in lung pathologies.

Characterization of pleural plaques is a unique way of determining mineral content in lung samples that the scientific literature has yet to recognize. The detection in pleural plaques of particles that are too large to be inhaled strengthens the arguments in Taunton et al. (2010) and of Wood et al. (2006) that secondary mineral precipitation or mineral phase transformations occur in the lung. A broader study of pleural plaques with extensive characterization of the starting material to which the subject is exposed would be greatly beneficial to this type of research and would investigate whether the secondary minerals are important to the health effects seen in those exposed to fibrous materials.

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References

Berry G. (1999) - Models for mesothelioma incidence following exposure to fibers in terms of timing and duration of exposure and the biopersistence of the fibers. *Inhalation Toxicology*, 11, 111-130.

Candy J.M., Oakley A.E., Klinowski J., Carpenter T.A., Perry R.H., Atack J.R., Perry E.K., Blessed G., Fairbairn A. and Edwardson J.A. (1986) - Aluminosilicates and senile plaque formation in Alzheimer's disease. *Lancet*, 1, 354-357.

Christen B., Wegmann W. and Vogt P. (1997) - Clinical pathology and histology of pleural plaques. *Indoor Built Environment*, 6, 79-85.

Clarke C.C., Mowat F.S., Kelsh M.A. and Robers M.A. (2006) - Pleural plaques: A review of diagnostic issues and possible nonasbestos factors. *Archives of Environmental & Occupational Health,* 61, 183-192.

Eastes W. and Hadley J.G. (1995) - Dissolution of fibers inhaled by rats. *Inhalation Toxicology*, 7, 179-196.

Gault M.H., Chafe L., Longerich L. and Mason R.A. (1993) - Calcium and calcium magnesium carbonate specimens submitted as urinary tract stones. *Journal of Urology*, 149, 244-249.

Gibbs A.R., Gardner M.J., Pooley F.D., Griffiths D.M., Blight B. and Wagner J.C. (1994) - Fiber levels and disease in workers from a factory predominantly using amosite. *Environmental Health Perspectives*, 102, 261-263.

Gold J., Amandusson H., Krozer A., Kasemo B., Ericsson T., Zanetti G. and Fubini B. (1997) - Chemical characterization and reactivity of iron chelator-treated amphibole asbestos. *Environmental Health Perspectives*, 105, 1021-1030.

Gunter M.E., Belluso E. and Mottana A. (2007) - Amphiboles: Environmental and health concerns. In: Hawthorne F.C., Oberti R., Della Ventura G., Mottana A. (eds) *Amphiboles crystal chemistry, occurrence, and health issues*, 67, 453-516.

Guthrie G.D. and Mossman B.T. (1993) - Health effects of mineral dusts. *Reviews in Mineralogy*, 28.

Hesterberg T.W. and Hart G.A. (2000) - Lung biopersistence and *in vitro* dissolution rate predict the pathogenic potential of synthetic vitreous fibers. *Inhalation Toxicology*, 12, 91-97.

Hiroshima K., Murai Y., Suzuki Y., Goldstein B. and Webster I. (1993) - Characterization of asbestos fibers in lungs and mesotheliomatous tissues of baboons following long-term inhalation. *American Journal of industrial Medicine*, 23, 883-901.

Hiroshima K. and Suzuki Y. (1993) - Characterization of asbestos bodies and uncoated fibers in lungs of hamsters. *Journal of Electron Microscopy*, 42, 41-47.

Hodgson J.T. and Darnton A. (2000) - The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Annals of Occupational*

Hygiene, 44, 565-601.

Hume L.A. and Rimstidt J.D. (1992) - The biodurability of chrysotile asbestos. *American Mineralogist*, 77, 1125-1128.

Jacob G. and Bohlig H. (1955) - Roentgenological complications in pulmonary asbestosis. *Fortschritte auf dem Gebiete der Rontgenstrahlen und der Nuklearmedizin*, 83, 515-525.

Jurinski J.B. and Rimstidt J.D. (2001) - Biodurability of talc. *American Mineralogist*, 86, 392-399.

Mansfield C.F. (1980) - A urolith of biogenic dolomite - another clue in the dolomite mystery. *Geochimica et Cosmochimica Acta*, 44, 829-831.

McDonald J.C. and McDonald A.D. (1997) - Chrysotile, tremolite, and carcinogenicity. *Annals of Occupational Hygiene*, 41, 699-705.

Norton M.R. and Gunter M.E. (1999) - Relationships between respiratory diseases and quartz-rich dust in Idaho, U.S.A. *American Mineralogist*, 84, 1009-1019.

Osbourne C.A., Oldroyd N.O. and Clinton C.W. (1986) - Etiopathogenesis of uncommon canine uroliths: Xanthine, carbonate, drugs, and drug metabolites. *Veterinary Clinics of North America Small Animal Practice*, 16, 217-225.

Parry W.T. (1985) - Calculated solubility of chrysotile asbestos in physiological systems. *Environmental Research*, 37, 410-418.

Pooley F.D. (1972) - Asbestos bodies, their formation, composition, and character. *Environmental Research*, 5, 363-379.

Porro F.W., Patton J.R. and Hobbs A.A. (1942) - Pneumoconiosis in the talc industry. American *Journal Roentgenology*, 47, 507-524.

Plumlee G.S., Morman S.A. and Ziegler T.L. (2006) - The Toxicological Geochemistry of Earth Materials: An Overview of Processes and the Interdisciplinary Methods Used to Understand Them. In Sahai N., Schoonen M.A.A. (eds) *Medical Mineralogy and Geochemsitry,* 5-57.

Ross M. and Nolan R.P. (2003) - History of discovery and use and asbestos-related disease in context with the occurrence of asbestos within ophiolite complexes. In Dilek Y. and Newcomb S. (eds) Ophiolite concept and the evolution of geological thought. *GSA Special Paper,* 373, 447-470.

Sanchez M.S. and Gunter M.E. (2006) - Quantification of amphibole content in expanded vermiculite products from Libby, Montana U.S.A. *American Mineralogist*, 91, 1448-1501.

Stephens M., Gibbs A.R., Pooley F.D. and Wagner J.C. (1987) - Asbestos induced pleural fibrosis: pathology and mineralogy. *Thorax*, 42, 583-588.

Sullivan P.A. (2007) - Vermiculite, Respiratory Disease, and Asbestos Exposure in Libby, Montana: Update of a Cohort Mortality Study. *Environmental Health Perspective*, 115, 579-585.

Suzuki Y. and Churg J. (1970) - Formation of the asbestos body: A comparative study with three different types of asbestos. *Environmental Research*, 3, 107-118.

Taunton A.E., Gunter M.E., Druschel G.K. and Wood S.A. (2010) - Geochemistry in the lung: Reaction-path modeling and experimental examination of rock-forming minerals under physiologic conditions. *American Mineralogist*, 95, 1624-1635.

Wagner J.C., Sleggs C.A. and Marchand P. (1960) - Diffuse pleural mesothelioma and asbestos exposure in the Northwestern Cape Province. *British Journal of Industrial Medicine*, 17, 260-271.

Wagner J.C., Berry G. and Pooley F.D. (1982) - Mesotheliomas and asbestos type in asbestos textile workers: a study of lung contents. *British Medical Journal*, 285, 603-606.

Wagner J.C., Moncrieff C.B., Coles R., Griffiths D.M. and Munday D.E. (1986) - Correlation between fibre content of the lungs and disease in naval dockyard workers. *British Journal of Industrial Medicine*, 43, 391-395.

Weir L.A., Gunter M.E. and Williams T.J. (2007) - The lung as an air sampler. *Geology Society of America Abstracts with Programs*.

Wood S.A., Taunton A.E., Normand C. and Gunter M.E. (2006) - Mineral-fluid interactions in the lungs: insights from reaction path modeling. *Inhalation Toxicology*, 18, 977-986.

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