International Chrysotile Association

For environmental
occupational health
safe and responsible use

# Compilation

## Recently Published Studies on Chrysotile Fibers – 2016

# TABLE OF CONTENTS

STUDY No. 1

# THE HEALTH RISK OF CHRYSOTILE ASBESTOS

**STUDY No. 1**

# THE HEALTH RISK OF CHRYSOTILE ASBESTOS

**Current Opinion in Pulmonary Medicine (2014) Vol. 20, No. 4, 389-392**

**David Bernstein**

This review clarifies the differences between the two mineral families (chrysotile and amphibole) referred to as “asbestos” The author summarizes the scientific basis for understanding the important differences in the toxicology and epidemiology of these two minerals. Biopersistence studies and sub-chronic inhalation toxicology studies have shown that exposure to chrysotile at up to 5,000 times the current threshold limit value (0.1 fibers/$cm^3$) produces no pathological response. These studies demonstrate as well that following short-term exposure the longer chrysotile fibers rapidly clear from the lung and are not observed in the pleural cavity. In contrast, short-term exposure to amphibole asbestos results quickly in the initiation of a pathological response in the lung and the pleural cavity.

The author concludes that the valuation of the toxicology and epidemiology studies of chrysotile indicates that it can be used safely under controlled use. In contrast, even short-term exposure to amphibole asbestos can result in disease.

REVIEW

# The health risk of chrysotile asbestos

*David M. Bernstein*

**Purpose of review**
The word asbestos is a poorly attributed term, as it refers to two very different minerals with very different characteristics. One is the serpentine mineral of which the white asbestos, chrysotile, is the most common. The other is the amphibole asbestos, which includes the blue asbestos crocidolite and the brown asbestos amosite. Although today chrysotile is the only type used commercially, the legacy of past use of amphibole asbestos remains. This review clarifies the differences between the two mineral families referred to as asbestos and summarizes the scientific basis for understanding the important differences in the toxicology and epidemiology of these two minerals.

**Recent findings**
Biopersistence and sub-chronic inhalation toxicology studies have shown that exposure to chrysotile at up to 5000 times the current threshold limit value (0.1 fibers/cm$^3$) produces no pathological response. These studies demonstrate as well that following short-term exposure the longer chrysotile fibers rapidly clear from the lung and are not observed in the pleural cavity. In contrast, short-term exposure to amphibole asbestos results quickly in the initiation of a pathological response in the lung and the pleural cavity.

**Summary**
Significant progress has been made in understanding the factors that influence inhalation toxicology studies of fibers and epidemiological studies of workers. Evaluation of the toxicology and epidemiology studies of chrysotile indicates that it can be used safely under controlled use. In contrast, even short-term exposure to amphibole asbestos can result in disease.

**Keywords**
amphibole asbestos, biopersistence, chrysotile, epidemiology, inhalation toxicology, lung, pleura

## INTRODUCTION

The word asbestos is associated with health risk and controversy. However, it is a poorly attributed term, as it refers to two very different minerals with very different characteristics. One is the serpentine mineral of which the white asbestos, chrysotile, is the most common. The other is the amphibole asbestos, which includes the blue asbestos crocidolite and the brown asbestos amosite. Although today chrysotile is the only type used commercially, the legacy of past use of amphibole asbestos remains. This review clarifies the differences between the two mineral families referred to as asbestos and summarizes the scientific basis for understanding the important differences in the toxicology and epidemiology of these two minerals.

Although mineralogists have long been aware of the structural and chemical differences between serpentine and amphibole asbestos, these characteristics have only more recently been taken into consideration by the toxicological and epidemiological literature.

## CHRYSOTILE AND AMPHIBOLE ASBESTOS

The most important characteristics that influence the toxicology of chrysotile are that it is soluble in acid [1] and formed as rolled or concentric thin sheets (7.3 Å thick) composed of silicate and brucite layers with the magnesium hydroxide part of each layer closest to the fiber surface [2–5]. The magnesium on the outside of the role is readily attacked by acid milieu such as occurs inside the alveolar macrophage (pH 4–4.5), and dissociates from the crystalline structure, leaving an unstable silicate sheet. This process results in the thin rolled sheet of the chrysotile fiber breaking apart and decomposing into smaller pieces. These pieces can then be readily

Consultant in Toxicology, Geneva, Switzerland

Correspondence to David M. Bernstein, Consultant in Toxicology, 1208 Geneva, Switzerland. Tel: +41 227350043; e-mail: davidb@itox.ch

**Curr Opin Pulm Med** 2014, 20:366–370

DOI:10.1097/MCP.0000000000000064

## KEY POINTS

- Asbestos is a poorly attributed term referring to two very different minerals.
- Chrysotile is an acid-soluble rolled thin sheet silicate with little biopersistence.
- Amphibole asbestos (crocidolite, amosite) are solid silicate fibers with negligible solubility and high biopersistence.
- Chrysotile produces little effect, and with controlled use it can be used safely.
- Amphibole asbestos is highly pathogenic and quickly initiates disease even after short-term exposure.

cleared from the lung by macrophages through lymphatic and mucociliary clearance.

With amphibole asbestos, the fibrils are formed as solid rods with the silica on the outside of the fibrils, which makes it very strong and durable [6,7]. The amphibole fibril has negligible solubility at any pH that might be encountered in an organism as a result of the lack of acid-soluble surface groups on the solid fibril [8], and thus persists in the lung.

## THE IMPORTANCE OF FIBER LENGTH AND BIOPERSISTENCE

The first line of defense to inhaled organisms, particles or fibers that reach the lung parenchyma is the macrophage [9]. The macrophage responds by phagocytizing the object, creating a vacuole around it and then lowering the pH to 4–4.5. Fibers that can be fully phagocytized by the macrophage can be cleared from the active region of the lung by mucociliary or lymphatic clearance. Shorter fibers can also directly enter into the lymphatics and be cleared. Fibers longer than the macrophage (~20–25 μm) act as an anchor preventing the macrophage from moving. Such fibers will remain in the lung unless they dissolve or disintegrate [10,11,12▪▪]. The acid of the macrophage facilitates the disintegration of the chrysotile fiber. The longer amphibole fibers are not affected by the acid and persist, resulting in the macrophage secreting lysozyme, neutral proteases, acid hydrolases and $O_2$ metabolites, potent mediators of inflammatory response. In addition, the longer amphibole fibers have been found to penetrate through the lung into the pleural cavity.

## TOXICOLOGY

The understanding of the toxicology of chrysotile has evolved in parallel with the factors for performing in-vitro and inhalation toxicology studies.

### In-vitro toxicology

In-vitro toxicology studies are often useful in exploring the possible mechanisms involved in the pathogenesis. However, the in-vitro test system is a static system, which cannot take into account the systemic dynamics that occur *in vivo* and that can influence the solubility and clearance of the fibers. In addition, very high doses are usually used with in-vitro studies, as almost no effect occurs at doses approaching even higher exposures *in vivo*. As an example, in a recent study [13], exposure was presented as 5 μg/cm$^2$ UICC chrysotile. No indication was provided of the number or size distribution of fibers in the test samples. Using data presented for UICC chrysotile [14], the dose was estimated to be 50 000 fibers/cell in the culture. In humans, even at high exposures, one fiber would be deposited in every 2000 alveoli per day [12▪▪]. Although in-vitro testing may be useful in investigating possible mechanisms of toxicity, as applied to the evaluation of fibers these test systems are of limited use for risk assessment [15].

### Biopersistence

Recent studies have shown that chrysotile fibers are rapidly cleared from the lung and do not reach the pleural cavity and do not result in a pathological response in either the lung or pleural cavity. This is in contrast to the amphibole asbestos fibers such as amosite and tremolite, which because of their insolubility at both neutral and acid pH are very biopersistent in the lung, result in the formation of interstitial fibrosis even after short-term exposure and quickly translocate to the pleural cavity [12▪▪].

As cited above, Kobell [1] in 1834 reported that one of the most important characteristics that differentiated chrysotile was that it was soluble in acid. The importance of this was not recognized until the 1990s when studies were performed to determine why newly developed high aluminium synthetic vitreous fibers were rapidly cleared from the lung [16]. The investigators found that under the acid conditions of the alveolar macrophage, the fiber quickly dissolved and broke apart. This combined with the fact that chrysotile is a rolled sheet silicate with a sheet thickness of 7.3 Å provided a basis for understanding why the long chrysotile fibers clear rapidly from the lung [12▪▪]. The rapid clearance of chrysotile is thought to be characterized not by congruent dissolution as with many synthetic vitreous fibers but rather with the loss of structural integrity of the serpentine sheet silicate and the subsequent disintegration into smaller pieces. Suquet [17] reported on the assessment of the structural damage produced by grinding or acid leaching

of chrysotile. The author reported that 'Acid leaching transformed chrysotile into porous, noncrystalline hydrated silica, which easily fractured into short fragments. If the acid attack was too severe, these fragments converted into shapeless material.'

Biopersistence studies of chrysotile have shown that it is not biopersistent in the lung and that it does not produce a pathological response following short-term exposure in either the lung or the pleural cavity [18–21,22▪].

### Inhalation toxicology

The early inhalation toxicology studies of asbestos have been often difficult to interpret. These studies were performed prior to the current understanding of animal physiology and the factors that influence fiber toxicology. The paradigm that has evolved concerning fiber toxicity is based upon three criteria: dose, dimensions and durability. Rodents, which are routinely used in toxicology studies, are mandatory nasal breathers and as such can only inhale fibers less than approximately 1 μm diameter. In addition, they are susceptible to lung overload, under exceedingly high exposure concentrations to relatively insoluble particles [23–27]. The fiber preparation procedures used in most early studies involved the use of heavily milled asbestos samples [28–31], which were then aerosolized using a procedure that involved grinding of the fibers as well [28], both of which greatly reduced the number of longer fibers present. Exposure was standardized based upon a gravimetric concentration of 10 mg/m$^3$, without consideration of the number of particles and longer fibers present. As a result, the exposure concentration in these studies has been calculated to range from 200 000 to 8600 000 fibers/cm$^3$, well in excess of what would be considered lung overload [12▪▪].

Studies taking into account both the animal physiology and the fiber characteristics that influence potential toxicity have shown that chrysotile even at exposure concentrations of 500 fibers(WHO)/cm$^3$ produces no pathological response [32].

## EPIDEMIOLOGY

Several important limitations underlie epidemiological studies of chrysotile. Although chrysotile is currently used largely in high-density cement products, the epidemiological and regulatory evaluation of chrysotile is based upon a cross-section of all uses in the past. The studies characterized as chrysotile only have been reviewed recently and provide further understanding of the difficulty in using these studies to evaluate chrysotile as used today [12▪▪]. As a result of the measurement techniques used at the time, there was little or no quantitative exposure information available on the types of fibers to which the workers were exposed. In addition, fiber exposure was estimated based upon the extrapolation from gravimetric or total particle number of samples without information available as to fiber diameter or length. The nature of industrial processes was used to suggest the type of fiber to which the workers were exposed. However, in many applications, chrysotile and amphibole asbestos were often used interchangeably depending upon availability, cost and effectiveness in the process. Equally important, work histories of employees were not as well documented as might occur today [33].

### Etiology

Although early studies correlated severity of illness in 'asbestos'-exposed workers with the dustier jobs [34–41] providing an exposure–response relationship, owing to the state of occupational hygiene measurements at the time, none of the studies were able to use exposure measurements that included fiber number or fiber type or the size distribution of the fibers [33].

As not only fiber biopersistence but also fiber length influences the relative potency of fibers, understanding the relative potency requires a precise measurement of the type of fiber exposed and the bivariate length and diameter size distribution of the fibers.

Taking into account these factors, the chrysotile epidemiology studies are often difficult to interpret. The importance of identifying even short-term exposure to amphibole because of the differential potency of chrysotile as compared with amphibole asbestos as well as the importance of the fibers longer than 20 μm in fiber pathogenesis was not taken into account in the studies.

The epidemiology studies characterized in the review by Hodgson and Darnton [42] as predominately chrysotile exposure [43–48] have been reviewed in light of current data, and information has been learned from the toxicology studies on the importance of fiber type and fiber length in producing a pathological response in the lung and pleural cavity. Most of the studies have been shown not to be chrysotile-only studies, but to have had compounding exposures to amphibole asbestos as well as other methodological difficulties in evaluating the results [12▪▪].

An evaluation of epidemiological studies of workers exposed to chrysotile as used in the production of high-density cement products, which provided as well differentiation as to when amphibole asbestos exposure also occurred, has shown that

**Table 1.** Epidemiological studies of workers exposed to chrysotile as used in the production of high-density cement products

| Cohort | Plant | Results |
|---|---|---|
| Weill *et al.*, 1979 [49] | Asbestos-cement manufacturing workers | No excess mortality was observed following exposure for 20 years to chrysotile asbestos at exposure levels equal to or less than 100 MPPCF years (corresponding to approximately 15 fibers/cm$^3$ × years). |
| Thomas *et al.*, 1982 [50] | Asbestos-cement factory that used chrysotile | No appreciably raised SMR for the causes of death investigated, including all causes, all neoplasms, cancer of the lung and pleura and cancers of the gastrointestinal tract |
| Gardner *et al.*, 1986 [51] | Asbestos cement factory | No excess of lung cancers or other asbestos-related excess death was reported, at mean fiber concentrations below 1 fiber/cm$^3$ |
| Ohlson and Hogstedt, 1985 [52] | Asbestos cement workers | No excess work-related mortality was observed at cumulative exposures estimated at about 10–20 fibers/cm$^3$ years |
| Sichletidis *et al.*, 2009 [53] | Workers exposed to relatively 'pure chrysotile' in an asbestos cement factory | SMR for lung cancer of 1.71 was attributed almost exclusively to cigarette smoking |
| Yano *et al.*, 2001 [54] | Workers exposed to amphibole-free chrysotile asbestos | Authors attributed effect to chrysotile, which was less than 0.1% of the total dust exposure, which ranged up to 320 mg/m$^3$ |

MPPCF, million particles per cubic foot of air; SMR, standardized mortality ratio.

chrysotile can be used safely when exposures are controlled. Studies of chrysotile as used in the production of high-density cement products are summarized in Table 1 [49–54].

Studies that have been interpreted as studies on chrysotile asbestos are, after careful review and understanding of the conditions and data presented, not representative of chrysotile exposure alone, but rather have numerous other elements as described above that were not fully taken into consideration.

## CONCLUSION

The use of the common term asbestos has long obscured our understanding of the health effects of the exposure to chrysotile in comparison to amphibole asbestos such as crocidolite and amosite asbestos.

Chrysotile, the only type currently used, has been shown to have little biopersistence in the lung and to produce no pathological response in both short-term and sub-chronic inhalation toxicology studies in either the lung or pleural cavity. In contrast, similar exposures of amphibole asbestos are highly pathogenic quickly producing interstitial fibrosis with fibers translocating to the pleural cavity and initiating pathological response there as well.

Most epidemiological studies have historically not well differentiated exposure to these very different fiber families due in part to the state of industrial hygiene measurements at the time the exposures took place. When taking into consideration the importance of even short-term exposure to amphibole asbestos, the studies of chrysotile cement workers clearly demonstrate that under controlled use of chrysotile, it can be used safely.

### Acknowledgements

*D.M.B. has appeared as an expert witness in litigation concerned with alleged health effects of exposure to chrysotile.*

### Conflicts of interest

*There are no conflicts of interest.*

## REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Kobell F. Ueber den schillernden Asbest von Reichenstein in Schlesien. J Prakt Chemie 1834; 2:297–298.
2. Whittaker EJW. The Structure of chrysotile. V. Diffuse reflexions and fibre texture. Acta Cryst 1957; 10:149–156.
3. Whittaker EJW. In Research report: chrysotile fibers –filled or hollow tubes? Mathematical interpretation may resolve conflicting evidence. Chem Eng News 1963; 41:34–35.
4. Tanji T, Yada K, Akatsuka Y. Alternation of clino- and orthochrysotile in a single fiber as revealed by high-resolution electron microscopy. Clay Clay Miner 1984; 32:429–432.
5. Titulaer MK, van Miltenburg JC, Jansen JBH, *et al.* Characterization of tubular chrysotile by thermoporometry, nitrogen sorption, drifts, and TEM. Clays Clay Miner 1993; 41:496–513.
6. Skinner HCW, Ross M, Frondel C. Asbestos and other fibrous materials – mineralogy, crystal chemistry, and health effects. New York (NY): Oxford University Press; 1988. p. 204.
7. Whittaker EJW. The crystal chemistry of the amphiboles. Acta Cryst 1960; 13:291–298.

## Diseases of the pleura


8. Speil S, Leineweber JP. Asbestos minerals in modern technology. Environ Res 1969; 2:166–208.
9. Fels AOS, Cohn ZA. The alveolar macrophage. J Appl Physiol 1986; 60:353–369.
10. Bernstein DM. Synthetic vitreous fibers: a review of toxicology, epidemiology and regulations. Crit Rev Toxicol 2007; 37:839–886.
11. Bernstein DM, Rogers RA, Sepulveda R, *et al.* Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure. Inhal Toxicol 2011; 23:372–391.
12. ■■ Bernstein D, Dunnigan J, Hesterberg T, *et al.* Health risk of chrysotile revisited. Crit Rev Toxicol 2013; 43:154–183.

This is a comprehensive review of the toxicology and epidemiology of the relative potency of chrysotile in comparison to amphibole asbestos.

13. Qi F, Okimoto G, Jube S, *et al.* Continuous exposure to chrysotile asbestos can cause transformation of human mesothelial cells via HMGB1 and TNF-α signaling. Am J Pathol 2013; 183:1654–1666.
14. Okayasu R, Wu L, Hei TK. Biological effects of naturally occurring and man-made fibres: in vitro cytotoxicity and mutagenesis in mammalian cells. Br J Cancer 1999; 79:319–324.
15. Bernstein D, Castranova V, Donaldson K, *et al.* Testing of fibrous particles: short-term assays and strategies. Inhal Toxicol 2005; 17:497–537.
16. Guldberg M, Jensen SL, Knudsen T, *et al.* High-alumina low-silica HT stone wool fibers: a chemical compositional range with high biosolubility. Regul Toxicol Pharmacol 2002; 35 (2 Pt 1):217–226.
17. Suquet H. Effects of dry grinding and leaching on the crystal structure of chrysotile. Clays Clay Miner 1989; 37:439–445.
18. Bernstein DM, Rogers R, Smith P. The biopersistence of Canadian chrysotile asbestos following inhalation. Inhal Toxicol 2003; 15:101–128.
19. Bernstein DM, Rogers R, Smith P. The biopersistence of Brazilian chrysotile asbestos following inhalation. Inhal Toxicol 2004; 16:745–761.
20. Bernstein DM, Rogers R, Smith P. The biopersistence of Canadian chrysotile asbestos following inhalation: final results through 1 year after cessation of exposure. Inhal Toxicol 2005; 17:1–14.
21. Bernstein DM, Chevalier J, Smith P. Comparison of Calidria chrysotile asbestos to pure tremolite: final results of the inhalation biopersistence and histopathology following short term exposure. Inhal Toxicol 2005; 17:427–449.
22. ■ Bernstein DM, Rogers R, Sepulveda R, *et al.* Evaluation of the deposition, translocation and pathological response of brake dust with and without added chrysotile in comparison to crocidolite asbestos following short-term inhalation: Interim results. Toxicol Appl Pharmacol 2014; 276:28–46.

This is an evaluation of the toxicity and biopersistence of chrysotile as used in brake pads in comparison with crocidolite asbestos following short-term inhalation.

23. Bolton RE, Vincent JH, Jones AD, *et al.* An overload hypothesis for pulmonary clearance of UICC amosite fibres inhaled by rats. Br J Ind Med 1983; 40:264–272.
24. Muhle H, Bellman B, Heinrich U. Overloading of lung clearance during chronic exposure of experimental animals to particles. Ann Occup Hyg 1988; 32:141–147.
25. Morrow PE. Possible mechanisms to explain dust overloading of the lung. Fundam Appl Toxicol 1988; 10:369–384.
26. Oberdorster G. Lung particle overload: implications for occupational exposures to particles. Regul Toxicol Pharmacol 1995; 21:123–135.
27. ILSI Risk Science Institute. The relevance of the rat lung response to particle overload for human risk assessment: a workshop consensus report. Inhal Toxicol 2000; 12:1–17.
28. Timbrell V, Hyett AW, Skidmore JW. A simple dispenser for generating dust clouds from standard reference samples of asbestos. Ann Occup Hyg 1968; 11:273–281.
29. Timbrell V, Rendall REG. Preparation of the UICC standard reference samples of asbestos. Powder Technol 1972; 5:279–287.
30. Pinkerton KE, Brody AR, McLaurin DA, *et al.* Characterization of three types of chrysotile asbestos after aerosolization. Environ Res 1983; 31:32–53.
31. Campbell WJ, Huggins CW, Wylie AG. Chemical and physical characterization of amosite, chrysotile, crocidolite, and nonfibrous tremolite for oral ingestion studies by the National Institute of Environmental Health Sciences [report of investigation 8452]. Avondale (MD): United States Department of The Interior, US Bureau of Mines; 1980.
32. Bernstein DM, Rogers R, Chevalier J, *et al.* The toxicological response of Brazilian chrysotile asbestos: a multidose sub-chronic 90 d inhalation toxicology study with 92 day recovery to assess cellular and pathological response. Inhal Toxicol 2006; 18:313–332.
33. Berman DW, Crump KS. Draft technical support document for a protocol to assess asbestos-related risk. Washington, DC: 20460: Office of Solid Waste and Emergency Response U.S. Environmental Protection Agency; 2003.
34. Ashcroft T. Epidemiological and quantitative relationships between mesothelioma and asbestos on Tyneside. J Clin Pathol 1973; 26:832–840.
35. Elmes PC, Wade OL. Relationship between exposure to asbestos and pleural malignancy in Belfast. Ann N Y Acad Sci 1965; 132:549–557.
36. Hain E, Dalquen P, Bohlig H, *et al.* Retrospective study of 150 cases of mesothelioma in Hamburg area (author's transl). Int Arch Arbeitsmed 1974; 33:15–37.
37. McDonald AD, Harper A, McDonald JC, *et al.* Epidemiology of primary malignant mesothelial tumors in Canada. Cancer 1970; 26:914–919.
38. McEwen J, Finlayson A, Mair A, *et al.* Mesothelioma in Scotland. Br Med J 1970; 4:575–578.
39. Newhouse ML, Thompson H. Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. Br J Ind Med 1965; 22:261–269.
40. Rubino GF, Scansetti G, Donna A, *et al.* Epidemiology of pleural mesothelioma in North-western Italy (Piedmont). Br J Ind Med 1972; 29:436–442.
41. Zielhuis RL, Versteeg JP, Planteijdt HT. Pleura mesothelioma and exposure to asbestos: a retrospective case-control study in the Netherlands. Int Arch Occup Environ Health 1975; 36:1–18.
42. Hodgson JT, Darnton A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann Occup Hyg 2000; 44:565–601.
43. Dement JM, Brown DP. Lung cancer mortality among asbestos textile workers: a review and update. Ann Occup Hyg 1994; 38:525–532.
44. McDonald AD, Fry JS, Woolley AJ, McDonald JC. Dust exposure and mortality in an American factory using chrysotile, amosite, and crocidolite in mainly textile manufacture. Br J Ind Med 1983; 40:368–374.
45. Piolatto G, Negri E, La Vecchia C, *et al.* An update of cancer mortality among chrysotile asbestos miners in Balangero, northern Italy. Br J Ind Med 1990; 47:810–814.
46. Liddell FDK, McDonald AD, McDonald JC. Dust exposure and lung cancer in Quebec chrysotile miners and millers. Ann Occup Hyg 1998; 42:7–20.
47. Hughes JM, Weill H, Hammad YY. Mortality of workers employed in two asbestos cement manufacturing plants. Br J Ind Med 1998; 44:161–174.
48. McDonald AD, Fry JS, Woolley AJ, McDonald JC. Dust exposure and mortality in an American chrysotile asbestos friction products plant. Br J Ind Med 1984; 41:151–157.
49. Weill H, Hughes J, Waggenspack C. Influence of dose and fiber type on respiratory malignancy risk in asbestos cement manufacturing. Am Rev Respir Dis 1979; 120:345–354.
50. Thomas HF, Benjamin IT, Elwood PC, *et al.* Further follow-up study of workers from an asbestos cement factory. Br J Ind Med 1982; 39:273–276.
51. Gardner MJ, Winter PD, Pannett B, *et al.* Follow up study of workers manufacturing chrysotile asbestos cement products. Br J Ind Med 1986; 43:726–732.
52. Ohlson CG, Hogstedt C. Lung cancer among asbestos cement workers. A Swedish cohort study and a review. Br J Ind Med 1985; 42:397–402.
53. Sichletidis L, Chloros D, Spyratos D, *et al.* Mortality from occupational exposure to relatively pure chrysotile: a 39-year study. Respiration 2009; 78:63–68.
54. Yano E, Wang ZM, Wang XR, *et al.* Cancer mortality among workers exposed to amphibole-free chrysotile asbestos. Am J Epidemiol 2001; 154:538–543.

ERRATUM

# The health risk of chrysotile asbestos: Erratum

During the editing process of the recent article by Bernstein [1], the conflicts of interest statement was wrongly amended from 'No conflicts of interest relevant to this article' to 'There are no conflicts of interest.' The publisher apologises for this error.

Dr Bernstein would like to take this opportunity to clarify that he works as a scientific consultant to the chrysotile asbestos industry and gives presentations worldwide on the science of chrysotile asbestos. In the last three years he has received payment for his consultancy services from: Honeywell, International Chrysotile Association and Zimbabwe National Chrysotile Taskforce.

Dr Bernstein received no payment, compensation or funding for the current article [1]. The article is solely his work and the opinions stated therein are his own.

## REFERENCE

1. Bernstein DM. The health risk of chrysotile asbestos. Curr Opin Pulm Med 2014; 20:366–370.

1070-5287 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins www.co-pulmonarymedicine.com

STUDY No. 2

# HEALTH RISK OF CHRYSOTILE REVISITED

**STUDY No. 2**

# HEALTH RISK OF CHRYSOTILE REVISITED

**Recent Results Cancer Res. 189, 79 (2011)**
**Crit Rev Toxicol, 2013; 43(2): 154–183**

**David Bernstein, Jacques Dunnigan, Thomas Hesterberg, Robert Brown, Juan Antonio Legaspi Velasco, Raúl Barrera, John Hoskins, and Allen Gibbs**

This review provides a basis for substantiating both kinetically and pathologically the differences between chrysotile and amphibole asbestos. Chrysotile, which is rapidly attacked by the acid environment of the macrophage, falls apart in the lung into short fibers and particles, while the amphibole asbestos persists creating a response to the fibrous structure of this mineral. Inhalation toxicity studies of chrysotile at non-lung overload conditions demonstrate that the long (>20$\mu$m) fibers are rapidly cleared from the lung, are not translocated to the pleural cavity and do not initiate fibrogenic response. In contrast, long amphibole asbestos fibers persist, are quickly (within 7 d) translocated to the pleural cavity and result in interstitial fibrosis and pleural inflammation. Quantitative reviews of epidemiological studies of mineral fibers have determined the potency of chrysotile and amphibole asbestos for causing lung cancer and mesothelioma in relation to fiber type and have also differentiated between these two minerals. These studies have been reviewed in light of the frequent use of amphibole asbestos. As with other respirable particulates, there is evidence that heavy and prolonged exposure to chrysotile can produce lung cancer. The importance of the present and other similar reviews is that the studies they report show that low exposures to chrysotile do not present a detectable risk to health. Since total dose over time decides the likelihood of disease occurrence and progression, they also suggest that the risk of an adverse outcome may be low with even high exposures experienced over a short duration.

Critical Reviews in Toxicology

http://informahealthcare.com/txc
ISSN: 1040-8444 (print), 1547-6898 (electronic)

Crit Rev Toxicol, 2013; 43(2): 154–183
 DOI: 10.3109/10408444.2012.756454

informa healthcare

REVIEW ARTICLE

# Health risk of chrysotile revisited

David Bernstein[1], Jacques Dunnigan[2], Thomas Hesterberg[3], Robert Brown[4], Juan Antonio Legaspi Velasco[5], Raúl Barrera[6], John Hoskins[7], and Allen Gibbs[8]

[1]*Consultant in Toxicology, Geneva, Switzerland,* [2]*University of Sherbrooke, Sherbrooke, QC, Canada,* [3]*Center for Toxicology and Environmental Health, Little Rock, Arkansas, USA,* [4]*Toxicology Services, Rutland, UK,* [5]*Academia Nacional de Medicina México,* [6]*Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico,* [7]*Independent Toxicologist, Haslemere, UK, and* [8]*Llandough Hospital, Penarth, UK*

**Abstract**

This review provides a basis for substantiating both kinetically and pathologically the differences between chrysotile and amphibole asbestos. Chrysotile, which is rapidly attacked by the acid environment of the macrophage, falls apart in the lung into short fibers and particles, while the amphibole asbestos persist creating a response to the fibrous structure of this mineral. Inhalation toxicity studies of chrysotile at non-lung overload conditions demonstrate that the long (>20 μm) fibers are rapidly cleared from the lung, are not translocated to the pleural cavity and do not initiate fibrogenic response. In contrast, long amphibole asbestos fibers persist, are quickly (within 7 d) translocated to the pleural cavity and result in interstitial fibrosis and pleural inflammation. Quantitative reviews of epidemiological studies of mineral fibers have determined the potency of chrysotile and amphibole asbestos for causing lung cancer and mesothelioma in relation to fiber type and have also differentiated between these two minerals. These studies have been reviewed in light of the frequent use of amphibole asbestos. As with other respirable particulates, there is evidence that heavy and prolonged exposure to chrysotile can produce lung cancer. The importance of the present and other similar reviews is that the studies they report show that low exposures to chrysotile do not present a detectable risk to health. Since total dose over time decides the likelihood of disease occurrence and progression, they also suggest that the risk of an adverse outcome may be low with even high exposures experienced over a short duration.

**Keywords**

Amphibole asbestos, cement products, chrysotile, epidemiology, health risk, inhalation toxicology, mining

**History**

Received 22 March 2012
Revised 19 November 2012
Accepted 21 November 2012

**Table of Contents**



Address for correspondence: David Bernstein, *Consultant in Toxicology,* Geneva, Switzerland. E-mail: davidb@itox.ch

## Introduction

Recent scientific studies have contributed to a more complete understanding of the health risk from chrysotile asbestos as used today in high-density products. Key to understanding this is the differentiation of exposure, dose and response of the serpentine mineral chrysotile in comparison to the amphibole asbestos types such as crocidolite, tremolite and amosite. This paper reviews scientific studies identified as chrysotile only or predominately chrysotile and discusses how the newer toxicological and epidemiological data provide a convergence in the understanding of the risk from chrysotile.

The association of asbestos exposure with disease dates from the turn of the twentieth century (McDonald & McDonald, 1996). The report by Wagner et al. (1960), reporting on 33 cases of mesothelioma, which the authors stated were primarily from the crocidolite mining area in the

DOI: 10.3109/10408444.2012.756454



North West Cape Province of South Africa (18 out of 33 cases), was instrumental in establishing a relationship to asbestos exposure. While the relationship Wagner et al. (1960) described concerned individuals working primarily in crocidolite mining, there was virtually no quantification of exposure at this time. Subsequently, Selikoff et al. (1984), reported on 632 insulation workers exposed to asbestos who entered the trade before 1943 and were traced through 1962; 45 died of cancer of the lung or pleura, whereas only 6.6 such deaths were expected. Three of the pleural tumors were mesotheliomas; there was also one peritoneal mesothelioma. The use of the generic term ''asbestos'' to describe both minerals, the serpentine chrysotile and members of the amphibole family (amosite, crocidolite, tremolite, anthophyllite and actinolite, of which only the first two were industrially important) and the lack of complete occupational histories are significant limitations in the early epidemiology studies, resulting in improper characterization of fiber-specific exposure. These factors further confused and effectively prevented differentiation in the association of disease to fiber mineral type. In addition, because of the common use of the name ''asbestos'' for either of the two mineral types and their similar uses, it was conceivable to imagine that all asbestos types could have similar potency. In essence, because the same name was used for these two very different minerals, the impetus was to equate rather than differentiate the two.

As a result of the frequent use of the all-inclusive term asbestos and the limitations in analysis and identification, most studies through the late 1990s provided little quantitative scientific basis for distinguishing between the effects of chrysotile as compared to those of amphibole asbestos. NIOSH (2011) in their Asbestos Roadmap, stated that ''Imprecise terminology and mineralogical complexity have affected progress in research. 'Asbestos' and 'asbestiform' are two commonly used terms that lack mineralogical precision. 'Asbestos' is a term used for certain minerals that have crystallized in a particular macroscopic habit with certain commercially useful properties''. And, ''The use of non-standard terminology or terms with imprecise definitions when reporting studies makes it difficult to fully understand the implications of these studies or to compare the results to those of other studies''.

## The differences in serpentine and amphibole asbestos

The physical and chemical properties which differentiate chrysotile which is a serpentine mineral from the amphibole asbestos types such as amosite and crocidolite have only recently been factored into the understanding of the toxicology and epidemiology of these minerals. The use of the common name asbestos for both of these mineral types further obscured the important differences between the serpentine and amphibole fibers. In addition, some of the earlier methods of characterization of the fibers were rudimentary in that length and width were generally not addressed, even if the fiber type was reported.

Chrysotile was first described by von Kobell (1834). The name chrysotile was derived by combining the Greek words for golden and fibrous. von Kobell described that chrysotile is distinguished by its behavior of being decomposed by acid. The curved structure of the Mg-analog of kaolinite was suggested by Pauling (1930) because of the misfit between the octahedral and tetrahedral sheets. The crystal structure of chrysotile asbestos was first determined by Warren & Bragg (1930). Subsequently, Noll & Kircher (1951) and Bates et al. (1950) published electron micrographs showing cylindrical and apparently hollow chrysotile fibers. Chrysotile is one of the three different polymorphs of serpentine (antigorite, lizardite and chrysotile) that are thought to be the result of different structural mechanisms which reduce strain in the formations (Evans, 2004; Veblen & Wylie, 1993; Wicks & O'Hanley, 1988).

Chrysotile has the approximate composition $Mg_3Si_20_5(OH)_4$ and is a sheet silicate composed of silicate and brucite layers. The silica layer is a tetrahedra in a pseudohexagonal network. Joined to this is a sheet of magnesium hydroxide octahedra, in which on one side, two out of every three hydroxyls are replaced by apical oxygens of the silica tetrahedral (Cressey & Whittaker, 1993). The different dimensions of these two components result in a structural mismatch in which the layers curl, concentrically or spirally. The fiber walls are made up of approximately 12–20 of these layers in which there is some mechanical interlocking. However, there is no chemical bonding as such between the layers. Each layer is about 7.3 Å. thick, with the magnesium hydroxide part of each layer closest to the fiber surface and the silicon–oxygen tetrahedra ''inside'' the curl (Whittaker, 1963, 1957; Tanji, 1985. Titulaer et al. (1993, Table 2) reported on the porous structure of chrysotile by transmission electron microscopy (TEM). Based upon a number of samples, the authors determined that the thickness of the chrysotile wall in the fibers ranged from 8 to 15 nm, with from 11 to 21 sheets in each tube wall.

The structure of chrysotile is shown in Figure 1 (as a rolled sheet although concentric sheets also occur). The cylinders are chrysotile fibrils which bunch together to form a chrysotile fiber. The magnesium is on the outside of the roll and, as discussed below, the magnesium layer is soluble in biological systems. The magnesium is readily attacked by the acid milieu inside the macrophage (pH 4–4.5), and dissociates from the crystalline structure, leaving the now unstable silicate sheet. This process causes the rolled sheet of the chrysotile fiber to break apart and decompose into smaller pieces. These pieces can then be readily cleared from the lung by macrophages through mucociliary and lymphatic clearance. Fibers cleared on the mucociliary escalator are cleared to the gut where they are attacked by the even stronger acid environment (hydrochloric acid, pH 1.2, Oze & Solt (2010)) of the stomach.

In contrast, the amphibole asbestos class of fibers is formed as solid rods/fibers (Skinner et al., 1988; Whittaker, 1960). The structure of an amphibole is a double chain of tetrahedral silicate with the silica on the outside of the fiber which makes it very strong and durable (Figure 2). There are five asbestiform varieties of amphiboles: anthophyllite asbestos, grunerite asbestos (amosite), riebeckite asbestos (crocidolite), tremolite asbestos and actinolite asbestos. Of these, crocidolite and amosite were the only amphiboles with

Crit Rev Toxicol, 2013; 43(2): 154–183

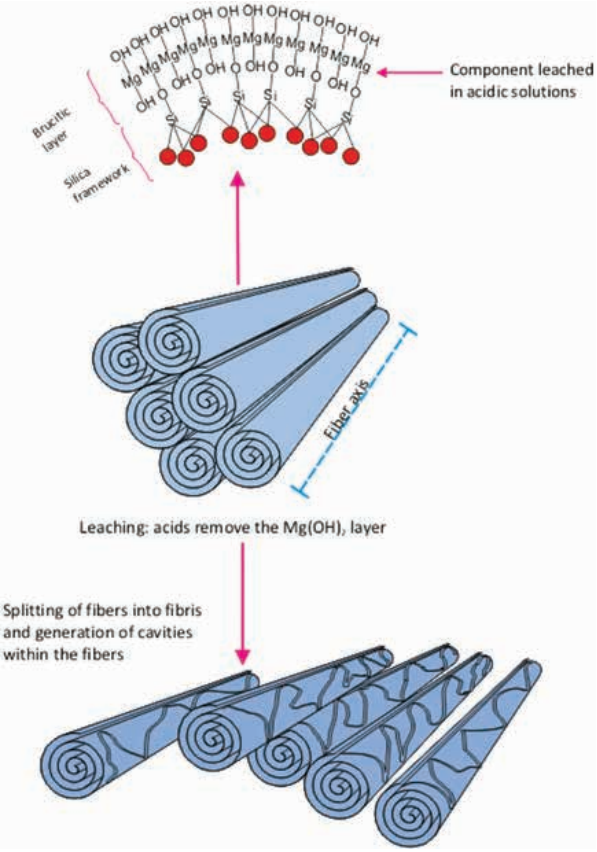



Figure 1. Schematic illustration of the chrysotile fiber. Chrysotile is a rolled sheet or concentrate rings of silicate with the magnesium on the outside of the sheet and the silica on the inside. The chrysotile fiber is acid soluble. Chrysotile has the formula $Mg_3Si_2O_5(OH)_4$. The fiber consists of magnesium hydroxide layers condensed onto silicon·oxygen tetrahedra. The fiber walls are made up of 11 to 21 such layers in which there is some mechanical interlocking. There is not any chemical bonding as such between the layers, however. Each layer is about 7.3 Å thick. The $Mg(OH)_2$ part of the molecule layers is closest to the fiber surfaces; the silicon–oxygen tetrahedra are inside. Under the acid conditions associated with the macrophage, the fiber structure is weakened and the long fibers break into short pieces which can be engulfed and cleared by the macrophages.

significant industrial uses (Virta, 2002). Tremolite, while not used commercially, has been found as a contaminant in other fibers or in other industrial minerals (e.g. chrysotile and talc). The chemical composition of the amphiboles fibers is more complex and the idealized chemical formulae of the five amphiboles are shown below. Although their structures are the same, this variability in composition is a direct consequence of the fact that the silicate framework can accommodate a mixture of many different ions (as determined by the host rock) in the space between the silicate ribbons which form the fibers (Speil & Leineweber, 1969).

Crocidolite $(Na_2Fe^{2+}_3Fe^{3+}_2)\ Si_8O_{22}(OH)_2$
Amosite $(Fe^{2+}, Mg)_7\ Si_8O_{22}(OH)_2$
Tremolite $Ca_2Mg_5\ Si_8O_{22}(OH)_2$
Anthophyllite $(Mg, Fe^{2+})_7\ Si_8O_{22}(OH)_2$
Actinolite $Ca_2(Mg, Fe^{2+})_5\ Si_8O_{22}(OH)_2$

The crystalline structure common to amphibole minerals consists of two ribbons of silicate tetrahedra placed back to back (Virta, 2002).

Due to the structural matrix of amphibole fibers, they have negligible solubility at any pH that might be encountered in an organism (Speil & Leineweber, 1969). Some associated surface contaminating metals such as iron can become ionized and can then be released from the fiber (Aust et al., 2011).

## *In-vitro* biodurability

The magnesium hydroxide part of each layer being closest to the fiber surface is reflected in the chemical characteristics of chrysotile, which has poor acid resistance compared to other asbestiform substances. The amphiboles, for example, in which the silicate oxygens are on the ''outside'' of the layers and the hydroxides are masked within, have better resistance to acids. Hargreaves & Taylor (1946) reported that if fibrous chrysotile is treated with dilute acid, the magnesia can be completely removed. The hydrated silica which remains, though fibrous in form, had completely lost the elasticity characteristic of the original chrysotile and had a structure that was ''amorphous'' or ''glassy'' in type. Wypych et al. (2005) examined what happens to natural chrysotile fibers when acid-leached under controlled conditions. The authors reported that the leached products consisted of layered hydrated disordered silica with a ''distorted'' structure resembling the silicate layer existing in the original minerals. Extensive characterization techniques confirmed the removal of the brucite-like sheets, leaving silica with an eminently amorphous structure. Suquet (1989) reported on the assessment of the structural damage produced by grinding or acid leaching of chrysotile. The author reported that ''Acid leaching transformed chrysotile into porous, non-crystalline hydrated silica, which easily fractured into short fragments. If the acid attack was too severe, these fragments converted into shapeless material''.

Seshan (1983) reported that following exposure to water, strong acids and simulated gastric juices, chrysotile asbestos underwent changes in the physical, chemical and surface properties. The authors reported that the surface becomes silica-like and that upon exposure to water and acid the magnesium is lost from the fibers. The authors also reported that upon acid exposure, the magnesium ions are leached out, leaving a magnesium-free silica network. In addition, the acid treatment also destroyed the X-ray diffraction pattern of chrysotile and changed its refractive index. In contrast, crocidolite asbestos remained unchanged.

Larsen (1989) evaluated different types of natural and synthetic fibers which had been subjected to systematic solubility tests *in vitro* in a physiological solution at 37 °C. Included in this evaluation were chrysotile and crocidolite. Solubility was evaluated by the measurement of silicon in a Gamble's solution similar in composition to lung fluid (without the organic components) using atomic absorption spectrophotometry. The authors reported that the dissolution values ranged from a few nanograms of silicon dissolved per $cm^2$ (chrysotile and crocidolite) to several thousands of $ng/cm^2$ silicon dissolved (glass wools) and that aramide and carbon fibers proved to be practically insoluble. For

DOI: 10.3109/10408444.2012.756454



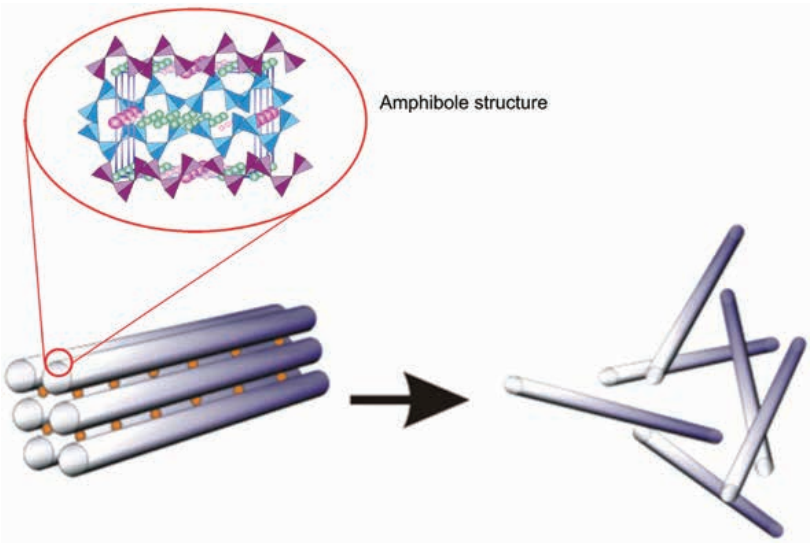



Figure 2. With amphiboles, the soluble cations shown as small circles are located between the fibers which are formed with double chain silicate. When the soluble cations dissolve as can happen in the lung, the amphibole fibers in these bundles are released as individual fibers. The double chain silicate amphibole fibers themselves are highly insoluble in both the lung fluids and in the macrophages.

chrysotile, the authors reported that after a 6-week shaking-table experiment (closed system) that 6 ng/cm$^2$ silicon and 160 ng/cm$^2$ magnesium had dissolved.

Oze & Solt (2010) investigated the biodurability of chrysotile and tremolite asbestos in simulated lung and gastric fluids. The simulated gastric fluid (SGF) was composed of HCl and NaCl solution at a pH 1.2 and the simulated lung fluid (SLF) was a modified Gamble's solution at pH 7.4 at 37 °C. The studies were performed under batch conditions using 0.01, 0.1 and 1 g of ground fiber in a 50 ml vial over 720 h in apparently static conditions. There was no discussion of the influence of the large number of fibers present in such quantities on fluid contact and whether the suspensions settled over time. The relative biodurabilities determined under these conditions were (from most to least) tremolite (SLF) > chrysotile (SLF) > tremolite (SGF) > chrysotile (SGF) when accounting for the greater surface area of chrysotile per mass or per fiber compared to tremolite. Silica release from chrysotile was 30–66 times greater under acid conditions as compared to neutral pH. The authors estimated that a chrysotile fiber will dissolve ~200 × faster in SLF and ~2.5 × faster in SGF compared to tremolite asbestos. The authors calculated that a 1 × 10 µm chrysotile fiber will completely dissolve in neutral pH in ~19 months while a tremolite fiber of equal shape will dissolve in ~4 years. At acid pH, a chrysotile fiber of the same dimensions will dissolve in ~33 h and a tremolite fiber will dissolve in ~9 months. The authors pointed out that these values represent approximate fiber lifetimes and do not account for changes in the surface area with respect to time, or for preferential dissolution sites such as crystal defects or edges. In addition, these times do not take into account the inflammatory processes in the lung that have been shown to occur with tremolite and their influence on dissolution rates.

In another study using a Gambles solution, Osmon-McLeod et al. (2011) assessed the durability of a number of fibers including long fiber amosite and long fiber chrysotile. In this study, the pH of the Gambles solution was adjusted to 4.5 to mimic that inside macrophage phagolysosomes, which the authors described as ''potentially the most degradative environment that a particle should encounter following lung deposition and macrophage uptake''. Fiber durability was assessed from the loss of mass of the fiber. The chrysotile was recovered with ~30% of original weight after the 24-week incubation. The amosite asbestos was recovered with ~75% of original weight. None of the carbon nano-tube samples included in the study showed a significant loss of mass by week 24 with one exception which was recovered at only ~70% of its original weight at all time-points from week 3 onward. The authors stated that for chrysotile, the percent recoveries reflect true mass loss, whereas the small mass loss for amosite asbestos over the 24-week period may be due to the loss of small fibers in the sample. The chrysotile showed no difference in average fiber width with incubation, but did show a marked decrease in length. At 0 weeks the chrysotile sample comprised a mixture of fibrils and ropes of fibrils, while at 10 weeks only small fibrils remained. The authors commented that it is probable that the measured loss of length accurately reflects fiber shortening in addition to the breaking up of large fiber bundles. Pathogenicity of these samples was also evaluated *in vivo* using a mouse model sensitive to inflammogenic effects of fibers. Osmon-McLeod et al. (2011) found that the data indicate that long fiber chrysotile showed ~70% mass loss and a marked decrease in length with long-term incubation in the Gambles solution, with a concomitant mitigation of the pathogenicity seen in mice injected with 0 weeks samples. Long fiber amosite that had been incubated for 10 weeks, however, also showed a loss of mass comparable to one of the long carbon nano-tubes at the same time-point, but no fiber shortening, and did not lose its pathogenicity.

These studies illustrate the differences in dissolution rates between chrysotile and amphibole asbestos under both neutral and acidic conditions and provide support for understanding the results of the inhalation studies discussed below.

## The relevance of early inhalation toxicology studies

The early inhalation toxicology studies of asbestos are often difficult to interpret. While they used rudimentary techniques

to quantify concentrations and in general were unable to measure the dimension of fibers, the early inhalation toxicology studies should not be completely disregarded as they did give some, although limited, information on possible worker exposures. Exposure concentration was determined using gravimetric techniques without consideration of fiber number or fiber length and diameter, and little consideration was given to the length and diameter distribution of the fibers to which the animals were exposed. To fluidize the fibers to facilitate aerosol generation, the fibers were usually ground extensively which shortened the length and produced a very large number of particles and shorter fibers (Timbrell et al., 1968).

In early inhalation studies, such as those reported by Vorwald et al. (1951), fiber dust concentrations in the exposure chamber were produced using a rotating paddle in a dust hopper. Aerosol concentrations were reported based upon light microscopy in the range of 30–50 million particles and fibers per cubic foot. This corresponds to approximately 500 000 particles and fibers/cm$^3$ if it were measured by TEM (Breysse et al., 1989). Subsequent studies such as those by Gross et al. (1967) based exposure on gravimetric concentration and reported a mean gravimetric concentration of 86 mg/m$^3$ (range 42–146 mg/m$^3$). There was no further characterization of the aerosol in this study. Following this, Wagner et al. (1974) reported on studies of UICC Canadian and Rhodesian chrysotile performed at a nominal concentration of 10 mg/m$^3$. This gravimetric concentration of 10 mg/m$^3$ became the standard concentration for subsequent studies by Wagner and other investigators through the 1980s with some investigators still reporting on studies at this exposure concentration more recently (e.g. Morris et al., 2004).

The historical chrysotile chronic inhalation studies are presented in Table A1 (Appendix). The exposure concentrations in all studies were based upon gravimetric determination. Of the 16 studies, six did not report the fiber concentration, eight reported estimates by phase contrast optical microscopy (PCOM) and three by scanning electron microscopy (SEM).

The two chrysotile samples used most often in these studies were either the UICC (Timbrell et al., 1968; Timbrell & Rendall, 1972) chrysotile or the NIEHS (Pinkerton et al., 1983) chrysotile. Both samples were ground extensively using large-scale milling machines.

The UICC chrysotile sample was milled using a ''Classic Mill designed by R. F. Bourne, at The Asbestos Grading Equipment Company, Johannesburg, South Africa'' (Timbrell et al., 1968). Timbrell & Rendall (1972) describe ''The Classic mill is an air swept attrition mill fitted with a disc rotor (16 inch diameter) which carries four beaters and is mounted on a horizontal shaft driven by an electric motor at speeds up to 5000 rpm''. The patent (Patent number GB 3,490,704) on the mill provides greater detail.

The characteristics of the NIEHS chrysotile can be obtained from the publication by Pinkerton et al. (1983). They refer to an NTIS report by Campbell et al. (1980) concerning the actual preparation of the sample. The NIEHS chrysotile was prepared from a grade 4 chrysotile used in the plastics industry, which was prepared by passing the material through a hurricane pulverizer. The hurricane pulverizer is an industrial high-speed impact hammer mill with a size classifier which recycled larger fibers/particles back into the device for continued milling (Perry & Chilton, 1973; Work, 1963).

Suquet (1989) assessed the structural damage produced by grinding and acid leaching of chrysotile and the surface state of ground and leached products. The author reported that ''Severe dry grinding converted chrysotile fibers into fragments cemented by a shapeless, non-crystalline material''. This comminution treatment apparently broke atomic bonds and produced strong potential reaction sites, which were able to adsorb $CO_2$ and $H_2O$ molecules from the atmosphere.

The number of fibers that would have been present in a chrysotile aerosol with a gravimetric concentration of 10 mg/m$^3$ has been estimated based upon a chronic inhalation study using NIEHS chrysotile (Hesterberg et al., 1993; Mast et al., 1995). In this study total fiber aerosol exposure was reported by SEM as 100 000 (World Health Organization) WHO* fibers/cm$^3$. If measured by TEM, this would have likely been more than 1 000 000 fibers/cm$^3$ (Breysse et al., 1989).

Exposure of rats to high aerosol concentrations of fibers creates a very different dose profile in the lung in comparison to human exposures. Rats are considerably smaller than humans and correspondingly rat lungs are more than 300 times smaller than human lungs. While the rat inhales proportionally less air per minute, the doses administered in some toxicology studies can result in unrealistic fiber lung burdens as compared to human exposure. In addition, for the rat which is a mandatory nasal breather, alveolar deposition is largely limited to fibers less than approximately 1 μm in diameter, while in humans this limit is approximately 3 μm (Morgan, 1995). For most asbestos fiber types, however, this difference is less important than for MMVF. The total chrysotile lung burden following 24 months of exposure in the Mast et al. (1995) study was $5.5 \times 10^{10}$ fibers/lung as measured by SEM (Bernstein, 2007). With extrapolation to that which would have been observed by TEM, the lung burden would have been $9.4 \times 10^{11}$ fibers/lung. This would correspond to an average of 2300 fibers per alveoli (assuming 10% deposition).

The gravimetric exposure concentrations ranged from 2 to 86 mg/m$^3$, which based upon the extrapolation described above (Breysse et al., 1989; Mast et al., 1995), corresponds to between 200 000 and 8 600 000 fibers/cm$^3$. The large majority of these earlier studies targeted 10 mg/m$^3$. The single study performed at the lowest concentration of 2 mg/m$^3$ had a comparative concentration group of 10 mg/m$^3$. In this study, the author's reported ''With a 2 mg/m$^3$ cloud the percentage retention of chrysotile is almost double that for a 10 mg/m$^3$ cloud'', which reflects the difficulty of evaluating dose response at these overload conditions.

This is illustrated in Wagner et al.'s (1974) study which had five exposure periods at the same exposure concentration of 10 mg/m$^3$. The exposure periods were (7 h/d, 5 wk) for either 1 d, 3, 6, 12 and 24 months with the animals maintained

*WHO fibres: defined as fibers >5 μm long, <3 μm wide and with length:width ratios >3:1; WHO (1985).

DOI: 10.3109/10408444.2012.756454



their lifetime. In the crocidolite exposed groups, the number of mesothelioma were 1 (1d grp), 1 (3m grp), 0 (6m grp), 2 (12m grp) and 0 for (24m grp). Thus, the 1 d of exposure produced more mesothelioma than the 24-month exposure most likely due to the effect of the high-exposure concentration, resulting with continued exposure in lung overload.

An asbestos exposure concentration of 10 mg/m$^3$ corresponds to more than 10 million times the American Conference of Industrial Hygienists (ACGIH) threshold limit value (TLV) of 0.1 fiber/cm$^3$ for asbestos.

The fiber size distribution and the ratio of longer fibers to shorter fibers and non-fibrous particle content are essential in determining the dose–response relationship to these fibers. Thus, it can become very difficult to use these studies for human risk assessment or even to compare the effects of one study with those of another.

The issue of using an equivalent fiber number for exposure was approached in a study reported by Davis et al. (1978) where chrysotile, crocidolite and amosite were compared on an equal mass and equal number basis. However, the fiber number was determined by phase contrast optical microscopy (PCOM) and thus the actual number, particularly of the chrysotile fibers, was probably greatly underestimated.

At such high exposure concentrations, it would be reasonable to expect that the number of particles and short fibers present in the exposure would be sufficient to overload the lung through impairment of macrophage function. These conditions which occurred in the earlier high gravimetric dose studies of ground chrysotile would be sufficient based upon studies with insoluble particles (Bolton et al., 1983; Morrow, 1988, 1992; Muhle et al., 1988; Oberdörster, 1995) to severely reduce the normal clearance of the chrysotile fibers from the lung and initiate a non-specific inflammatory and proliferative response which has been shown to lead for innocuous dusts to fibrosis and cancer. The following section discuses studies at several orders of magnitude above regulatory levels but without approaching the extremes discussed above.

## The correlation of fiber length and biopersistence to chronic toxicity

The association that long fibers (20–50 μm) have with both lung and peritoneal disease, as opposed to shorter ball-milled fibers (3 μm or less), was reported as early as 1951 (Vorwald et al., 1951).

The importance of fiber length in the pathogenicity of fibers in the pleural cavity was investigated by Stanton (1972, 1973) in a series of studies on the relationship of fiber length and characteristics to their pathogenicity in on the pleural surface. The fibers were evaluated using a highly artificial exposure by implantation in gelatin, and placing them on the pleural mesothelial surface. The authors reported that in this system, carcinogenicity was related to ''durable'' fibers longer than 10 μm.

Davis et al. (1986) evaluated the toxicological response in chronic inhalation and interperitoneal injection studies to samples of either short ($\sim<5$ μm) or long ($\sim>10$ μm) amosite asbestos with equal airborne mass concentration. The authors reported that in the inhalation study with LFA the long fiber caused the development of widespread pulmonary fibrosis and one-third of the animals developed pulmonary tumors that were mesotheliomas. In the group with short fiber amosite no fibrosis or pulmonary or mesothelioma tumors were found in any animal.

Poland et al. (2008) reported on a study in which carbon nanotubes were compared with short fiber and long fiber amosite asbestos following intraperitoneal injection. The amosite samples were prepared by Davis et al. (1986) for use in the studies discussed above. 50 mg of each material was injected into the peritoneal (abdominal) cavity of mice and the cavity systematically lavaged at 24 h or 7 d post exposure with physiological saline. The long fiber amosite developed inflammatory and granulomatous changes while the short fiber amosite did not.

In a study investigating the biopersistence of synthetic mineral fibers (SMFs), Hammad et al. (1988) found that fibers <5 μm in length had the longest retention following short-term inhalation, with longer fibers clearing more rapidly and fibers >30 μm in length clearing very rapidly. He proposed that clearance of mineral wools is a result of biological clearance and the elimination of fibers by dissolution and subsequent breakage. However, there was no relationship between these phenomena and long-term toxicological effects.

Adamson (1993, 1994) exposed mice to long and short crocidolite asbestos and found that long fibers (>20 μm), which were deposited in bronchiolar regions induced fibrosis and a proliferative response while short fibers (<1 μm), which reached the alveoli did not induce fibrosis and a proliferative response.

Lippmann (1990), McClellan et al. (1992), WHO (1988), and Goodglick & Kane (1990) reviewed as well the importance of fiber length to the potential of a fiber to induce a pathogenic effect.

In an analysis that provided the basis for the European Commission's directive on synthetic vitreous fibers (SVF), Bernstein et al. (2001a,b) reported that a good correlation exists for SVFs between the biopersistence of fibers longer than 20 μm and the pathological effects following either chronic inhalation or chronic intraperitoneal injection studies. This analysis showed that it was possible using the clearance half-time of the fibers longer than 20 μm as obtained from the inhalation biopersistence studies to predict the number of fibers longer than 20 μm remaining after 24 months of chronic inhalation exposure (Bernstein et al., 2007). These studies, however, only included SVFs.

Berman et al. (1995) statistically analyzed the results of 13 separate animal inhalation studies, which exposed animals to nine different asbestos types. Due to limitations in the characterization of asbestos structures in the original studies, new exposure measures were developed from samples of the original dusts, which were regenerated and analyzed by TEM. The authors reported that while no univariate model was found to provide an adequate description of the lung tumor responses in the inhalation studies, the measure most highly correlated with tumor incidence was the concentration of structures (fibers) ≥20 μm in length. However, using multivariate techniques, measures of exposure were identified which adequately described the lung tumor responses. The authors reported that

Table 1. Capabilities and limitations of analytical techniques used for asbestos measurements (reproduced from Berman & Crump, 2003)†.

| Parameter | Midget impinger | Phase contrast microscopy | Scanning electron microscopy | Transmission electron microscopy |
|---|---|---|---|---|
| Range of magnification | 100 | 400 | 2000–10 000 | 5000–20 000 |
| Particles counted | All | Fibrous structures‡ | Fibrous structures‡ | Fibrous Structures‡,§ |
| Minimum diameter (size) Visible | 1 μm | 0.3 μm | 0.1 μm | <0.01 μm |
| Resolve internal structure | No | No | Maybe | Yes |
| Distinguish mineralogy¶ | No | No | Yes | Yes |

†The capabilities and limitations in this table are based primarily on the physical constraints of the indicated instrumentation. Differences attributable to the associated procedures and practices of methods in common use over the last 25 years are highlighted in Table 2.
‡Fibrous structures are defined here as particles exhibiting aspect ratios (the ratio of length to width) greater than 3 (Walton, 1982).
§TEM counts frequently resolve individual fibrous structures within larger, complex structures. Based on internal structure, several different counting rules have been developed for handling complex structures. See the discussion of methods presented below.
¶Most SEM and TEM instruments are equipped with the capability to record selected area electron diffraction (SAED) spectra and perform energy dispersive X-ray analysis (EDXA), which are used to distinguish the mineralogy of structures observed.

> Structures contributing to lung tumor risk appear to be long (≥5 μm) thin (0.4 μm) fibers and bundles, with a possible contribution by long and very thick (≥5 μm) complex clusters and matrices. Potency appears to increase with increasing length, with structures longer than 40 μm being about 500 times more potent than structures between 5 and 40 μm in length. Structures <5 μm in length do not appear to make any contribution to lung tumor risk.

This analysis found no difference in the potency of chrysotile and amphibole regarding the induction of lung tumors. However, the authors stated that the mineralogy appears to be important in the induction of mesothelioma, with chrysotile being less potent than amphibole. These results, however, should be viewed in the context of the inhalation toxicology studies evaluated by Berman et al. (1995, Table 1), the majority of which were performed at very high concentrations (10 mg/m$^3$). As discussed above, the overload effect from these very high exposure concentrations would be expected to produce similar tumorigenic response in the lung for chrysotile and amphibole.

Recent studies on the serpentine asbestos, chrysotile, have shown that it is not very biopersistent in the lung (Bernstein et al., 2003, 2004, 2005a,b, 2011). As serpentine is a naturally occurring mined fiber, there appear to be some differences in biopersistence depending upon from where it is mined. However, chrysotile lies on the soluble end of this scale and ranges from the least biopersistent fiber to a fiber with biopersistence in the range of glass and stonewools. It remains less biopersistent than refractory ceramic fibers and special purpose glasses and more than an order of magnitude less biopersistent than amphibole asbestos (Bernstein, 2007). A 90 d sub-chronic inhalation toxicity study of chrysotile in rats showed that at an exposure concentration 5000 times greater than the US-ACGIH TLV of 0.1 f(WHO)/cm$^3$, chrysotile produced no significant pathological response or sustained inflammatory response (Bernstein et al., 2006).

Some earlier studies have shown chrysotile to clear less rapidly than in the studies performed using the EC protocol. An example is the study by Coin et al. (1992) in which rats were exposed for 3 h to a NIEHS chrysotile aerosol of 10 mg (respirable)/m$^3$ and then followed for a period of 29 d. The authors reported that through 3 weeks after cessation of exposure, fibers greater than 16 μm in length were cleared slowly, if at all.

While a brief description is provided, the details of the aerosol exposure to the NIEHS chrysotile which was used in the Coin et al. (1992) study are not described directly in the publication. However, the characteristics of the exposure aerosol and the preparation methods can be derived from an earlier publication by Pinkerton et al. (1983) referenced by Coin and a non-published report by Campbell et al. (1980) referenced by Pinkerton et al.

These publications describe that the chrysotile used by Coin et al. (1992) was prepared from a grade 4 chrysotile used in the plastics industry which was prepared by passing the material through a hurricane pulverizer. The hurricane pulverizer is an industrial high-speed impact hammer mill with a size classifier which recycled larger fibers/particles back into the device for continued milling (Perry & Chilton, 1973; Work, 1962).

The aerosol used in the Coin et al. (1992) study was generated from this ground material as described by Pinkerton et al. (1983) using a Timbrell generator (Timbrell, 1968). The stainless steel blades of this generator are known to further pulverize fiber samples. While the original chrysotile sample had 13.9% fibers longer than 19.9 μm (Campbell et al., 1980), the final aerosolized sample used in the Coin et al. (1992) study had 1.8% fibers longer than 19.9 μm (Pinkerton et al., 1983). For fibers ≥ 16 μm in length, Coin et al., only present the data graphically. Visual extrapolation from Figure 5 of Coin et al. indicates that there were approximately 2, 2, 5 and $4 \times 10^5$ fibers L ≥ 16 μm (measured by SEM) present at 1, 8, 15 and 29 d post-exposure, respectively, (no error bars were indicated and no tables of the values given). In addition, the Coin et al. (1992) study used a single exposure and examined sub-groups on animals for 3 weeks. The mean number of fibers found in the control animals was $7 \times 10^5$ WHO fibers per animal and $3 \times 10^3$ fibers ≥ 16 μm per animal, indicating contamination. No standard deviation is given, however, so the extent of this contamination remains unknown. Coin does not state how this contamination occurred. In the chrysotile studies performed following the EC protocol, animals were exposed for 5 d and then followed for 1 year post-exposure. In the EC protocol studies, no WHO fibers (including fibers

DOI: 10.3109/10408444.2012.756454



with $L > 20\,\mu m$) were observed in the lungs of any of the control animals.

## Non-overload studies that evaluate the toxicity of chrysotile

As discussed above, the early toxicology studies were difficult to interpret. Concentration was determined using gravimetric techniques without consideration of fiber number or fiber length and diameter and little consideration was given to the dose, and the length and diameter distribution of the fibers to which the animals were exposed.

### Chronic inhalation toxicity studies

While well-designed chronic inhalation toxicology studies limiting particle overload effects of SVFs have been performed, few chronic inhalation toxicology studies of asbestos have been performed taking this into account.

Davis et al. (1986) reported on the only chronic inhalation study that evaluated the pathogenicity of long versus short amosite asbestos. The short fiber amosite sample was produced so that almost all fibers were less than 5 μm in length with 70 WHO fibers/cm$^3$ in the exposure atmosphere. The LFA had 2060 WHO fibers/cm$^3$ with approximately half of this longer than 10 μm. The mass concentration of both groups was similar. The authors reported that following 12 months of exposure that significantly more short fiber amosite was present in the lung as compared to long fibers. The long fibers caused the development of widespread fibrosis, however, with the short fibers no fibrosis was found in any animal. In addition, one-third of the animals treated with long fibers developed pulmonary tumors or mesothelioma while no pulmonary neoplasms were found in the animals treated with short fibers. In parallel intraperitoneal injection studies also reported by Davis et al. (1986), the long fiber amosite produced mesothelioma in 95% of the animals treated while the short fiber amosite produced one mesothelioma over the same period.

McConnell et al. (1999) reported on a chronic inhalation study on amosite asbestos in hamsters in which the number of particles and shorter fibers were reduced while maintaining the number of fibers longer than 20 μm in the test atmosphere. The amosite aerosol concentration ranged from 10 to 69 long fibers (>20 μm)/cm$^3$ with exposure levels selected based upon a previous, multi-dose 90 d sub-chronic inhalation study (Hesterberg et al., 1999). At the high-dose amphibole amosite asbestos exposure of 263 WHO fibers/cm$^3$ (69 fibers $L > 20\,\mu m$/cm$^3$) 20% of the animals developed mesotheliomas with 82% of the animals developing mesothelial hyperplasia.

### Sub-chronic inhalation toxicity studies

The 90 d sub-chronic inhalation toxicity study has been used extensively in regulatory evaluation. The use of this and other shorter term studies for the evaluation of the toxicity and potential carcinogenicity of fibers was reviewed by an ILSI Risk Science Institute Working Group (Washington, DC) (Bernstein et al., 2005c). This working group was sponsored by the ILSI Risk Science Institute and the US Environmental Protection Agency Office of Pollution Prevention and Toxics(Washington, DC). The working group stated that current short-term testing methods, defined as 3 months or less in exposure duration, evaluate a number of endpoints that are considered relevant for lung diseases induced by fibers such as asbestos. Sub-chronic studies to assess biomarkers of lung injury (e.g. persistent inflammation, cell proliferation and fibrosis) are considered to be more predictive of carcinogenic potential than *in vitro* measures of cellular toxicity. Of particular importance in the evaluation of fiber toxicity using the 90 d sub-chronic inhalation toxicity study is the association reported by the Working Group based upon the available inhalation toxicology studies that:

> All fibers that have caused cancer in animals via inhalation have also caused fibrosis by 3 month. However, there have been fibers that have caused fibrosis but not cancer. Therefore, *in vivo* studies that involve short-term exposure of rat lungs to fibers and subsequent assessment of relevant endpoints, notably fibrosis, are probably adequately conservative for predicting long-term pathology – that is, will identify fibers that have a fibrogenic or carcinogenic potential (Bernstein et al., 2005c).

Bellmann et al. (2003) reported on a calibration study which compared the toxicity of a range of SVFs with different biosolubilities in a 90 d sub-chronic inhalation toxicity study. One of the SVFs tested was a calcium–magnesium-silicate (CMS) fiber, a relatively biosoluble fiber, for which the stock preparation had a large concentration of non-fibrous particles in addition to the fibers. In this study, due to the method of preparation, the aerosol exposure concentration for the CMS fiber was 286 fibers/cm$^3$ length < 5 μm, 990 fibers/cm$^3$ length > 5 μm and 1793 particles/cm$^3$, a distribution which is not observed in the commercial product. The total CMS exposure concentration was 3069 particles & fibers/cm$^3$. The authors pointed out that ''The particle fraction of CMS that had the same chemical composition as the fibrous fraction seemed to cause significant effects''. For the CMS fiber, the authors reported that the number of polymorphonuclear leukocytes in the bronchoalveolar lavage fluid was higher and interstitial fibrosis was more pronounced than had been expected on the basis of biopersistence data. In addition, the interstitial fibrosis persisted through 14 weeks after cessation of the 90 d exposure. This effect was attributed to the large number of non-fibrous particles in the exposure aerosol – 50% of the aerosol was composed of non-fibrous particles and short fibers.

By comparison, after chronic inhalation exposure of rats to another CMS fiber, X607 fiber, which had considerably fewer non-fibrous particles present (particles with an aspect ration of < 3:1), no lung tumors or fibrosis was detected (Hesterberg et al., 1998). This provides support for the argument that it was the large non-fibrous component of the CMS used in the Bellmann study and the resulting lung overload that caused the pathogenicity observed with this relatively biosoluble fiber. A similar overload mechanism might explain the results of earlier chrysotile inhalation studies, in which animals were exposed to much higher levels of non-fibrous particles and short (<5 μm) fibers.

Bernstein et al. (2006) reported on the toxicological response of a commercial Brazilian chrysotile following

exposure in a multi-dose sub-chronic 90 d inhalation toxicity study, which was performed according to the protocols specified by the US EPA (2001) and the European Commission (EUR 18748 EN, 1999).

In this study, male Wistar rats were exposed to two chrysotile levels at mean fiber aerosol concentrations of 76 fibers with $L>20\,\mu m/cm^3$ (3 413 total* fiber/$cm^3$ and 536 WHO fiber/$cm^3$) or 207 fibers $L>20\,\mu m/cm^3$ (8941 total fiber/$cm^3$; 1429 WHO fiber/$cm^3$). The animals were exposed using a flow-past, nose-only exposure system for 5 d per week, 6 h/d, during 13 consecutive weeks followed by a subsequent non-exposure period of 92 d. Animals were sacrificed after cessation of exposure and after 50 and 92 d of non-exposure recovery. At each sacrifice, the following analyses were performed on sub-groups of rats: lung burden; histopathological changes; cell proliferation; inflammatory cells in the broncho–alveolar lavage; clinical biochemistry and confocal microscopic analysis.

Exposure to chrysotile for 90 d followed by 92 d of recovery, at a mean exposure of 76 fibers with $L>20\,\mu m/cm^3$ (3413 total fiber/$cm^3$) resulted in no fibrosis (Wagner score 1.8–2.6) at any time-point. At an exposure concentration of 207 fibers $L>20\,\mu m/cm^3$ (8941 total fiber/$cm^3$), slight fibrosis was observed. In comparison with other studies, the lower dose of chrysotile produced less inflammatory response than the biosoluble synthetic vitreous CMS fiber referred to above, and considerably less than amosite asbestos (Bellmann et al., 2003).

These similarly designed 90 d inhalation toxicity studies show that the pathological response from exposure to chrysotile is similar or less than that of SVFs.

### Shorter term inhalation toxicity studies

In a short-term exposure study in rats (6 h/d, 5 d) with the amphibole tremolite asbestos at an exposure concentration of 100 long fibers ($>20\,\mu m$)/$cm^3$ and 2016 total fiber/$cm^3$, extensive inflammatory response was observed immediately after the end of the 5 d exposure and interstitial fibrosis developed within 28 d after cessation of the 5 d exposure (Bernstein et al., 2005b).

In a recent study by Bernstein et al. (2010, 2011), the pathological response and translocation of a commercial chrysotile product similar to that which was used through the mid-1970s in a joint compound intended for sealing the interface between adjacent wall boards was evaluated in comparison to amosite asbestos. This study was unique in that it presented a combined real-world exposure and was the first study to investigate whether there were differences between chrysotile and amosite asbestos fibers in time course, size distribution and pathological response in the pleural cavity. Rats were exposed by inhalation for 5 d (6 h/d) to either sanded joint compound consisting of both chrysotile fibers and sanded joint compound particles or amosite asbestos.

The mean fiber number was 295 fibers/$cm^3$ for chrysotile and 201 fibers/$cm^3$ for amosite. The mean number of WHO fibers in the chrysotile fibers and sanded joint compound particle atmosphere was 1496 fibers/$cm^3$, which was more than 10 000 times the OSHA occupational exposure limit of 0.1 fibers/$cm^3$. The amosite exposure atmosphere had fewer shorter fibers, resulting in a mean of 584 WHO fibers/$cm^3$.

An important part of the Bernstein et al. (2010, 2011) study was to design procedures for evaluation of the pleural space while limiting procedural artifacts. These methods included examination of the diaphragm as a parietal pleural tissue and the *in situ* examination of the lungs and pleural space obtained from freeze-substituted tissue in deeply frozen rats. The diaphragm was chosen as a representative parietal pleural tissue because at necropsy it could be removed within minutes of sacrifice with minimal alteration of the visceral lung surface. The area of the diaphragm chosen for examination included an important lymphatic drainage site (stomata) on the diaphragmatic surface. The use of both confocal microscopy and SEM enabled the identification of fibers as well as examination of the pleural space, *in situ*, for possible inflammatory response. The examination of the pleural space *in situ* including the lung, visceral pleura and parietal pleura in rats deeply frozen immediately after termination provided a non-invasive method for determining fiber location and inflammatory response.

No pathological response was observed at any time-point in the chrysotile fibers and sanded joint compound particles exposure group. The long chrysotile fibers ($L>20\,\mu m$) cleared rapidly ($T_{1/2}$ of 4.5 d) and were not observed in the pleural cavity. In contrast, a rapid inflammatory response occurred in the lung following exposure to amosite resulting in Wagner grade 4 interstitial fibrosis within 28 d and which persisted through 90 d (histopathology was evaluated through 90 d post exposure as the animals were allocated to the confocal analyses from 181 to 365 d post exposure). Long amosite fibers had a biopersistence of $T_{1/2}>1000$ d in the lung and were observed in the pleural cavity within 7 d post exposure. By 90 d, the long amosite fibers were associated with a marked inflammatory response on the parietal pleura. This study provides support that in contrast to amosite asbestos, exposure to chrysotile fibers and joint compound particles following short-term inhalation would not initiate an inflammatory response in the lung, and that the chrysotile fibers present following this exposure do not migrate to, or cause an inflammatory response in the pleural cavity, the site of mesothelioma formation.

These studies provide further confirmation of the differences between exposure to chrysotile alone and to chrysotile mixed in a joint compound and amphibole asbestos.

### What do the toxicology studies indicate?

The more recent toxicology studies summarized above demonstrate that chrysotile asbestos has a relatively short biopersistence and does not result in pathological response even through 90 d of exposure (Bernstein et al., 2006). These studies also confirm the difference between chrysotile and amphibole asbestos which is highly persistent in the lung and results in a fibrotic response even after 5 d of exposure (Bernstein et al., 2005b, 2010, 2011).

This is mirrored in pathological response to chrysotile and amphibole asbestos following both short-term (5 d of

*Total fibers: all objects with a length:diameter aspect ratio greater than 3:1

DOI: 10.3109/10408444.2012.756454



exposure) (Bernstein et al., 2005b, 2010, 2011) and long-term (90 d of exposure) repeated dose inhalation exposure to well-defined chrysotile aerosols in the rat (Bernstein et al., 2006) and following chronic exposure to amosite in the hamster (McConnell et al., 1999).

Following such exposures, chrysotile asbestos produces neither a pathological response in the lung nor in the pleural cavity at doses up to 5000 times the US TLV for chrysotile. In the 90 d exposure study (Bernstein et al, 2006), at an exposure concentration more than 14 000 times the TLV, slight fibrosis was observed. In addition, the chrysotile fibers clear rapidly from the lung and are not observed at the visceral pleural surface, neither in the pleura nor on the parietal pleural surface.

The amphibole asbestos fibers tremolite and amosite have thus far been evaluated. In the lung, immediately following a 5 d exposure, the amphibole fibers have been shown to produce extensive inflammation with granuloma formation. With 28 d after cessation of exposure, interstitial fibrosis (Wagner grade 4) was observed with both tremolite and amosite. Both of these fibers were poorly cleared from the lung with the fibers longer than 20 μm persisting through the end of the study (365 d post exposure) (Bernstein et al., 2005b, 2010, 2011).

The pleural transfer was also evaluated for amosite asbestos. Within 2 weeks following cessation of the 5 d exposure, amphibole fibers were observed at the visceral pleural surface and were associated with extensive inflammation and fibrotic development. Amphibole fibers were observed penetrating the visceral pleura and extending in the pleural cavity. Inflammation was also observed on the parietal pleural surface (Bernstein et al., 2010, 2011).

The study by Osmon-McLeod et al. (2011), which reported that long fiber chrysotile showed ~70% mass loss and a marked decrease in length with long-term incubation in a Gamble's solution which was adjusted to mimic that inside macrophage phagolysosomes provides a basis for understanding the rapid clearance of chrysotile.

These studies strongly suggest that even short exposures to amphibole can influence the pathological development in the lung and pleural cavity and provide a new perspective in understanding and differentiating the results presented in epidemiology studies of chrysotile and amphibole asbestos exposed cohorts.

## Epidemiology studies

While chrysotile is currently used largely in high-density cement products, the epidemiological and regulatory evaluation of chrysotile is based upon a cross section of all uses in the past. Of particular importance for understanding the implications of the current use of chrysotile are those studies characterized as chrysotile only. Those studies characterized as chrysotile only are reviewed below in light of the toxicological studies, which indicate the importance of even short-term exposure to amphibole asbestos in causing disease.

The early case-control studies of mesothelioma provided relationships of occupational exposure to asbestos (Ashcroft, 1973; Elmes & Wade, 1965; Hain et al., 1974; McDonald et al., 1970; McEwen et al., 1970; Newhouse & Thompson, 1965; Rubino, 1972; Zielhuis et al., 1975). However, due to the state of occupational hygiene measurements at the time, none of the studies were able to use exposure measurements which included fiber number or fiber type. The associations to disease were attributed to the fiber most used without consideration of the criteria that have been understood more recently to determine fiber potency: biopersistence and fiber length. In addition, the lack of complete occupational histories is a significant limitation in the early epidemiology studies, resulting in improper characterization of fiber-specific exposure.

Berman & Crump (2003) summarized the various limitations that likely influence the epidemiological evaluations and that had to be addressed in order to assess the uncertainty in the available epidemiology studies. These included:

- limitations in air measurements and other data available for characterizing historical exposures;
- limitations in the manner that the character of exposure (i.e. the mineralogical types of fibers and the range and distribution of fiber dimensions) was delineated;
- limitations in the accuracy of mortality determinations or incompleteness in the extent of tracing of cohort members;
- limitations in the adequacy of the match between cohort subjects and the selected control population and
- inadequate characterization of confounding factors, such as smoking histories for individual workers.

In addition, the capabilities and limitations of the analytical techniques used for determining the asbestos exposure measurements in these epidemiological studies were summarized as shown in Table 1. Midget impinger (MI) and phase contrast microscopy (PCM) were the two analytical techniques used to derive exposure estimates in the majority of epidemiology studies from which the existing risk factors were derived. However, the MI and PCM measurements did not determine fiber length which has been shown to be related to biological activity.

With few exceptions, little to no quantitative sampling was conducted prior to the 1960s when exposure concentrations were generally considered to be higher than those monitored more recently, due to lack of use of dust control equipment at the time and procedures to reduce dust levels that were introduced only later. For most studies, therefore, early exposures had to be estimated by extrapolation from later measurements (Berman & Crump, 2003).

In particular, as a result of the measurement techniques, there was often little quantitative exposure information on the types of fibers to which workers were exposed. The nature of the industrial process may have suggested the type of fiber used. However, in the past there was little attempt to differentiate serpentine from amphibole asbestos, and as a result amphibole was often substituted or mixed with serpentine without detailed documentation. The use of amphibole in place of serpentine resulted from such factors as availability, cost and effectiveness in the process. In addition, work histories of employees were not always as well documented as might occur today (Berman & Crump, 2003).

While all uncertainty factors are important in assessing the difference between chrysotile and amphiboles, the differentiation of the fiber type in the exposure atmosphere is obviously

critical in determining possible effects associated with each type of fiber. Of equal importance is the number of fibers in the exposure atmosphere with length greater than approximately 20 μm, that is, those fibers which are not readily phagocytized and removed from the lung by macrophages and which therefore have greatest potential in producing disease if they do not readily break apart or dissolve in the lung fluids.

An additional issue which is often not well addressed is that of possible exposures to asbestos either prior to employment or concurrent to employment in the industry under study and consequently the fiber types to which the individuals were exposed.

## Evaluation of epidemiology studies considered in earlier evaluations

Hodgson & Darnton (2000) reviewed asbestos exposed cohorts which gave information on exposure levels from which (as a minimum) a cohort average cumulative exposure could be estimated. In another review, Berman & Crump (2008) also assessed the health risks associated with ''asbestos'' exposure also using the cohorts in which they determined that there was sufficient information to estimate exposure.

In both of these evaluations, the authors classified the cohorts by asbestos fiber type based on what was reported in the cited publications. That is whether they considered the cohort exposed to chrysotile alone, a mixture of chrysotile with amphibole asbestos, or to amphibole asbestos alone. These assessments were made from the then currently available literature and presented potential biases based upon the published data.

These studies are reviewed here in light of current data and the information learned from the toxicology studies on the importance of fiber type and fiber length in producing a pathological response in the lung and the pleural cavity.

### *Studies characterized as predominately chrysotile exposure*

It is interesting to note that the authors of very few of the epidemiology studies on asbestos were able to state that there was no amphibole exposure present in the cohort. Hodgson & Darnton (2000) considered the following studies which were characterized as predominately chrysotile exposure (Table 2) and stated that very small quantities of amphibole fiber were ignored as being important to the findings in some cohorts (South Carolina, New Orleans plant 2, CT).

Similarly, Berman & Crump (2008) considered the same cohorts as being exposed to chrysotile and considered other possible exposure either within the plant in question, or before or concurrent to employment as not important.

Table 2. Epidemiological studies characterized as predominately chrysotile exposure by Hodgson & Darnton (2000).

| Study | Referred to as: |
|---|---|
| Dement et al. (1994) and McDonald et al. (1983) | South Carolina |
| Piolatto et al. (1990) | Balangero Italian mine and mill |
| Liddell et al. (1997) | Quebec |
| Hughes et al. (1987) | New Orleans (plant 2, y) |
| McDonald et al. (1984) | Connecticut |

At the time the exposures took place, in none of these cohorts were the type of fibers to which workers were exposed actually determined from air samples, and in none of these studies were the fiber length distributions of the fibers determined in the workplace. While some investigators have attempted to recreate the work environment, experience with fiber aerosol generation in animal toxicology studies strongly indicates that accurately recreating all the factors which influence fiber size and distribution would be very difficult.

The results from Hodgson & Darnton (2000) for these studies for lung cancer and mesothelioma are presented in Table 3.

### *Fiber lung burdens: Charleston, South Carolina, and Quebec*

The analysis of the types and numbers of fibers found in lung tissue of individuals exposed to asbestos provides the most robust indicator of past exposure. While in general, such analyses were not performed, in two of the above-mentioned studies, fiber lung burdens were analyzed to determine the type and quantity of fibers present in the samples analyzed.

The lung burden analyses provide an indication to which fibers the workers were exposed. The samples were usually taken from lung biopsy sections or at necropsy and were often from paraffin blocks. As an example, in the Sebastien et al. (1989) study, the samples analyzed were around 1 g (personal communication, P. Sebastien). As such, only a small portion of the lung was analyzed.

Sebastien et al. (1989) reported in the analysis of 161 lung tissue samples taken at necropsy from asbestos textile workers in Charleston, South Carolina and Quebec miners and millers, both exposed to chrysotile. The authors reported that while chrysotile, tremolite, amosite, crocidolite, talc-anthrophyllite and other fiber types (included rutile, micas, iron, silica and unidentified silicates) fibers were found in both cohorts tremolite predominated. Non-trivial concentrations (>0.1 f/μg) of amosite and crocidolite were measured in 32% of specimens from Charleston, SC and 9% from Thetford, VT. The analysis indicted that in Charleston, commercial amphiboles were detected only in cases hired before 1940; no crocidolite was detected in cases hired after 1940. In Thetford, concentrations greater than 0.1 f/μg were measured in five cases.

Churg et al. (1984) analyzed the fiber lung content from six cases with mesothelioma derived from a series of approximately 90 autopsies of long-term workers in the Quebec chrysotile industry. These six cases represented all the mesotheliomas present in the series of 90 cases. The authors reported that the patients with mesothelioma having only chrysotile ore components had a much higher ratio of tremolite group amphiboles (9.3) than chrysotile fibers (2.8) compared to the control group. This was not true for one patient in whom amosite was found.

Pooley & Mitha (1986) in reporting on the determination and interpretation of the levels of chrysotile in lung tissue included result from the South Carolina textile workers in their Table 2 which compared the calculated mean values mass per 1000 fibers of asbestos obtained from lung tissue

DOI: 10.3109/10408444.2012.756454

Table 3. Studies characterized as predominately chrysotile exposure (Hodgson & Darnton, 2000).

| Study | Exposure estimates | Smoking histories | Fiber specificity | Lung cancer risk (% expected lung cancer per fiber/cm$^3$ year)† $R_L$ (95% CI) | Mesothelioma risk (% total expected mortality per fiber/cm$^3$ year)† $R_{M\ \text{age adjusted}}$ 95%CI |
|---|---|---|---|---|---|
| South Carolina: Dement et al. (1994) and McDonald et al. (1983) | MI measurements 1930–1975<br>In 1968 and 1971, both impinger and PCM samples were collected (a total of 986 samples) | Based on two surveys conducted by the U S Public Health Service in 1964 and 1971 and on data collected by the company | Chrysotile textile plant. Crocidolite yarn was used in small quantities to make tape or braided packing from 1950s until 1975 | Women 6.7 (3.6, 11)<br>Men 4.6 (2.9, 6.7) | Women 0 (0.0, 0.35)<br>Men 0.013 (0.0016, 0.047) |
| Balangero: Piolatto et al. (1990) | Fiber levels were measured by PCM in 1969. In order to estimate earlier exposures, information on daily production, equipment changes, number of hours worked per day, etc. were used to create conditions at the plant during earlier years. PCM samples were obtained under these simulated conditions and combined with work histories to create individual exposure histories | No information on smoking | Chrysotile mine and mill with presence of Balangeroite fiber | 0.03 (−0.11, 0.24) | 0.0025 (0.0003, 0.009) |
| Quebec: Liddell et al. (1997) | MI measurements<br>Conversions between dust levels and PCM concentrations were derived from side-by-side samples | Smoking history was obtained in 1970 by a questionnaire administered to current workers, and to proxies of those who had died after 1950 | 1. Chrysotile mine and mill at the town of Asbestos<br>2. Factory at the town of Asbestos that, in addition to processing chrysotile, had also processed some crocidolite<br>3. Chrysotile mining and milling company complex near Thetford Mines (evidence of greater amounts of tremolite in the ore at | 0.06 (0.042, 0.079) | 0.0009 (0.0006, 0.0013) |

(continued)

Table 3. Continued

| Study | Exposure estimates | Smoking histories | Fiber specificity | Lung cancer risk (% expected lung cancer per fiber/cm$^3$ year)† $R_L$ (95% CI) | Mesothelioma risk (% total expected mortality per fiber/cm$^3$ year)† $R_{M\ \text{age adjusted}}$ 95%CI |
|---|---|---|---|---|---|
| | | | Thetford Mines)<br>4. Number of smaller mines and mills also in the vicinity of Thetford Mines | | |
| New Orleans (plant 2): Hughes et al. (1987) | MI measurements initiated in the early 1950s<br>Levels estimated from initial samples in the 1950s were also assumed to hold for all earlier periods because no major dust control measures had been introduced prior to that time<br>In plant 2, the revised estimates tended to be about one-third of the previous estimates through the 1940s and about one-half the previous estimates thereafter | Based upon a cross sectional study of over 95% of workers employed in these plants in 1969. Information concerning the smoking habits of earlier workers in these plants is not available | Plant 1: Some amosite was used from the early 1940s until the late 1960s, constituting about 1% of some products, and crocidolite was used occasionally for approximately 10 years beginning in 1962<br>Plant 2: Utilized only chrysotile, except that pipe production, which began in 1946 and was housed in a separate building, produced a final product that contained about 3% crocidolite | 0.81 (0.21, 1.6) | 0 (0, 0.033) |
| Connecticut: McDonald et al. (1984) | Dust levels from impinger measurements were available for the years 1930, 1935, 1936 and 1939. There was little other exposure information available until the 1970s. No conversion from MPPCF to fiber/cm$^3$ value was suggested by the authors | No information on smoking | Plant that manufactured asbestos friction products. The plant began operation in 1913 and used only chrysotile until 1957, when a little anthophyllite was used. Also, a small amount of crocidolite (about 400 pounds) was handled experimentally between 1964 and 1972 | 0.80 (0.029, 1.8) | 0 (0, 0.016) |

†Risk estimates as determined by Hodgson & Darnton (2000).

DOI: 10.3109/10408444.2012.756454



extracts. They reported that South Carolina textile plant cases had 0.032 ng/$10^3$ fibers of chrysotile compared with 1.19 ng/$10^3$ fibers crocidolite and 2.098 ng/$10^3$ fibers amosite. In addition, the South Carolina control lung tissues had 0.015 ng/$10^3$ fibers chrysotile and 0.725 ng/$10^3$ fibers amosite.

Case et al. (2000) evaluated asbestos fiber type and length in lungs of fibers longer than 18 μm in length in chrysotile textile from the South Carolina cohort and chrysotile miners/milers from the Thetford Mines portion of the Quebec cohort. Lung samples were obtained from either deparaaffinized paraffin blocks or formalin fixed tissues and were chemically digested in commercial bleach. The authors stated that the lung retained fiber measurements were limited in inference as the results represented only the fraction of internal dose that was retained until death. In addition, they could not be certain to what degree the groups of chrysotile miners/millers and textile workers were representative of the cohorts from which they were derived. The results obtained closely paralleled those reported by Sebastian et al. (1989). The Case et al. (2000) results indicated that the ''chrysotile only'' textile workers had a high proportion of individuals with lung tissue containing amosite and/or crocidolite. The results did not support a role of the fiber length alone in explaining the greater lung cancer risk in textile workers. The authors concluded that ''this subset of the Charleston textile workers does not support the hypothesis that this is a pure chrysotile cohort'' (WHO, 1998). In addition, they stated that ''the exposure experience of textile workers is clearly unique and should not be used to assess risk of lung cancer in miners, cement workers or friction products workers, regardless of fiber type''.

In these two cohorts, the hypothesis that exposure was to chrysotile only is not supported from the lung burden measurements.

### *Discussion of the predominately chrysotile epidemiology studies*

In addition to the analysis of lung burden in the two studies presented above, each of the studies characterized as predominately chrysotile have been examined for the presence of amphibole asbestos in the exposure and the evaluation of other factors in the study design which could have influenced the results.

*South Carolina cohort.* In the analyses presented by Hodgson & Darnton (2000) and Berman & Crump (2008), the South Carolina cohort stands out as the study which reports a carcinogenic potential attributed to the use of ''chrysotile'' in the textile plant. The South Carolina cohort (Dement & Brown, 1994; Hein et al., 2007) is very interesting because it involved the use of textile grade chrysotile fibers. The authors acknowledge that small quantities of crocidolite (approximately 2000 pounds) were used in the plant in separate processes and concluded that this use was isolated and did not influence possible exposures in the textile plant. Dement et al. (1982) reported on a study of this factory and observed a large excess of lung cancer corresponding to an standardized mortality ratio (SMR) of 500 at 100 fiber-years/$cm^3$ which was reported as statistically significant as compared to the control cohort This study is in pronounced contrast to any other study where there was exposure only to chrysotile. As presented in the above section, the lung burden measurements on workers from this cohort indicate that both amosite and crocidolite were present in the workers' lungs.

In reviewing this study, the following important factors which would influence the results are apparent:

(1) Very close proximity to US Navy base which used large amounts of amosite
(2) Close proximity to other facilities using potentially toxic materials
(3) Possible prior use of amphiboles

(1) *Very close proximity to US Navy base which used large amounts of amosite*

The plant (General Asbestos & Rubber Co. known as GARCO) was located in North Charleston within a few hundred meters of the US Navy base in Charleston (Figure 3). This base was very active leading up to and during WWII and as Dement mentions employed 29 000 people building and repairing military ships. The Navy base opened in 1909 and during the war years, 1359 vessels were worked at the shipyard: damaged ships were repaired, combat vessels overhauled and 253 warships were constructed and launched. Nearly every military ship at the time was insulated using large quantities of amphibole asbestos (Balzer & Cooper, 1968; Bowles & Barsigian, 1954; Bowles & Stoddard, 1933; Virta, 2005). This process also involved the use of potentially toxic substances* in addition to the extensive use of amphibole asbestos. Dement et al. do not consider this important and do not factor into the analysis the possible influence of the emissions from the base nor the industrial area immediately adjacent to the GARCO plant.

(2) *Close proximity to other facilities using potentially toxic materials*

Close proximity to other facilities using potentially toxic materials is of importance as the predominate finding in the Dement et al. study is lung cancer with a potential of other substances contributing to possible causality.

There is no consideration of the Naval Weapons Station Charleston which occupies 17 000 acres of land – seven times larger than the Naval Shipyard site which was commissioned in 1941 and located on the western shore of the Cooper River just north of the GARCO plant. The Naval Weapons Station Charleston had a production capacity for more than 60 million pounds of conventional ordnance. Among other industries that could affect the health of the Charleston workers was the Rollins Chemical Company established in 1914 in South Charleston. Adjoining the Rollins plant on the west was the Warner–Klipstein plant, starting in 1915 as a producer of chlorine and chlorine products. This plant, reorganized in 1928 as the Westvaco Chlorine Products Corporation, became an important manufacturer of caustic, chlorine and

*OSHA 29 CFR Part 1915: coal tar pitch volatile, 4-nitrobiphenyl, alpha-naphthylamine, methyl chloromethyl ether, 3,3′-dichlorobenzidine (and its salts), bis-chloromethyl ether, beta-naphthylamine, benzidine, 4-aminodiphenyl, ethyleneimine, beta-propiolactone, 2-actylaminofluorene, 4-dimethylaminoazobenzene, nitrosodimethylamine, vinyl chloride, inorganic arsenic, lead, benzene, acrylonitrile, ethylene oxide, formaldehyde, asbestos.


Figure 3. Map of North Charleston showing the location of the Textile plant (GARCO) and the US Navy Yard. The distance from GARCO to the Navy Yard is a few hundred meters. The width of the map is approximately 3.5 km.

chlorinated compounds. The Carbide and Carbon Chemicals Company moved to South Charleston from Clendenin in 1925 and began operations in buildings acquired from the Rollins Chemical Company. Currently it is a division of Union Carbide Corporation, the company was a producer of more than 400 chemicals, plastics and fibers from derivatives of natural gas and petroleum.

(3) *Amphibole asbestos exposure in the cohort population*

In a report predating Dement et al. (1994), Dreesen et al. (1938) stated that ''Approximately 90% of the asbestos used in these plants is obtained from Canada. The remaining 10% comes from Arizona or South Africa, and, infrequently, from Russia and Australia''. While no specifics on fiber type were provided, South Africa was a large supplier of the blue and brown amphibole asbestos, crocidolite and amosite asbestos while Australia supplied crocidolite asbestos.

As presented above, the environment within Charleston had unique sources of pollutants from industrial and military operations that would very likely influence the cancer and mortality incidence of the region. This is reflected in the much higher mortality rate in Charleston compared to the US average.

Dement et al. supports the use of the US mortality rates stating ''it is difficult to estimate the exact number of persons ever employed at this plant; however, this is likely to exceed 10 000 prior to 1965''. They do not consider the larger number of persons that worked just a short distance from the plant at the Naval ship yard.

The US mortality rate was reported by the authors as 39 per 100 000 over the period 1950–1969. The US National Cancer Institute (Devesa et al., 1999) provides the mortality rate for Charleston over the period 1950–1969 as 101.5 which is 2.6 times the rate used in Dement et al. (1982). As GARCO provided housing for its employees in North Charleston and considering the proximity of this neighborhood to the Navy base and other installations, it is likely that the local mortality rate was even higher than 101.5. While the issue of which rate would be most appropriate is difficult to reconstruct, the available information indicates that the rate used underestimates the control background level.

Another issue which is not addressed in the Dement et al. (1982) study is that of prior and or concurrent exposures or exposures through family members. It would not be unreasonable to expect that GARCO employees and or

DOI: 10.3109/10408444.2012.756454



family members had prior work experience in the military or in other industries. A brief internet search of recently published death summaries (The Post and Courier, Charleston, SC) shows individuals such as:

- Marine Corps and Merchant Marines veteran and retired supervisor for GARCO.
- Long term employee of GARCO Mill and a retired owner/operator of – Garage for 26 years. He also served his country in the US Army. He was an automobile enthusiast and loved racing and working on vehicles.
- Army veteran, retired employee of GARCO
- Occupation: GARCO, retired Contractor, self-employed military: US Merchant Marine, WW II veteran
- Formerly worked at GARCO, the Charleston Navy Exchange and the former Geer Drug Company
- Machinist with GARCO and a retired employee with the Charleston Naval Shipyard
- Navy veteran, retired employee with GARCO

Hein et al. (2007) stated that in addition to a lack of smoking histories for all of the cohort members that the findings reported were subject to additional limitations including incomplete lifetime work histories and high rates of loss to follow-up, especially among female workers. The idea that the population studied worked uniquely at GARCO is neither supported in the Dement et al. (1982) nor the Hein et al. (2007) publications.

*Other factors influencing lung cancer incidence*

Dement et al. (1982) state that one of the most important factors which need to be considered in evaluating the occupational contribution to observed mortality patterns are cigarette smoking patterns among the cohort. They showed in Table 9 that the prevalence of cigarette smoking among 292 out of the 768 asbestos study cohort members was similar to that of the US white adult males (1965). For the other 475 cohort members, no information on smoking was provided. This was based upon a classification of current smoker, past smoker or non-smoker. However, no information was provided on the smoking incidence in the asbestos cohort and how this compares to the US white adult males. For those workers who had also been in the military, the military rates of tobacco and alcohol use have been reported as higher than those found in comparable civilian sectors (Ballweg & Brey, 1989; Bray et al., 1989, 1991; Conway et al., 1989; US DHHS, 1989).

The authors determined a conversion from the MI measurements in millions of particles per cubic foot of air (MPPCF), to membrane filter counts, measured as fibers longer than 5 μm/cm$^3$ using concurrent samples by these two methods in plant operations collected during 1968–1971. The authors reported that for textile operations, except preparation, a conversion of 3 fiber/cm$^3$ for 1 MPPCF was used while for preparation a conversion of 8 fiber/cm$^3$ was used. The 95% confidence limits on these conversions were estimated as 3 fiber/cm$^3$ (CI 2.5–3.5) and as 8 fiber/cm$^3$ (CI 5–9).

In subsequent analyses of occasional samples of air filters from the South Carolina plant the authors reported that, ''Only two fibers of the 18 840 fiber structures (0.01%) were found to be amphiboles and the remainder were chrysotile based on morphology'' (Stayner et al., 2008). As presented above, several studies have analyzed the fiber content of lungs from workers and have shown the presence of significant quantities of amphiboles. Stayner et al. (2008) did not report the presence of even tremolite fibers, this was perhaps due to using a physical morphology based analysis rather than chemical based identification techniques (EDAX, or the Addison & Davies, 1990).

Green et al. (1997) examined pulmonary fiber burdens in a necropsy population in 39 former workers from the South Carolina textile plant and 31 controls. The authors reported that the grade of pulmonary fibrosis correlated better with the tremolite asbestos concentration than the chrysotile concentration. They also found that the geometric mean concentrations for amosite and crocidolite asbestos were higher in the textile plant workers than in the controls. They reported that 28% of the textile asbestos workers and 13% of the controls had values of crocidolite or amosite asbestos in their lungs which exceeded 1 million fibers per g dry lung [a value considered above background for that lab at that time]. These amphibole concentrations could easily explain the small number of mesotheliomas which occurred in the cohort.

The above information strongly suggests that The South Carolina textile workers were exposed to amphiboles and other causative agents (pollutants, smoking) either directly or indirectly which confounds the understanding of what exposure produced the lung cancer and mesothelioma.

Based upon the more recent inhalation toxicology studies of amphiboles, that even short exposures to amphibole asbestos in the South Carolina textile plant or through prior or para-occupational exposure could have significantly impacted the results. The recent work by Bernstein et al. (2010) has confirmed that amphibole asbestos fiber types are much more potent than chrysotile asbestos and that with such a differential in response, even small amphibole exposure could have had a significant influence on the findings reported in the South Carolina cohort. McDonald et al. (1983) attributed the cancer incidence to the small amount of tremolite present in the mine. Analyses have shown that the tremolite was present in quantities of less than 1% and showed that the amphibole accumulated with time in the lung while the chrysotile did not. With a larger potential for exposure to amphibole asbestos and other pollutants than originally perceived in the South Carolina cohort, it is clear that the South Carolina cohort was not a pure chrysotile cohort as originally postulated.

*Piolatto et al. (1990).* Piolatto et al. (1990) reported on the analyses of a cohort of asbestos workers from the Balangero mine in Italy. The authors reported that

> examination of several samples of chrysotile from the mine ruled out the presence of contamination with fibrous amphiboles at detectable concentrations. A fibrous silicate (balangeroite) was characterised, however, consisting of brown, rigid and brittle xyloid fibers with a complex structure similar to gageite, usually intergrown with chrysotile.

The Balangeroite fiber was reported as accounting for 0.2–0.5% of the total mass of samples of chrysotile as commercialized from the Balangero mine. There is no

mention of the actual concentration in the mine pit. The authors stated as well that ''Nothing is at present known about its adverse effects, although they can be suspected on the basis of its fiber dimensions being similar to those of amphiboles''.

Silvestri et al. (2001) summarized information on work practice, fiber concentration and health-related effects in the workers at the Balangero mine and in the population of the surrounding area. The authors stated that in addition to chrysotile, Balangeroite, a fibrous magnesium-iron silicate first discovered at Balangero is present in the ore and that it is very similar, from a morphological point of view, to amphiboles. From its opening in 1930 there were no exposure controls at the mine until the 1960s and no standard was imposed until 1986 when the European directive was implemented in Italy. The authors cited a report from the 1940s that ''The damage is not so bad for the trees and plants, but rather for the cows, as the dust is often so deep on the grass that they can't pasture''. Estimated exposure concentrations in the mine exceeded 50 fiber/ml; in the crushing area 120 fibers/ml; in the fiber selection area 235 fibers/ml and in the bagging area 80 fibers/ml. By 1989 with controls, they were 0.19 fibers/ml in the mine; 0.54 fibers/ml in the crushing area; 0.93 fibers/ml in the fiber selection area and 0.78 fibers/ml in the bagging area.

The percentage of Balangeroite fiber was similar to that of tremolite in Quebec. The difference however, is that the tremolite occurs in separate veins in Quebec (Williams-Jones et al., 2001) while as reported above the Balangeroite fiber was ''usually intergrown with chrysotile''. Balangeroite has been classed as an ''iron-rich asbestiform'' fiber with structural, biochemical, and perhaps most important biodurability characteristics similar to crocidolite (Gazzano et al., 2005; Groppo et al., 2005; Turci et al., 2005). There is no report of lung-retained fiber analyses from workers at the Balangero mine (Case & McDonald, 2008).

*Liddell et al. (1997).* Liddell et al. (1997) reported on the mortality experience of a cohort of ~11 000 workers from Quebec chrysotile miners and millers. The cohort extended over a long period of observations (a birth cohort 1891–1920) and the several updates reported at different intervals since 1971. In the last update published, Liddell et al. (1997) reported that high exposures have led to excesses, increasing with degree of exposure, of mortality from all causes, and from lung cancer and stomach cancer. However, at exposures below 300 (million particles per cubic foot) × years, (mpcf.y), equivalent to roughly 1000 (fibers/cm$^3$) × years (which is equivalent to an exposure of 80 fibers/cm$^3$ over a period of 10 years such as might have occurred in the 1940s) the findings were as follows: there were no discernible associations of degree of exposure and SMRs, whether for all causes of death or for all the specific cancer sites examined. The authors concluded that from the viewpoint of mortality that exposure in this industry to less than 300 mpcf.y has been essentially innocuous.

The issue of the possible presence and impact of contamination of the chrysotile ore with tremolite had been addressed by McDonald & McDonald (1995) in which preliminary investigations had suggested as important in the aetiology of mesothelioma. In the area of Thetford Mines, there were some 15 geographically dispersed mines and mills falling into two clearly definable groups: 5 in a circumscribed central area and 10 located in a peripheral area. Lung burden analysis (Sebastien et al., 1989) of 58 members of the cohort in the central area and 25 in the peripheral area had shown that the geometric mean concentration of tremolite was almost four times higher in the central area than in the peripheral area.

*Hughes et al. (1987).* The plants in this study started operation in the 1920s and produced asbestos cement building materials. There is little exposure data prior to the 1950s. Starting in 1952, air sampling data was collected using MIs (with measurements made in MPPCF). In plant 2, totally 248 measurements were made during the 1950s, and more than 1100 during the 1960s. Weill et al. (1979) reported that the original study population consisted of workers who were employed continuously in the months before January 1970 in either of the two asbestos cement building materials plants in New Orleans, LA. These plants opened in the early 1920s and were in operation at the time of the study. The authors reported that the predominant fiber used was chrysotile. In addition, crocidolite was used in the pipe department of the second plant (where it constituted 3% of the product). In the first plant, amosite was used (1% of various products), and crocidolite was used infrequently in the manufacture of corrugated bulkheads. In addition, they stated that ''silicate'' was used in both plants. Hughes et al. (1987) reported that plant 2 consisted of four separate buildings, each one manufacturing different products. Pipe production, which opened in 1946, used crocidolite in addition to chrysotile. The authors stated that all other areas used chrysotile only. Amosite was never used. Jones et al. (1989) stated that there was ''a systematic use of crocidolite in the pipe production area of plant 2, although chrysotile was the primary fiber in both plants''. There are no lung burden measurements available from workers in the study.

*McDonald et al. (1984).* McDonald et al. (1984) reported that this factory was established in 1913 and manufactured a number of asbestos-related products over the years. The authors reported that chrysotile from mainly Canada was used until 1957, when some anthophyllite was added in making paper discs and bands. In addition, they reported that approximately 400 lb of crocidolite was used experimentally on a few occasions in the laboratory during 1964 and 1972. The overall quality of anthophyllite and crocidolite used within the factory was not specified further. In addition, the authors reported that the situation was complicated by the fact that the plant under study developed from an earlier asbestos textile plant some 10 miles away which manufactured woven brake linings from 1905 until 1939. Effort was made from the work history records found to eliminate from the cohort people who worked in certain numbered departments (28–50) in the woven brake lining plant. Prior to the 1970s, the few measurements available on exposure were made by impinger and reported in mpcf. Subsequently, measurements were made using membrane filters (without identification of fiber

DOI: 10.3109/10408444.2012.756454 

type on the filter). There was no report of lung burden measurements in this study.

## Chrysotile epidemiological cohort studies

This section provides an evaluation of epidemiological studies of workers exposed to chrysotile which provided as well differentiation when amphibole asbestos exposure also occurred.

### *Chrysotile high-density cement studies*

Weill et al. (1979) reported on an investigation on 5645 asbestos-cement manufacturing workers. Dust exposures were based on total airborne particulate measurements using the MI at various locations throughout both plants and were recorded in MPPCF. No excess mortality was observed following exposure for 20 years to chrysotile asbestos at exposure levels equal to or less than 100 MPPCF years (corresponding to approximately 15 fibers/cm$^3$ × years). The authors stated:

> . . . However, the demonstration that low cumulative and short-term exposures did not produce a detectable excess risk for respiratory malignancy may be of assistance in the development of regulatory policy, because a scientifically defensible position based on these data is that there are low degrees of exposure not associated with a demonstrable excess risk

The authors also assessed the influence of fiber type on the risk of respiratory malignancy. Workers with exposure to chrysotile only ($n = 4201$) were compared with two groups of workers exposed to crocidolite asbestos in addition to chrysotile: those with steady employment in the pipe plant ($n = 1004$) and those with intermittent exposure to crocidolite through occasional maintenance work in that area ($n = 235$). Persons with exposure to amosite asbestos ($n = 205$) were excluded from analysis. The authors observed that the additional exposure to crocidolite asbestos enhanced the risk for respiratory malignancy, particularly for those workers exposed intermittently in maintenance jobs which were characterized by high exposure concentrations of dust for short periods of time.

Thomas et al. (1982) reported on a cohort within an asbestos-cement factory that used chrysotile. Some crocidolite was used in the factory prior to 1936 and thereafter only chrysotile was used. A total of 1970 workers were traced, and their mortality experience was examined. No information was available on smoking habits. Dust measurements were not made prior to 1968. Pre-1968 exposure concentrations were estimated as ranging from 0.1 fiber/cm$^3$ at the cement machine to 20+ fiber/cm$^3$ on the beater floor and at hard waste grinding. Since 1968 dust controls reduced exposure to below 2 fibers/cm$^3$. The authors reported that there was no appreciably raised SMR for the causes of death investigated, including all causes, all neoplasms, cancer of the lung and pleura and cancers of the gastrointestinal tract (standard errors were not reported). The authors indicate: ''Thus the general results of this mortality survey suggest that the population of the chrysotile asbestos-cement factory studied are not at any excess risk in terms of total mortality, all cancer mortality, cancers of the lung and bronchus or gastrointestinal cancers''. Two pleural mesotheliomas were observed in men who had worked at the factory before 1936 and had been exposed to crocidolite.

Gardner et al. (1986) reported on a cohort study carried out on 2167 subjects employed between 1941 and 1983 at an asbestos cement factory in England. The production process used chrysotile asbestos only, except for a small amount of amosite asbestos during 4 months in 1976. No excess of lung cancers or other asbestos-related excess death was reported, at mean fiber concentrations below 1 fiber/cm$^3$, although higher levels had probably occurred in certain areas of the asbestos-cement factory. One death was observed from pleural mesothelioma and one with asbestosis mentioned as an associated cause on the death certificate, however, neither was considered by the authors to be linked to asbestos exposure at the factory.

Ohlson & Hogstedt (1985) reported on a cohort study of 1176 asbestos cement workers in a Swedish plant using chrysotile asbestos. Only a few exposure measurements were available for the 1950s and 1960s. These indicated a dust level of 10 mg/m$^3$ before the 1970s and half that amount during the 1970s. The fiber concentrations averaged 1 fiber/cm$^3$ based on several hundred samples from five sets of measurements between 1970 and 1976. The fiber concentration at earlier times was estimated to have been twice that level, 2 fiber/cm$^3$ in accordance with the total dust measurements. The highest value was 8 fibers/cm$^3$ recorded during 45 min in 1970 in the asbestos bag barn. The vast majority of asbestos used was chrysotile although 630 tons of amosite were used between 1949 and 1951 and 400 tons of crocidolite in 1962. Smoking habits were not known for the entire cohort. In a sub-sample of the cohort 40% were smokers, 24% never-smokers and 36% ex-smokers. The authors stated that while the distribution was close to the national average, the participants in a voluntary health survey may not have been representative of the whole cohort. No excess work-related mortality was observed at cumulative exposures estimated at about 10–20 fibers/cm$^3$ years.

Yano et al. (2001) reported on cancer mortality among workers exposed to amphibole-free chrysotile asbestos in China. The plant studied opened in 1939 and since 1958 greatly expanded in the size and variety of products with 6000 tons of raw asbestos used in 1996. The authors stated that in the 1970s, the products were classified into textiles, asbestos cement products, friction materials, rubber products and heat resistant materials. This study is included in this section as it included cement products even though other products were manufactured as well. The authors reported that the adjusted relative risk of lung cancer was 8.1 (95% confidence interval (CI): 1.8, 36.1) for workers exposed to high versus low levels of asbestos. The authors stated that they ''compared the various sections of the asbestos plant for three groups of workers exposed to high, intermediate and low levels of asbestos fibers''. The few aerosol measurements performed are presented in Table 4 reproduced from Yano et al. (Table 1). The authors point out that there was an apparent discordance between the concentrations of airborne dust and fibers.

Crit Rev Toxicol, 2013; 43(2): 154–183

Table 4. Concentrations of fiber and dust for workers in major sections of the Chongqin, China, asbestos plant, by job category, 1999. (Reproduced from Yano et al's)*.

| Job category | Fiber (fibers/ml (range)) | Dust (mg/m3 (range)) |
|---|---|---|
| Raw material (opening) | 6.5 (5.8–7.5) | 8.8 (6.1–12.3) |
| Raw material (bagging) | 12.6 (5.2–58.4) | 18.2 (14.5–22.4) |
| Rubber plate† | 2.8 (2.6–3.1) | 237.5 (176.0–320.5) |
| Textile | 4.5 (0.7–17.0) | 22.4 (15.8–35.5) |
| Asbestos cement‡ | 0.1 | 22.3 |

*Geometric mean (range) for three to five workers exposed in each asbestos plant section.

†In the rubber plate section, workers were engaged mainly in dumping mica and various raw materials into a pit in a small room without ventilation.

‡In the asbestos cement section, the number of workers in the dusty environment was small, and only one worker who was engaged in dumping raw materials into the pit was monitored.

The authors also reported that there were two cases of malignant mesothelioma, one pleural and the other peritoneal, in the asbestos cohort which are discussed below. They concluded that these results suggest that heavy exposure to pure chrysotile asbestos alone, with negligible amphibole contamination, can cause lung cancer and malignant mesothelioma in exposed workers, however, they do not define further the exposure characteristics. There are considerable inconsistencies in this study. The authors report that there are no consistent industrial hygiene measurements over the history of the study. They state that the respirable dust concentration was measured once every 4 years. Yano et al. do not present any information on what fiber types were on these filters and more importantly, the fiber concentration measurements (0.1–58 fibers/cm$^3$) account for a very small part of the 6.1–320 mg/m$^3$ dust burden. In a recent animal inhalation toxicology study, a chrysotile exposure of 1500 fiber/cm$^3$ has a gravimetric weight of 2.6 mg/m$^3$ (Bernstein et al., 2010). In the Yano et al. (2001) paper, the highest fiber concentration was 58.4 fiber/cm$^3$, which would correspond to approximately 0.1 mg/m$^3$. Even assuming in Table 5 that the ''Raw material (opening)'' category was pure chrysotile (which has not been verified in the publication), 1 fiber/cm$^3$ would weigh 1.35 mg/m$^3$ assuming no other particulate matter present. For the rubber plate category, fibers accounted for 3.8 out of 238 mg/m$^3$; for the textile category, fibers accounted for 6 out of 22 mg/m$^3$; for the asbestos cement category, fibers accounted for 0.14 out of 22.3 mg/m$^3$. Other than stating that in the rubber plate section, workers were engaged mainly in dumping mica and various raw materials into a pit in a small room without ventilation, there is no discussion about composition of the ''dust'' which ranged in mass concentration from 6.1 to 320.5 mg/m$^3$. To put these exposures in perspective, the ACGIH TLV for nuisance dusts is 10 mg/m$^3$ (total dust), 3 mg/m$^3$ (respirable fraction), for mica 3 mg/m$^3$, and for latex rubber 0.0001 mg/m$^3$. There is no indication that the control cohort had similar exposures, as there is no presentation of what the control was exposed to. Considering that the dose makes the poison, this very high unaccounted dose, which was clearly not chrysotile, should be of major concern. This study is clearly not a pure chrysotile exposure as based upon the mass concentration presented in Table 5, 99.9% of the exposure was to something else. Even on the small biopsy samples there is no lung burden analysis, which has always been the bottom line in determining the fibers present to which workers were exposed. Yano et al. (2001) state that a pleural mesothelioma death was reported which occurred 13.8 years after first exposure. This would suggest some prior exposure. If the exposure occurred prior to employment, as suggested by Yano et al. (2009), then this case should not have been included in this study. In a follow-up to this study Wang et al. (2012) reported that ''asbestos dust concentrations were measured periodically in the different workshops, but fiber concentrations and personal samples were not available until 1999''. Additionally, Yano et al. (2009) stated that the analysis of the lungs indicated that the vast majority of these asbestos fibers present were tremolite with some occasional chrysotile fibers. This would clearly suggest that small asbestos fiber component of the exposure was not to pure chrysotile but to chrysotile contaminated with tremolite.

The purity of Chinese chrysotile was evaluated by Tossavainen et al. (2001) who reported on the analysis for amphibole fibers in 10 chrysotile bulk samples originating from six Chinese chrysotile mines. In addition, the asbestos fiber content in lung tissue from seven deceased workers of the Shenyang asbestos plant using these raw materials was determined. The authors reported that all of the bulk samples contained amphibole fibers as an impurity in concentrations ranging from 0.002 and 0.310 wt%. Tremolite fibers were detected in every sample but anthophyllite fibers were present only in the sample originating from the dolomite-hosted deposit. In the lung, anthophyllite (71%), tremolite (9%) and chrysotile (10%) were found as the main fiber types. The authors noted that all except one of the mines studied were located in western China, and that nearly all of the bulk Chinese chrysotile comes from mines in this region. Yano et al. (2001) reported on a mine that was West/South West China.

Sichletidis et al. (2009) reported on an investigation into the mortality rate among workers exposed to relatively ''pure chrysotile'' in an asbestos cement factory in Greece. The asbestos cement plant was opened in 1968 and the investigation covered all 317 workers. The plant used 2000 tons of chrysotile annually. Regular asbestos fiber measurements were made and the day and cause of death were recorded among active and retired workers. Asbestos fiber concentrations were always below permissible levels. Fifty-two workers died during the study. The cause was cancer in 28 subjects, with 16 of those cases diagnosed as lung cancer. No case of mesothelioma was reported. The overall mortality rate was significantly lower than that of the Greek general population, SMR was 0.71 (95% CI 0.53–0.93). Mortality due to cancer was increased (SMR: 1.15, 95% CI 0.77–1.67), mainly due to lung cancer mortality (SMR: 1.71, 95% CI 0.98–2.78), but not significantly. The authors stated that the SMR for lung cancer of 1.71 was attributed almost exclusively to cigarette smoking. The authors concluded that occupational exposure to relatively pure chrysotile within permissible levels was not associated with a significant increase in lung cancer or with mesothelioma. Decreased overall mortality of workers

DOI: 10.3109/10408444.2012.756454



indicates a healthy worker effect, which – together with the relatively small cohort size – could have prevented the detection of small risks.

*Chrysotile studies not specifically of cement products*

Berry & Newhouse (1983) reported on a mortality (1942–1980) study carried out in a factory manufacturing friction material. Chrysotile was the only type of asbestos used except during two well-defined periods before 1945 when crocidolite asbestos was used as well, and over 99% of the population was traced. Compared with national death rates, there was no detectable excess of deaths due to lung cancer, gastrointestinal cancer or other cancers. The exposure levels were relatively low, with only 5% of men having a cumulative exposure of 100 fiber-years/ml. This was due, in part, to the inclusion of several short-term workers but was also a consequence of good environmental control in the factory during the past 30 years. The authors state: ''The experience at this factory over a 40-year period showed that chrysotile asbestos was processed with no detectable excess mortality''. The authors also reported on a case control study that was carried out on the 11 deaths due to mesothelioma which showed that eight of the workers had been exposed to crocidolite asbestos and another was possibly exposed intermittently to crocidolite asbestos. The other two had been employed for most of their working lives outside the factory, and their mesotheliomas could not be definitely attributed to exposure to chrysotile.

Newhouse & Sullivan (1989) reported on a further analysis of the Berry & Newhouse (1983) cohort though an additional seven years. The authors confirmed that there were no excess deaths from lung cancer or other asbestos related cancers, or from chronic respiratory disease. After 1950, hygienic control was progressively improved at this factory, and from 1970, the authors reported that the levels of asbestos did not exceeded 0.5–1.0 fiber/cm$^3$. The authors stated: ''It is concluded that with good environmental control, chrysotile asbestos may be used in manufacture without causing excess mortality''. At this time there were 13 deaths attributed to mesothelioma and of these, 11 had known contact with crocidolite asbestos. Of the remaining two, one had an uncertain diagnosis and in the other the occupational history was not well established.

The importance of tremolite asbestos contamination in chrysotile dust and talc was evaluated by Roggli et al. (2002a) who examined the association of the development of mesothelioma to contaminating tremolite fibers present in chrysotile dust and talc. The authors examined 312 cases of mesothelioma, for which fiber burden analyses of lung parenchyma had been performed by means of SEM. The amount of tremolite asbestos, non-commercial amphibole asbestos, talc and chrysotile was determined. Of the 312 cases, 166 had tremolite asbestos with 81 of these above background levels. Fibrous talc was identified in 193 cases with a strong correlation to the tremolite content ($p < 0.0001$). Chrysotile was identified in only 32 cases, but still correlated strongly with the tremolite content ($p < 0.0001$). Non-commercial amphibole fibers (tremolite, actinolite and/or anthophyllite) were the only fiber types found above background in 14 cases. The authors concluded that tremolite asbestos in lung tissue samples from mesothelioma victims derived from both talc and chrysotile and that tremolite asbestos accounts for a considerable fraction of the excess fiber burden in end-users of asbestos products.

In another study, Roggli et al. (2002b) evaluated the type of occupational exposure in correlation with asbestos fiber content and type in 1445 cases of mesothelioma with known exposure history. Of these, 268 cases had lung fiber burden analysis. Fiber analyses were performed on formalin-fixed or paraffin embedded lung tissue specimens by using techniques described in Roggli et al. (1992). The authors stated that samples usually included lung parenchyma abutting against the visceral pleura, with each sample typically weighing 0.25 to 0.35 gm (wet weight) and as little as 0.1 gm or less of wet tissue. Lung tissue was processed for digestion by using the sodium hypochlorite technique. Asbestos bodies were determined by light microscopy and fiber analysis by SEM with fiber morphology as determined by SEM and elemental composition assessed by EDXA. The cases were classified into 23 exposure categories which included occupational as well as non-occupational exposures although there was a substantial overlap in exposure types. The authors reported that all but one of the occupational categories analyzed had above-background levels of commercial amphiboles and that commercial amphiboles are responsible for most of the mesothelioma cases observed in the USA.

Carel et al. (2007), a study led by the International Agency for Research on Cancer, examined the risk of lung cancer following occupational exposure to asbestos and man-made vitreous fibers in a multicenter case-control study in Europe. Two regions were studied in this program, six Central and Eastern European countries and the UK, during the period 1998–2002. Comprehensive occupational and socio-demographic information was collected from 2205 newly diagnosed male lung cancer cases and 2305 frequency matched controls. Adjustment was made in the odds ratios (OR)* to take into account other relevant occupational exposures and tobacco smoking. The OR for asbestos exposure was 0.92 (95% CI 0.73–1.15) in Central and Eastern Europe and 1.85 (95%CI 1.07–3.21) in the UK. Similar ORs were found for exposure to amphibole asbestos. The OR for MMVF exposure was 1.23 (95%CI 0.88–1.71) with no evidence of heterogeneity by country. The Central and Eastern European asbestos industry had been reliant upon Russia for supplying asbestos in the 30–50 years prior, when exposure would have been important for determining this outcome. Russia, then as now, uses chrysotile asbestos commercially. While not discussed directly in this publication, the differences in the ORs are readily understood by the fact that the UK was the largest importer and user of amphibole per capita in the world. Commercial (non-military) asbestos production in the Soviet Union was of chrysotile alone (Kashansky et al., 2001). Carel et al.'s (2007) study clearly demonstrated that when chrysotile

*Odds Ratio (OR): The odds ratio is a relative measure of risk, telling us how much more likely it is that someone who is exposed to the factor under study will develop the outcome as compared to someone who is not exposed; an OR of 1 or less indicates no effect. Even if the OR is greater than 1, if the lower bound of the 95 % confidence interval (CI) is 1 or less then the OR is not different statistically from 1.

alone was used as in Central and Eastern Europe, there is no measurable excess of lung cancer risk.

South Africa, like Australia, represents a very particular situation in the history of asbestos use. Both countries have historically been the major sources of amphiboles (crocidolite and amosite in South Africa), and have used these varieties of asbestos locally along with chrysotile, which was also mined in both South Africa and Australia. In both these countries, the number of mesothelioma cases has been much higher than anywhere else in the world. White et al. (2008) have indicated that 23% of cases in South Africa were found in persons never employed in mining. These cases, however, were found associated with living in neighborhoods close to amphibole mining facilities, predominately one area with crocidolite mines, thus associated with environmental exposure. The authors concluded that:

> No cases [of mesothelioma] were associated with South African chrysotile. Consequently, in the vast majority of cases of mesothelioma, environmental exposure to asbestos occurred in the Northern Cape Province, in proximity to mines, mills and dumps where crocidolite was processed. Crocidolite appears more mesotheliomagenic than amosite, and chrysotile has not been implicated in the disease. This is true for both occupationally and environmentally exposed individuals.

The association of amphibole asbestos with lung disease was evaluated by Schneider et al. (2010) who reported on the measurement of asbestos fiber content of the lungs as it was associated with diffuse interstitial fibrosis (DPF). The asbestos fiber burden was determined in patients with DPF who had a history of asbestos exposure in which their biopsies did not meet established criteria for asbestosis. This was compared to the fiber burden in confirmed asbestosis cases. The fiber burden analysis was performed using SEM and EDXA of lung parenchyma from 86 patients with DPF and 163 patients with asbestosis. The correlation of the number of asbestos fibers found for a quantitative degree of fibrosis was reported. Schneider et al. (2010) reported that the fibrosis scores of the asbestosis cases correlated best with the number of uncoated commercial amphibole fibers.

### Chrysotile epidemiological reviews

As reviewed above, most exposures in the past even when characterized as pure chrysotile would be more accurately described as predominantly chrysotile exposure. Pierce et al. (2008) have analyzed the cumulative exposure-response data reported for predominantly chrysotile-exposed cohorts in the published literature to identify an actual ''no-effect'' exposure level for chrysotile-related lung cancer and mesothelioma. From over 350 published studies, 14 were found to meet the inclusion criteria in which lung cancer risk was stratified by cumulative chrysotile exposure and four studies were found for mesothelioma. The authors reported that

> The preponderance of the cumulative ''no-effects'' exposure levels for lung cancer and mesothelioma fall in a range of approximately 25–1000 fibers per cubic centimeter per year (f/cc-yr) and 15–500 f/cc-yr, respectively, and a majority of the studies did not report an increased risk at the highest estimated exposure.

The authors detailed as well that a number of sources of uncertainty affected these no-effect levels. These included uncertainty in the cumulative exposure estimates, conversion of dust counts to fiber data and use of national age-adjusted mortality rates. The authors also explained that there were numerous potential biases in the data including, for example, smoking was rarely controlled for and amphibole exposure did in fact occur in a majority of the studies, which would bias many of the reported ''no-effect'' exposure levels toward lower values.

Paustenbach et al. (2004) reviewed the potential environmental and occupational health hazards associated with the presence of chrysotile asbestos in brake linings and pads. This review, covering studies and observations published over several decades, demonstrated that in general, exposures have been minimal and did not show any demonstrable risk when chrysotile was used in brake linings and pads. The authors reported that only the friction materials manufacturing workers in the UK who were exposed to crocidolite while making railroad engine brake linings were found to have an increased relative risk of mesothelioma. In addition, the authors reviewed 20 published studies evaluating asbestos exposure or asbestos-related health effects in friction product manufacturing workers. The authors found that these studies indicated that friction product manufacturing workers were historically exposed to concentrations of chrysotile fibers perhaps 10–50 times greater than those of brake mechanics, however, the risk of asbestosis, mesothelioma and lung cancer, if any, was not apparent, except for those friction materials manufacturing workers who had some degree of exposure to amphibole asbestos during their careers.

Kanarek (2011) presented a review of asbestos and associated mesothelioma including case series, case-control and cohort epidemiology in which he stated that chrysotile is the ''exclusive or overwhelming fiber exposure''. However, the presentation of each case presents little if any data in support of this view. In the discussion, he states that ''This review sought to search the world epidemiology literature on mesothelioma to catalogue the case-series, cohort and case-control studies in which the asbestos exposure appeared to be overwhelmingly to the chrysotile type''. However, if the individual studies are examined closely, they appear not to be exclusively of chrysotile exposure. As an example, one of the studies cited in support is by Aguilar-Madrid et al. (2010) that reported on a study in which they carried out a case-control study of malignant pleural mesothelioma in 472 workers insured by the Mexican Institute of Social Security, all Valley of Mexico residents, with 119 incident cases and 353 controls. Unfortunately, in the study there was no measure of exposure in any work environment in which asbestos was used. The authors ''estimated'' exposure in four categories based upon comparison with other studies. As a result there was no knowledge available on which fibers were used in the work environments. However, for ''asbestos'' workers, the use of amphibole types (especially crocidolite, or mixtures containing amphiboles) was widespread in Mexico up to the 1990s,

DOI: 10.3109/10408444.2012.756454


particularly in the manufacture of fibro-cement pipes. As it is well known that clinical diagnosis of mesothelioma can be some 40–45 years after onset of exposure, mesothelioma cases that are diagnosed in 2010 may well relate to exposure conditions prevailing back in the 1970s. For this reason, it is almost certain that more new cases will be diagnosed in the near future. Because there was no measure of which fibers were used and their concentrations, in this study it is impossible to distinguish effects from chrysotile versus those from amphibole asbestos. In addition, the recent confirmation of mesothelioma cases following exposure to naturally occurring erionite, which outcrops over an area of central Mexico, will produce difficulties in attributing cause to occupational cases (Ilgren et al., 2008a,b; Kliment et al., 2009). In another example, Mancuso et al. (1983, 1988) is cited stating that exposure of railroad workers was exclusively to chrysotile. However, as explained by Gibbs & Pooley (2008), subsequent tissue analyses have shown the presence of amosite and crocidolite in the rail workers lungs.

Similarly, Smith & Wright (1996) also postulated that chrysotile asbestos is the main cause of pleural mesothelioma. In the studies cited, the authors often state that exposure was predominately to chrysotile without providing specific data as to how much amphibole was present. As discussed above, more recent inhalation toxicology studies demonstrate that even short 5 d exposures to amphiboles can result in significant pathological response in the lung and pleura.

Yarborough (2006) reviewed all available epidemiological studies to determine if chrysotile was a cause of mesothelioma. This review was prompted by the long-standing debate over the potential contribution of chrysotile to mesothelioma risk. Yarborough undertook an extensive review of the epidemiological cohort studies in order to evaluate the extent of the evidence related to free chrysotile fibers, with particular attention to confounding by other fiber types, job exposure concentrations, and consistency of findings. A total of 71 asbestos cohorts exposed to free asbestos fibers were reviewed. The authors concluded that the data ''does not support the hypothesis that chrysotile, uncontaminated by amphibolic substances, cause mesothelioma''.

## Use and exposures in the past and today

Historically, the two minerals groups, chrysotile and amphibole asbestos, were often used interchangeably in industrial applications. In some situations one was preferential to the other in terms of process. Often cost and availability were the overriding factors in determining which mineral was used. Additionally, industrial associations were often instrumental in determining which fiber was used. As an example, in the UK many of the mining operations in South Africa were either owned or associated with a UK company and as such, the UK became the largest importer of amphibole asbestos in the world.

Dust levels were not well controlled in the mines, and some applications for which the minerals were used, such as open spraying, also resulted in very high exposure concentrations (Esmen & Corn, 1998; Gibbs, 1994).

A review of the epidemiological studies described as chrysotile only show that implementation of workplace controls reduce the exposure concentration in these applications to low levels. As an example: Silvestri et al. (2001) summarized information on work practice, fiber concentration and health-related effects in the workers at the Balangero mine reported that by 1989 with controls, exposure concentrations were 0.19 fibers/ml in the mine; 0.54 fibers/ml in the crushing area; 0.93 fibers/ml in the fiber selection area and 0.78 fibers/ml in the bagging area.

Concerning the Quebec miners and millers, Liddell et al. (1998) stated that ''On the other hand, modern dust conditions are well below the average even of dust category one and so there can be considerable confidence that the risk of lung cancer as a result of such exposure has become vanishingly small''.

Today the situation is remarkably different. Only chrysotile is used commercially. In the past, some chrysotile mines had veins of tremolite running through the ore body, which were excavated with the chrysotile. Today, the tremolite veins when present are easily differentiated from chrysotile because they are of a different color and can be identified and avoided in those few mines that have such veins (Williams-Jones et al., 2001).

The Cana Brava chrysotile mine in Brazil routinely has the chrysotile analyzed to assess the presence of amphiboles. The reports from the Institute of Occupational Medicine in Edinburgh (Karbownik & Clark, 1997, 2005, 2006, 2007, 2008, 2009, 2012) as well as a laboratory in Brazil (Zamataro & Franzini 2012) have shown that there is no detectable amphibole asbestos in the chrysotile.

The chrysotile from the Calidria (New Idria, CA) chrysotile mine has also been assessed for the presence of amphibole asbestos (Coleman, 1996; Pooley, 2003). Ilgren (2004) summarized these results stating that ''Only very rarely have non-asbestiform 'non-friable' amphibole (so-called cleavage fragment) minerals been found in the New Idria serpentine body but away from the ore zone''.

Two reports (Kashansky et al., 2001; Tossavainen et al., 1996) found no tremolite in air samples from the Uralasbest mine in Asbest, Russia, which is the largest mine currently in production. Tossavainen et al. (2000) reported on the pulmonary mineral fibers concentrations in 24 chrysotile miners, millers, and product manufacturers from workers at the Uralasbest mine. The authors reported that while ''the mean and range of pulmonary chrysotile concentrations were about the same as reported previously from the Canadian mining and milling industry. In the Russian samples, the mean concentration of tremolite fibers was less by at least one order of magnitude''. The authors also reported that no amosite or crocidolite fibers were detected in any tissue sample with coated ferruginous bodies relatively rare (<1% of counted fibers).

Finley et al. (2012) reported on the evaluation of tremolite asbestos exposures associated with the use of chrysotile-containing commercial products. The authors conservatively estimated the cumulative tremolite asbestos exposures as: career auto mechanic: 0.0279 f/cc-year; non-occupational use of joint compound: 0.0006 f/cc-year; non-occupational use of vermiculite-containing gardening products: 0.0337 f/cc-year; home-owner removal of Zonolite insulation: 0.0002 f/cc-year. They also reported that these exposures are far below the

lowest-observed-adverse-effect level that they determined for tremolite.

In the past even when no effort was made to avoid mining the tremolite veins, the percentage of tremolite was very small and measurements in one study showed it never amounted to more than 0.24% found in one out of eight chrysotile samples analyzed, while the other seven samples contained no tremolite (detection limit of $0.002 < 0.0002\%$ using SEM, the most sensitive of the analytical methods used) (Addison & Davies, 1990).

Such levels of tremolite asbestos would be important if the chrysotile exposure was at very high concentrations and included a significant number of longer fibers which persisted over many years. In actual practice in the past, even when exposures to chrysotile were very high in chrysotile mining and milling, much of the tremolite asbestos has short length and low aspect ratio; with effects from exposure to tremolite asbestos only reported following long-term exposures at very high concentrations (McDonald et al., 1997).

Studies have reported that chrysotile as mined in the past without differentiation of the possible tremolite asbestos exposure, will not produce mesotheliomas in those exposed to current or recently regulated exposure concentrations, and certainly not in those exposed at environmental levels (Churg, 1988). With the awareness of industry of the tremolite issue specific measures have been introduced to avoid any tremolite veins in those few mines in which they occur.

In addition, in mines today, the use of water control spraying technology has greatly limited ambient dust levels to which the workers are exposed during mining and closed-circuit systems greatly reduce dust levels during milling (Bragg, 2001) and (Safe Use of Chrysotile Asbestos: A Manual on Preventive and Control Measures, 1993 and The Basics of Chrysotile Asbestos Dust Control, 2008. 4th edition, Published by the Chrysotile Institute, Montreal, QC, Canada (jmarcleblond@2011ica.com).

Today, the vast majority of chrysotile is used in high-density cement products (Virta, 2006). In these products, chrysotile is integrally bound into the cement particles and matrix with little or no opportunity for release as individual fibers. The industry also has instituted extensive training and educational programs on how to limit dust levels to assure personal protection not only in the mining sectors, but also in use (installation, maintenance, repair and disposal) in the construction trades.

## Discussion

While the safe use of asbestos mandates that exposures be controlled, the extensive literature base clearly differentiates the dose response of chrysotile as compared to amphibole asbestos and demonstrates that controlled use of chrysotile is not associated to a significant risk while even short exposure to amphibole asbestos can produce cancer.

The studies by Dement et al. (1982) and Yano et al. (2001) which have been interpreted as studies on chrysotile asbestos are, after careful review and understanding of the conditions and data presented, not representative of chrysotile exposure alone but rather have numerous other elements as described above which were not fully taken into consideration.

The importance of amphibole point sources, either industrial or environmental to the incidence of mesothelioma has been documented in a number of studies. The studies by Musti et al. (2009) and Barbieri et al. (2012) show the relationship of increased mesothelioma risk in individuals without occupational or domestic or household exposure who lived near an asbestos plant in an urban area that had documented use of amphibole asbestos over 50 years. Kurumatani & Kumagai (2008) investigated the magnitude of the risk among residents who lived near a former large asbestos cement pipe plant that used crocidolite and chrysotile. The authors reported that residents, who had lived within a 300 m radius of the plant, had a SMR for mesothelioma of 13.9 (5.6–28.7) for men and 41.1 (15.2–90.1) for women. Case & Abraham (2009) examined the mesothelioma risk in two American counties, Jefferson Parish, Louisiana and El Dorado County, California. Jefferson Parish, LA, was chosen as the prototype of legacy exposures on the basis of historical evidence of crocidolite use in asbestos plants with known mesotheliomas in the workforce, known shipyards with amosite use in the same area, and the presence of crocidolite-containing scrap in over 1400 properties. El Dorado, CA, was chosen due to the presence of naturally occurring amphibole exposures. The authors reported that the industrial use legacy exposure area was high in mesothelioma incidence and mortality in Jefferson Parish as a result of crocidolite and amosite exposure, while a clear increase in incidence or mortality was not observed in the naturally occurring asbestos area of El Dorado County. Pan et al. (2005) examined the mesothelioma incidence of people living near ultramafic rock deposits which are the principal source of asbestos. The authors reported that some occupations such as shipyard worker, boilermaker, insulator, plumber, pipefitter and steamfitter, and industries such as shipping, construction and Navy had higher occupational exposure to asbestos and were strongly associated with an increased risk of malignant mesothelioma. They also reported that residential proximity to ultramafic rock deposits shows an independent and dose–response association with mesothelioma risk.

The world production of asbestos in 1960 was around 2 million tons, and remained at 2 million tons in 2010 (Virta, 2006, 2011). However, while in the early 1960s production included all major types (chrysotile, crocidolite and amosite), due to their recognized toxicity, the United States has not imported amosite since 1985 and has not imported crocidolite since about 1995 (Virta, 2006). The mining of crocidolite and amosite in South Africa ended in 1997 and 1992, respectively, and the mining of crocidolite in Australia and Bolivia ended in 1983 and 1968 (Virta, 2006). Ilgren et al. (2012) have reported on plants in which crocidolite asbestos is still used in Bolivia. The authors reported that there was no increase in the incidence of mesothelioma in associated populations. Ilgren et al. attributed this to the specific characteristics of the Bolivian crocidolite which has a larger fiber width distribution than other crocidolite asbestos, with considerably fewer Stanton fibers (longer than 8 μm and thinner than 0.25 μm) (Stanton et al., 1981; van Orden et al., 2012).

DOI: 10.3109/10408444.2012.756454



Unfortunately, because of procrastination by some governments in implementing regulation of amphiboles (e.g. France, Décret n°94-645 du 26 juillet 1994), the remaining amphiboles inventories were allowed to be used in some factories up to the mid-1990s. In addition, due to the large use in past years of amphiboles by some countries and their relative insolubility, a significant background level of amphibole asbestos remains (in the environment, buildings and devices). With the characteristic long latency associated with onset of asbestos-related cancer, especially with mesothelioma, a high incidence of this particular cancer of the pleura may be expected in those countries for the next two or three decades due to the extended use of amphiboles. As observed in both the recent inhalation toxicology studies and in the epidemiology studies, even a short exposure to amphiboles can result in lung cancer and or mesothelioma.

The carcinogenic potency of amphibole asbestos has been established both epidemiologically and toxicologically, leading to it being no longer used in commerce. In 1989, a group of international experts convened by the WHO in Oxford (UK) had recommended that these asbestos varieties should be prohibited immediately, and that the use of chrysotile should be controlled and regulated at a permissible exposure limit (PEL) of 1 fiber/cm$^3$ in the workplace. The workplace PEL has since been lowered in some countries to 0.1 fiber/cm$^3$ (e.g. ACGIH TLV 0.1 f/cm$^3$; European OEL 0.1 f/cm$^3$; Pohanish, (2008)).

Today, the remaining practical concern is whether chrysotile can be produced and used safely, and if indeed this regulation carries a reasonable assurance that workers are adequately protected. Based upon the current science reviewed above, in absence of amphibole asbestos, the use of chrysotile at current Québec PELs in the workplace has not been associated with a statistically detectable increase in risk as observed epidemiologically. From these published studies, it can be seen that chrysotile can be used safely in the manufacturing of cement high-density applications. The International Labour Organization has issued a Code of Practices entitled ''Safety in the Use of Asbestos'' (ILO, 1984), which addresses all pertinent issues regarding the modern and responsible use of asbestos.

Erosion of surface deposits over millennia means that chrysotile is a ubiquitous component of the particulate matter in the air. The WHO (1985) estimates the background exposure to chrysotile as between 0.01 and 0.001 fiber per milliliter of air. The risk to health from exposure to chrysotile at this background level based upon the toxicology and epidemiology studies is certainly not significant. Industrial and other exposure at the high end of this range has been labeled acceptable by the Ontario Royal on Asbestos, not significant by the WHO, and ''. . . further control not justified'' by the Royal Society in London (UK).

In the area of occupational health, and specifically with regard to the use of chrysotile asbestos, regulatory agencies in all countries have the responsibility to set workplace exposure limits that will reduce the risk to workers to the lowest possible level. That this exercise should be based on the most recent scientific assessment available would seem obvious.

## Conclusion

This review provides an important basis for substantiating both kinetically and pathologically the differences between chrysotile and amphibole asbestos. Chrysotile which is rapidly attacked by the acid environment of the macrophage, falls apart in the lung into short fibers and particles, while the amphibole asbestos persist creating a response to the fibrous structure of this mineral.

Chrysotile is mineralogically distinct from the amphiboles with a very different chemical structure. The thin rolled or concentric sheets that form the chrysotile fiber leads to the ability of the lung/macrophage system to decompose the chrysotile fibers once inhaled as seen in the biopersistence studies of commercial chrysotiles. This effect is substantiated by both mineralogical and in-vitro studies.

The short-term inhalation toxicity studies of chrysotile that have been performed at non-lung overload conditions demonstrate that the long (>20 μm) fibers are rapidly cleared from the lung, are not translocated to the pleural cavity and do not initiate any fibrogenic response. This is in marked contrast to the long amphibole asbestos fibers which persist through the rat's lifetime, are quickly (within 7 d) translocated to the pleural cavity and result in interstitial fibrosis and pleural inflammation. Following sub-chronic inhalation at a mean exposure of 76 fibers $L > 20\,\mu m/cm^3$ (3413 total fibers/cm$^3$) resulted in no fibrosis at any time point and no difference with controls in BrdU response or biochemical and cellular parameters. The long chrysotile fibers were observed to break apart into small particles and smaller fibers.

Recent quantitative reviews of epidemiological studies of mineral fibers have determined the potency of chrysotile and amphibole asbestos for causing lung cancer and mesothelioma in relation to fiber type and have also differentiated between these two minerals. The most recent analyses also concluded that it is the longer, thinner fibers that have the greatest potency as has been reported in animal inhalation toxicology studies. The epidemiology studies on chrysotile have been reviewed and effects are evaluated in light of the frequent use of amphibole asbestos.

The studies reporting on the use of chrysotile alone in high-density cement products as well as other applications and the implementation of controls in mining and manufacturing provide a framework for establishing safe use.

As with other respirable particulates, there is evidence that heavy and prolonged exposure to chrysotile can produce lung cancer. The importance of the present and other similar reviews is that the studies they report show that low exposures to chrysotile do not present a detectable risk to health. Since total dose over time decides the likelihood of disease occurrence and progression, they also suggest that the risk of an adverse outcome may be low with even high exposures experienced over a short duration.

## Declaration of interests

The preparation of this review was supported by a grant from the International Chrysotile Association, Washington, DC, USA, in cooperation with The Canadian Chrysotile Association, Montréal, QC, Canada. The affiliation of the authors is as shown on the cover page and includes university,

government institute, hospital and corporate affiliations as well as independent toxicology consultants. The review is the professional work product of the authors and may not necessarily represent the views of the corporate sponsors. Two of the authors, David Bernstein and Allen Gibbs have appeared as expert witnesses in litigation concerned with alleged health effects of exposure to chrysotile. Jacques Dunnigan has served as an expert witness on the health effects of chrysotile before the Commission de la santé et sécurité du travail du Quebec/Workers Compensation Board of Québec.

## References


Adamson IY, Bakowska J, Bowden DH. (1993). Mesothelial cell proliferation after instillation of long or short asbestos fibers into mouse lung. Am J Pathol, 142, 1209–16.

Adamson IY, Bakowska J, Bowden DH. (1994). Mesothelial cell proliferation: a nonspecific response to lung injury associated with fibrosis. Am J Respir Cell Mol Biol, 10, 253–8.

Addison J, Davies LS. (1990). Analysis of amphibole asbestos in chrysotile and other materials. Ann Occup Hyg, 34, 159–75.

Aguilar-Madrid G, Robles-Pérez E, Juárez-Pérez CA, et al. (2010). Case-control study of pleural mesothelioma in workers with social security in Mexico. Am J Ind Med, 53, 241–51.

Ashcroft T. (1973). Epidemiological and quantitative relationships between mesothelioma and asbestos on Tyneside. J Clin Pathol, 26, 832–40.

Aust AE, Cook PM, Dodson RF. (2011). Morphological and chemical mechanisms of elongated mineral particle toxicities. J Toxicol Environ Health B Crit Rev, 14, 40–75.

Ballweg JA, Bray RM. (1989). Smoking and tobacco use by US military personnel. Mil Med, 154, 165–8.

Balzer JL, Cooper WC. (1968). The work environment of insulating workers. Am Ind Hyg Assoc J, 29, 222–7.

Barbieri PG, Mirabelli D, Somigliana A, et al. (2012). Asbestos fibre burden in the lungs of patients with mesothelioma who lived near asbestos-cement factories. Ann Occup Hyg. 2012. [Epub ahead of print]. doi:10.1093/annhyg/mer126.

Bates TF, Sand LB, Mink JF. (1950). Tubular crystals of chrysotile asbestos. Science, 111, 512–3.

Bellmann B, Muhle H, Creutzenberg O, et al. (2003). Calibration study on subchronic inhalation toxicity of man-made vitreous fibers in rats. Inhal Toxicol, 15, 1147–77.

Berman DW, Crump KS. (2003). Draft technical support document for a protocol to assess asbestos-related risk [report]. Washington (DC): Office of Solid Waste and Emergency Response US Environmental Protection Agency.

Berman DW, Crump KS. (2008). A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. Crit Rev Toxicol, 38, 49–73.

Berman DW, Crump KS, Chatfield EJ, et al. (1995). The sizes, shapes, and mineralogy of asbestos structures that induce lung tumors or mesothelioma in AF/HAN rats following inhalation. Risk Anal, 15, 181–95.

Bernstein DM. (2007). Synthetic vitreous fibers: a review toxicology, epidemiology and regulations. Crit Rev Toxicol, 37, 839–86.

Bernstein D, Castranova V, Donaldson K, et al. (2005c). Testing of fibrous particles: short-term assays and strategies. Inhal Toxicol, 17, 497–537.

Bernstein DM, Chevalier J, Smith P. (2005b). Comparison of Calidria chrysotile asbestos to pure tremolite: final results of the inhalation biopersistence and histopathology following short term exposure. Inhal Toxicol, 17, 427–49.

Bernstein DM, Riego-Sintes JM, Ersboell BK, et al. (2001a). Biopersistence of synthetic mineral fibers as a predictor of chronic inhalation toxicity in rats. Inhal Toxicol, 13, 823–49.

Bernstein DM, Riego-Sintes JM, Ersboell BK, et al. (2001b). Biopersistence of synthetic mineral fibers as a predictor of chronic intraperitoneal injection tumour response in rats. Inhal Toxicol, 13, 851–75.

Bernstein DM, Rogers R, Chevalier J, et al. (2006). The toxicological response of Brazilian chrysotile asbestos: a multidose sub-chronic 90 d inhalation toxicology study with 92 day recovery to assess cellular and pathological response. Inhal Toxicol, 18, 313–32.

Bernstein DM, Rogers RA, Sepulveda R, et al. (2010). The pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite asbestos following short term inhalation exposure – interim results. Inhal Toxicol, 22, 937–62.

Bernstein DM, Rogers RA, Sepulveda R, et al. (2011). Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure. Inhal Toxicol, 23, 372–91.

Bernstein DM, Rogers R, Smith P. (2003). The biopersistence of Canadian chrysotile asbestos following inhalation. Inhal Toxicol, 15, 101–28.

Bernstein DM, Rogers R, Smith P. (2004). The biopersistence of Brazilian chrysotile asbestos following inhalation. Inhal Toxicol, 16, 745–61.

Bernstein DM, Rogers R, Smith P. (2005a). The biopersistence of Canadian chrysotile asbestos following inhalation: final results through 1 year after cessation of exposure. Inhal Toxicol, 17, 1–14.

Berry G, Newhouse ML. (1983). Mortality of workers manufacturing friction materials using asbestos. Br J Ind Med, 40, 1–7.

Bolton RE, Vincent JH, Jones AD, et al. (1983). An overload hypothesis for pulmonary clearance of UICC amosite fibres inhaled by rats. Br J Ind Med, 40, 264–72.

Bowles O, Barsigian FM. (1954). Asbestos, In: McGann PW, ed. Minerals yearbook 1951, Bureau of Mines. United States Government Printing Office, 167–76. Available from: http://digital.library.wisc.edu/1711.dl/EcoNatRes.MinYB1951.

Bowles O, Stoddard BH. (1933). Asbestos, In: Kiessling OE, ed. Minerals yearbook 1932–33, United States Government Printing Office, 1933, 745–52. Available from: http://digital.library.wisc.edu/1711.dl/EcoNatRes.MinYB193132.

Bragg GM. (2001). Fiber release during the handling of products containing chrysotile asbestos using modern control technology. In: Nolan RP, Langer AM, Ross M, et al., eds. The health effects of chrysotile asbestos: contribution of science to risk-management decisions. Ottawa, Canada: Canadian Mineralist, Spec. Publ. 5, 111–14.

Bray RM, Guess LL, Marsden ME. (1989). Prevalence, trends, and correlates of alcohol use, nonmedical drug use, and tobacco use among US military personnel. Mil Med, 154, 1–11.

Bray RM, Marsden ME, Peterson MR. (1991). Standardized comparisons of the use of alcohol, drugs, and cigarettes among military personnel and civilians. Am J Public Health, 81, 865–9.

Breysse PN, Cherrie JW, Addison J, et al. (1989). Evaluation of airborne asbestos concentrations using TEM and SEM during residential water tank removal. Ann. Occup Hyg, 33, 243–56.

Campbell WJ, Huggins CW, Wylie AG. (1980). Chemical and physical characterization of amosite, chrysotile, crocidolite, and nonfibrous tremolite for oral ingestion studies by the national institute of environmental health sciences [report of investigation 8452]. Avondale (MD): United States Department of The Interior, US Bureau of Mines.

Carel R, Olsson AC, Zaridze D, et al. (2007). Occupational exposure to asbestos and man-made vitreous fibers and risk of lung cancer: a multicentre case-control study in Europe. Occup Environ Med, 64, 502–8.

Case BW, Abraham JL. (2009). Heterogeneity of exposure and attribution of mesothelioma: trends and strategies in two American counties. J Phys: Conf Ser, 151, 012008.

Case BW, Dufresne A, McDonald AD, et al. (2000). Asbestos fibre type and length in lungs of chrysotile textile and production workers: fibers longer than 18 mum. Inhal Toxicol, 12, 411–8.

Case BW, McDonald C. (2008). Chrysotile, tremolite, balangeroite and mesothelioma: similar situations? Occup Environ Med, 65, 815–9

Churg A. (1988). Chrysotile, tremolite and malignant mesothelioma. Chest, 93, 621–8.

Churg A, Wiggs B, Depaoli L, et al. (1984). Lung asbestos content in chrysotile workers with mesothelioma. Am Rev Respir Dis, 130, 1042–5.

Coin PG, Roggli VL, Brody AR. (1992). Deposition, clearance, and translocation of chrysotile asbestos from peripheral and central regions of the rat lung. Environ Res, 58, 97–116.

DOI: 10.3109/10408444.2012.756454




Coleman RG. (1996). New Idria serpentinite: a land management dilemma. Environ Eng Geoscience, 2, 9–22.

Conway TL, Trent LK, Conway SW. (1989). Physical readiness and lifestyle habits among US navy personnel during 1986, 1987, and 1988. Technical Report No 89–23. San Diego, California: Naval Health Research Center.

Cressey BA, Whittaker EJW. (1993). Five-fold symmetry in chrysotile asbestos revealed by transmission electron microscopy. Mineral Mag, 57, 729–32.

Davis JM, Addison J, Bolton RE, et al. (1986). The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. Br J Exp Pathol, 67, 415–30.

Davis JM, Beckett ST, Bolton RE, et al. (1978). Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. Br J Cancer, 37, 673–88.

Davis JM, Jones AD. (1988). Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. Br J Exp Pathol, 69, 717–37.

Dement JM, Brown DP. (1994). Lung cancer mortality among asbestos textile workers: a review and update. Ann Occup Hyg, 38, 525–32, 412.

Dement JM, Harris Jr RL, Symons MJ, et al. (1982). Estimates of dose-response for respiratory cancer among chrysotile asbestos textile workers. Ann Occup Hyg, 26, 869–87.

Devesa SS, Grauman DJ, Blot WJ, et al. (1999). Atlas of cancer mortality in the United States 1950–1994 [NIH Publication No. 99-4564]. Bethesda (MD): National Institutes of Health, National Cancer Institute.

Dreessen WC, Dallavalle JM, Edwards TI, et al. (1938). A study of asbestosis in the asbestos textile industry [Public Health Bulletin No. 211]. Bethesda (MD): National Institute of Health, 91–125.

Elmes PC, Wade OL. (1965). Relationship between exposure to asbestos and pleural malignancy in Belfast. Ann N Y Acad Sci, 132, 549–57.

Esmen NA, Corn M. (1998). Airborne fiber concentrations during splitting open and boxing bags of asbestos. Toxicol Ind Health, 14, 843–56.

European Commission Joint Research Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau. (1999). Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF). Bernstein DM, Riego-Sintes JMR, eds, Vol. EUR 18748 EN, April 93. Available from: http://ecb.ei.jrc.it/DOCUMENTS/Testing- Methods/mmmfweb.pdf.

Evans BW. (2004). The serpentinite multisystem revisited: chrysotile is metastable. Int Geol Rev, 46, 479–506.

Finley BL, Pierce JS, Phelka AD, et al. (2012). Evaluation of tremolite asbestos exposures associated with the use of commercial products. Crit Rev Toxicol, 42, 119–46.

Gardner MJ, Winter PD, Pannett B, et al. (1986). Follow up study of workers manufacturing chrysotile asbestos cement products. Br J Ind Med, 43, 726–32.

Gazzano E, Riganti C, Tomatis M, et al. (2005). Potential toxicity of nonregulated asbestiform minerals: balangeroite from the western Alps. Part 3: depletion of antioxidant defenses. J Toxicol Environ Health Part A, 68, 41–9.

Gibbs GW. (1994). The assessment of exposure in terms of fibres. Ann Occup Hyg, 38, 477–87, 409–10.

Gibbs AR, Pooley F. (2008). Mineral fibers analysis and asbestos related diseases. In: Craighead JE, Gibbs AR, eds. Asbestos and its diseases. New York: Oxford University Press, 299–316.

Goodglick LA, Kane AB. (1990). Cytotoxicity of long and short crocidolite asbestos fibers in vitro and in vivo. Cancer Res, 50, 5153–63.

Green FHY, Harley R, Vallyathan V, et al. (1997). Exposure and mineralogic correlates of pulmonary fibrosis in chrysotile asbestos workers. Occup Environ Med, 54, 549–59.

Groppo C, Tomatis M, Turci F, et al. (2005). Potential toxicity of nonregulated asbestiform minerals: balangeroite from the western Alps. Part 1: identification and characterization. J Toxicol Environ Health Part A, 68, 1–19.

Gross P, Cralley LJ, DeTreville RT. (1967). ''Asbestos'' bodies: their nonspecificity. Am Ind Hyg Assoc J, 28, 541–2.

Hain E, Dalquen P, Bohlig H, et al. (1974). [Retrospective study of 150 cases of mesothelioma in Hamburg area (author's transl)]. Int Arch Arbeitsmed, 33, 15–37.

Hammad Y, Simmons W, Abdel-Kader H, et al. (1988). Effect of chemical composition on pulmonary clearance of man-made mineral fibers. Ann Occup Hyg, 22, 769–79.

Hargreaves A, Taylor WH. (1946). An X-ray examination of decomposition products of chrysotile asbestos and serpentine. Mineral Mag, 27, 204–16.

Hein MJ, Stayner LT, Lehman E, et al. (2007). Follow-up study of chrysotile textile workers: cohort mortality and exposure-response. Occup Environ Med, 64, 616–25.

Hesterberg TW, Axten C, McConnell EE, et al. (1999). Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the Syrian golden hamster. Part I. Results of a subchronic study and dose selection for a chronic study. Inhal Toxicol, 11, 747–84.

Hesterberg TW, Hart GA, Chevalier J, et al. (1998). The importance of fiber biopersistence and lung dose in determining the chronic inhalation effects of X607, RCF1, and chrysotile asbestos in rats. Toxicol Appl Pharmacol, 153, 68–82.

Hesterberg TW, Miiller WC, McConnell EE, et al. (1993). Chronic inhalation toxicity of size-separated glass fibers in Fischer 344 rats. Fundam Appl Toxicol, 20, 464–76.

Hodgson JT, Darnton A. (2000). The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann Occup Hyg, 44, 565–601.

Hughes JM, Weill H, Hammad YY. (1987). Mortality of workers employed in two asbestos cement manufacturing plants. Br J Ind Med, 44, 161–74.

Ilgren EB. (2004). Coalinga chrysotile a short fibre, amphibole free, chrysotile Part V – lack of amphibole asbestos contamination. Indoor Built Environ, 13, 375–82.

Ilgren EB, Breña MO, Larragoitia JC, et al. (2008a). A reconnaissance study of a potential emerging Mexican mesothelioma epidemic due to fibrous zeolite exposure. Indoor Built Environ, 17, 496–515.

Ilgren E, Chatfield E. (1997). Coalinga fibre – a short, amphibole-free chrysotile. Part 1: Evidence for lack of fibrogenic activity. Indoor Built Environ, 6, 264–76.

Ilgren E, Chatfield E. (1998). Coalinga fibre – a short, amphibole-free chrysotile. Part 2: Evidence for lack of tumorigenic activity. Indoor Built Environ, 7, 18–31.

Ilgren EB, Pooley FD, Larragoitia JC, et al. (2008b). First confirmed erionite related mesothelioma in North America. Indoor Built Environ, 17, 567–8.

Ilgren E, Ramirez R, Claros E, et al. (2012). Fiber width as a determinant of mesothelioma induction and threshold Bolivian crocidolite: epidemiological evidence from Bolivia mesothelioma demography and exposure pathways. Ann Respir Med. Available from: www.slm-respiratory.com.

ILO. (1984). Safety in the use of asbestos: an ILO code of practice. Geneva: International Labour Office.

Jones RN, Diem JE, Hughes JM, et al. (1989). Progression of asbestos effects: a prospective longitudinal study of chest radiographs and lung function. Br J Ind Med, 46, 97–105.

Kanarek MS. (2011). Mesothelioma from chrysotile asbestos: update. Ann Epidemiol, 21, 688–97.

Karbownik J, Clark S. (1997, 2005, 2006, 2007, 2008, 2009, 2012). Determination of presence of amphibole asbestos fibres in four bulk samples of chrysotile [report to client, project no: 610]. Edinburgh, UK: IOM Consulting Limited.

Kashansky SV, Domnin SG, Kochelayev VA, et al. (2001). Retrospective view of airborne dust levels in workplace of a chrysotile mine in Ural, Russia. Ind Health, 39, 51–6.

Kliment CR, Clemens K, Oury TD. (2009). North American erionite-associated mesothelioma with pleural plaques and pulmonary fibrosis: a case report. Int J Clin Exp Pathol, 2, 407–10.

Kobell F. (1834). Ueber den schillernden Asbest von Reichenstein in Schlesien. J Prakt Chemie, 2, 297–8.

Kurumatani N, Kumagai S. (2008). Mapping the risk of mesothelioma due to neighborhood asbestos exposure. Am J Respir Crit Care Med, 178, 624–9.

Larsen G. (1989). Experimental data on in vitro fibre solubility. IARC Sci Publ, 90, 134–9.

LeBouffant L, Daniel H, Henin JP, et al. (1987). Experimental study on long-term effects of inhaled MMMF on the lung of rats. Ann Occup Hyg, 31, 765–90.

Legifrance. (1994). Décret no 94-645 du 26 juillet 1994 modifiant le décret no 88-466 du 28 avril 1988 relatif aux produits contenant de l'amiante, JORF n°173 du 28 juillet 1994 page 10907. Available from: http://www.legifrance.gouv.fr.

Liddell FD, McDonald AD, McDonald JC. (1997). The 1891–1920 birth cohort of Quebec chrysotile miners and millers: development from 1904 and mortality to 1992. Ann Occup Hyg, 41, 13–36.

Liddell FDK, McDonald AD, McDonald JC. (1998). Dust exposure and lung cancer in Quebec chrysotile miners and millers. Ann Occup Hyg, 42, 7–20.

Lippmann M. (1990). Effects of fiber characteristics on lung deposition, retention, and disease. Environ Health Perspect, 88, 311–7.

Mancuso TF. (1983). Mesothelioma among machinists in railroad and other industries. Am J Ind Med, 4, 501–13.

Mancuso TF. (1988). Relative risk of mesothelioma among railroad machinists exposed to chrysotile. Am J Ind Med, 13, 639–57. Erratum in: (1989). Am J Ind Med, 15, 125.

Mast RW, McConnell EE, Anderson R, et al. (1995). Studies on the chronic toxicity (inhalation) of four types of refractory ceramic fiber in male Fischer 344 rats. Inhal Toxicol, 7, 425–67.

McClellan RO, Miller FJ, Hesterberg TW, et al. (1992). Approaches to evaluating the toxicity and carcinogenicity of man-made fibers: summary of a workshop held November 11–13, 1991, Durham, North Carolina. Regul Toxicol Pharmacol, 16, 321–64.

McConnell EE, Axten C, Hesterberg TW, et al. (1999). Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the Syrian golden hamster. Part II. Results of chronic exposure. Inhal Toxicol, 11, 785–835.

McDonald AD, Case BW, Churg A, et al. (1997). Mesothelioma in Quebec chrysotile miners and millers: epidemiology and aetiology. Ann Occup Hyg, 41, 707–19.

McDonald AD, Fry JS, Woolley AJ, et al. (1983). Dust exposure and mortality in an American factory using chrysotile, amosite, and crocidolite in mainly textile manufacture. Br J Ind Med, 40, 368–74.

McDonald AD, Fry JS, Woolley AJ, et al. (1984). Dust exposure and mortality in an American chrysotile asbestos friction products plant. Br J Ind Med, 41, 151–7.

McDonald AD, Harper A, McDonald JC, et al. (1970). Epidemiology of primary malignant mesothelial tumors in Canada. Cancer, 26, 914–19.

McDonald JC, Liddell FD. (1979). Mortality in Canadian miners and millers exposed to chrysotile. Ann NY Acad Sci, 330, 1–9.

McDonald JC, McDonald AD. (1995). Chrysotile, tremolite and mesothelioma. Science, 267, 775–6.

McDonald JC, McDonald AD. (1996). The epidemiology of mesothelioma in historical context. Eur Respir J, 9, 1932–42.

McEwen J, Finlayson A, Mair A, et al. (1970). Mesothelioma in Scotland. Br Med J, 4, 575–8.

Morgan A. (1995). Deposition of inhaled asbestos and man-made mineral fibres in the respiratory tract. Ann Occup Hyg, 39, 747–58.

Morris GF, Notwick AR, et al. (2004). Development of lung tumors in mutant p53-expressing mice after inhalation exposure to asbestos. Chest, 125, 85S–6S.

Morrow PE. (1988). Possible mechanisms to explain dust overloading of the lung. Fundam Appl Toxicol, 10, 369–84.

Morrow PE. (1992). Dust overloading of the lungs: update and appraisal. Toxicol Appl Pharmacol, 113, 1–12.

Muhle H, Bellman B, Heinrich U. (1988). Overloading of lung clearance during chronic exposure of experimental animals to particles. Ann Occup Hyg, 32, 141–7.

Muhle H, Pott F, Bellmann B, et al. (1987). Inhalation and injection experiments in rats to test the carcinogenicity of MMMF. Ann Occup Hyg, 31, 755–64.

Musti M, Pollice A, Cavone D, et al. (2009). The relationship between malignant mesothelioma and an asbestos cement plant environmental risk: a spatial case-control study in the city of Bari (Italy). Int Arch Occup Environ Health, 82, 489–97.

Newhouse ML, Sullivan KR. (1989). A mortality study of workers manufacturing friction materials: 1941–86. Br J Ind Med, 46, 176–9.

Newhouse ML, Thompson H. (1965). Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. Br J Ind Med, 22, 261–9.

NIOSH. (2011). Asbestos fibers and other elongate mineral particles: state of the science and roadmap for research [revised April 2011, publication number 2011-159, current intelligence bulletin 62]. Washington (DC): Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health.

Noll W, Kircher H. (1951). Über die Morphologie von Asbesten und ihren Zusammenhang mit der Kristallstruktur. Neues Jb. Mineral., Mh. 1951, 219–40.

Oberdörster G. (1995). Lung particle overload: implications for occupational exposures to particles. Regul Toxicol Pharmacol, 21, 123–35.

Ohlson CG, Hogstedt C. (1985). Lung cancer among asbestos cement workers. A Swedish cohort study and a review. Br J Ind Med, 42, 397–402.

Osmond-McLeod MJ, Poland CA, Murphy F, et al. (2011). Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres. Part Fibre Toxicol, 8, 15.

Oze C, Solt K. (2010). Biodurability of chrysotile and tremolite asbestos in simulated lung and gastric fluids. Am Mineral, 95, 825–31.

Pan XL, Day HW, Wang W, et al. (2005). Residential proximity to naturally occurring asbestos and mesothelioma risk in California. Am J Respir Crit Care Med, 172, 1019–25.

Pauling L. (1930). The structure of the chlorites. Proc Nat Acad Sci USA, 16, 578–82.

Paustenbach DJ, Finley BL, Lu ET, et al. (2004). Environmental and occupational health hazards associated with the presence of asbestos in brake linings and pads (1900 to present): A 'state-of-the-art review'. J Toxicol Environ Health, Part B, 7, 33–110.

Perry RH, Chilton CH, eds. (1973). Chemical engineers' handbook. 5th ed. New York (NY): McGraw-Hill. TIC: 242591.

Pierce JS, McKinley MA, Paustenbach DJ, et al. (2008). An evaluation of reported no-effect chrysotile asbestos exposures for lung cancer and mesothelioma. Crit Rev Toxicol, 38, 191–214.

Pinkerton KE, Brody AR, McLaurin DA, et al. (1983). Characterization of three types of chrysotile asbestos after aerosolization. Environ Res, 31, 32–53.

Piolatto G, Negri E, La Vecchia C, et al. (1990). An update of cancer mortality among chrysotile asbestos miners in Balangero, northern Italy. Br J Ind Med, 47, 810–4.

Pohanish RP, ed. (2008). Sittig's handbook of toxic and hazardous chemicals and carcinogens. 5th ed. Norwich (NY): William Andrews, 272.

Poland CA, Duffin R, Kinloch I, et al. (2008). Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol, 3, 423–8.

Pooley F. (2003). Personal communication of a report prepared under contract to KCAC of the examination of chrysotile asbestos samples from the asbestos mine and processing plant of KCAC, Inc., 1991. Cited in Bernstein DM, Chevalier J, Smith P. (2005). Comparison of calidria chrysotile asbestos to pure tremolite: Final results of the inhalation biopersistence and histopathology examination following short-term exposure. Inhal Toxicol, 17, 427–49.

Pooley FD, Mitha R. (1986). Determination and interpretation of the levels of chrysotile asbestos in lung tisue. In: Wagner JC, ed. Biological effects of chrysotile. Accomplishments in Oncology Vol. 1. No. 2. Philadelphia: Lippincott, 12–18.

Roggli VL, Pratt PC, Brody AR. (1992). Analysis of tissue mineral fiber content. In: Roggli VL, Greenberg SD, Pratt PC, eds. Pathology of asbestos-associated diseases. Chap. 11. Boston (MA): Little, Brown, 299–345.

Roggli VL, Sharma A, Butnor KJ, et al. (2002b). Malignant mesothelioma and occupational exposure to asbestos: a clinicopathological correlation of 1445 cases. Ultrastruct Pathol, 26, 55–65.

Roggli VL, Vollmer RT, Butnor KJ, et al. (2002a). Tremolite and mesothelioma. Ann Occup Hyg, 46, 447–53.

Rubino GF, Scansetti G, Donna A, et al. (1972). Epidemiology of pleural mesothelioma in North-western Italy (Piedmont). Br J Ind Med, 29, 436–42.

Schneider F, Sporn TA, Roggli VL. (2010). Asbestos fiber content of lungs with diffuse interstitial fibrosis: An analytical scanning electron microscopic analysis of 249 cases. Arch Pathol Lab Med, 134, 457–61.

DOI: 10.3109/10408444.2012.756454




Sebastien P, McDonald JC, McDonald AD, et al. (1989). Respiratory cancer in chrysotile textile and mining industries: exposure inferences from lung analysis. Br J Ind Med, 46, 180–7.

Selikoff IJ, Churg J, Hammond EC. (1984). Landmark article April 6, 1964: Asbestos exposure and neoplasia. By Irving J. Selikoff, Jacob Churg, and E. Cuyler Hammond. JAMA, 252, 91–5.

Seshan K. (1983). How are the physical and chemical properties of chrysotile asbestos altered by a 10-year residence in water and up to 5 days in simulated stomach acid? Environ Health Perspect, 53, 143–8

Sichletidis L, Chloros D, Spyratos D, et al. (2009). Mortality from occupational exposure to relatively pure chrysotile: a 39–year study. Respiration, 78, 63–8.

Silvestri S, Magnani C, Calisti R, et al. (2001). The experience of the Balangero chrysotile asbestos mine in Italy: health effects among workers mining and milling asbestos and the health experienceof persons living nearby. In: Nolan RP, Langer AM, Ross M, Wicks FJ, Martin RF, eds. The health effects of chrysotile asbestos: contribution of science to risk-management decisions. Ottawa, Canada: Canadian Mineralogist, Spec. Publ. 5, 177–86.

Skinner HCW, Ross M, Frondel C. (1988). Asbestos and other fibrous materials – mineralogy, crystal chemistry, and health effects. New York (NY): Oxford University Press, 204.

Smith AH, Wright CC. (1996). Chrysotile asbestos is the main cause of pleural mesothelioma. Am J Ind Med, 30, 252–66.

Speil S, Leineweber JP. (1969). Asbestos minerals in modern technology. Environ Res, 2, 166–208.

Stanton MF. (1973). Some etioligical considerations of fibre carcinogenesis. In: Bogovski P, Gilson JC, Timbrell V, Wagner JC, eds. Biological effects of asbestos. Lyon: WHO IARC, 289–94.

Stanton MF, Layard M, Tegeris A, et al. (1981). Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. J Natl Cancer Inst, 67, 965–75.

Stanton MF, Wrench C. (1972). Mechanisms of mesothelioma induction with asbestos & fiberous glass. J Natl Cancer Inst, 48, 797–821.

Stayner L, Kuempel E, Gilbert S, et al. (2008). An epidemiological study of the role of chrysotile asbestos fiber dimensions in determining respiratory disease risk in exposed workers. Occup Environ Med, 65, 613–9.

Suquet H. (1989). Effects of dry grinding and leaching on the crystal structure of chrysotile. Clays Clay Miner, 37, 439–45.

Thomas HF, Benjamin IT, Elwood PC, et al. (1982). Further follow-up study of workers from an asbestos cement factory. Br J Ind Med, 39, 273–6.

Timbrell V, Hyett AW, Skidmore JW. (1968). A simple dispenser for generating dust clouds from standard reference samples of asbestos. Ann Occup Hyg, 11, 273–81.

Timbrell V, Rendall REG. (1972). Preparation of the UICC standard reference samples of asbestos. Powder Technol, 5, 279–87.

Titulaer MK, van Miltenburg JC, Jansen JBH, et al. (1993). Characterization of tubular chrysotile by thermoporometry, nitrogen sorption, drifts, and TEM. Clays Clay Miner, 41, 496–513.

Tossavainen A, Kotilainen M, Takahashi K, et al. (2001). Amphibole fibres in Chinese chrysotile asbestos. Ann Occup Hyg, 45, 145–52.

Tossavainen A, Kovalevsky E, Vanhala E, et al. (2000). Pulmonary mineral fibers after occupational and environmental exposure to asbestos in the Russian chrysotile industry. Am J Ind Med, 37, 327–33.

Tossavainen A, Riala R, Kamppi R, et al. (1996). Dust measurements in the chrysotile mining and milling operations of Uralasbest Company, Asbest, Russia [summary report]. Helsinki: Institute of Occupational Health, 220.

Turci F, Tomatis M, Gazzano E, et al. (2005). Potential toxicity of nonregulated asbestiform minerals: balangeroite from the western Alps. Part 2: oxidant activity of the fibers. J Toxicol Environ Health Part A, 68, 21–39.

US DHHS. (1989). Reducing the health consequences of smoking: 25 years of progress. A report of the Surgeon General, 1989 [DHHS Publication No (CDC) 89-8411]. Rockville (MD): Public Health Service, Centers for Disease Control, Office on Smoking and Health.

US EPA. (2001). US EPA health effects test guidelines OPPTS 870.8355 guideline for combined chronic toxicity/carcinogenicity testing of respirable fibrous particles [EPA 712-C-01-352, July]. Washington (DC): US Environmental Protection Agency.

Van Orden DR, Lee RL, Sanchez MS, et al. (2012). The size distribution of airborne Bolivian crocidolite fibers [case report]. The Annals of Respiratory Medicine. Monroeville (PA): RJ Lee Group, Inc. Available from: www.slm-respiratory.com [last accessed 26 Jul 2012].

Veblen DR, Wylie AG. (1993). Mineralogy of amphiboles and 1:1 layer silicates. In: Guthrie Jr GD, Mossman BT, eds. Health effects of mineral dusts. Washington (DC): Mineralogical Society of America, Reviews in Mineralogy, Vol. 28, 61–137.

Virta RL. (2002). Asbestos: geology, mineralogy, mining, and uses. Prepared in cooperation with Kirk-Othmer encyclopedia of chemical technology. USGS Open file 02-149. Online Edition. New York (NY): Wiley-Interscience, a division of John Wiley & Sons, Inc.

Virta RL. (2005). Mineral commodity profiles - asbestos [US Geological Survey circular 1255-KK]. Reston (VA): US Geological Survey.

Virta RL. (2006). Worldwide asbestos supply and consumption trends from 1900 through 2003 [circular 1298]. Reston (VA): US Geological Survey.

Virta RL. (2011). 2010 minerals yearbook – asbestos. US Geological Survey Minerals Yearbook – 2010. Reston (VA): US Geological Survey.

von Kobell F. (1834). Ueber den schillernden Asbest von Reichenstein in Schlesian: Jour. Prakt. Chemie, 2, 297–8.

Vorwald AJ, Durkan TM, Pratt PC. (1951). Experimental studies of asbestosis. AMA Arch Ind Hyg Occup Med, 3, 1–43.

Wagner JC, Berry G, Skidmore JW, et al. (1974). The effects of the inhalation of asbestos in rats. Br J Cancer, 29, 252–69.

Wagner JC, Berry G, Skidmore JW, et al. (1980). The comparative effects of three chrysotiles by injection and inhalation in rats. In: Wagner JC, ed. Biological Effects of Mineral Fibers. IARC Publication 30. Lyon: International Agency Research on Cancer, 363–73.

Wagner JC, Sleggs CA, Marchand P. (1960). Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. Br J Ind Med, 17, 260–71.

Walton WH. (1982). The nature, hazards, and assessment of occupational exposure to airborne asbestos dust: a review. Ann Occup Hyg, 25, 117–247.

Wang X, Yano E, Qiu H, et al. (2012). A 37-year observation of mortality in Chinese chrysotile asbestos workers. Thorax, 67, 106–10.

Warren BE, Bragg WL. (1930). The structure of chrysotile, $H_4Mg_3Si_2O_9$. Z Krystallographie, 76, 201–10.

Weill H, Hughes J, Waggenspack C. (1979). Influence of dose and fiber type on respiratory malignancy risk in asbestos cement manufacturing. Am Rev Respir Dis, 120, 345–54.

White N, Nelson G, Murray J. (2008). South African experience with asbestos related environmental mesothelioma: is asbestos fiber type important? Regul Toxicol Pharmacol, 52, S92–6

Whittaker EJW. (1957). The structure of chrysotile. V. Diffuse reflexions and fibre texture. Acta Cryst, 10, 149–56.

Whittaker EJW. (1957). The Structure of Chrysotile. V. Diffuse Reflexions and Fibre Texture. Acta Cryst, 10, 149–56.

Whittaker EJW. (1960). The crystal chemistry of the amphiboles. Acta Cryst, 13, 291–98.

Whittaker EJW. (1963). in Research report: Chrysotile Fibers –Filled or Hollow Tubes? Mathematical interpretation may resolve conflicting evidence. Chem Eng News, 41, 34–35, September 30, 1963.

WHO. (1985). Reference methods for measuring airborne man-made mineral fibers (MMMF): WHO/EURO MMMF reference scheme. Copenhagen: WHO.

WHO. (1988). Environmental health criteria 77: man-made mineral fibres. Vol. 77. Geneva: WHO.

Wicks FJ, O'Hanley DS. (1988). Serpentine minerals: structures and petrology. In: Bailey S, ed. Hydrous phyllosilicates (exclusive of micas). Washington, DC: Mineralogical Society of America, Reviews in Mineralogy, Vol. 19, 91–167.

Williams-Jones AE, Normand C, Clark JR, et al. (2001). Controls of amphibole formation in chrysotile deposits: evidence from the Jeffrey Mine, Asbestos, Quebec. In: Nolan RP, Langer AM, Ross M, Wicks FJ, Martin RF, eds. The health effects of chrysotile asbestos: contribution of science to risk-management decisions. Ottawa, Canada: Canadian Mineralogist, Spec. Publ. 5, 89–104.

Work LT. (1962). Size reduction gets a new stature. Ind Eng Chem, 54, 52–4.

Wypych F, Adad LB, Mattoso N, et al. (2005). Synthesis and characterization of disordered layered silica obtained by selective leaching of octahedral sheets from chrysotile. J Colloid Interface Sci, 283, 107–12.

Yano E, Wang ZM, Wang XR, et al. (2001). Cancer mortality among workers exposed to amphibole-free chrysotile asbestos. Am J Epidemiol, 154, 538–43.

Yano E, Wang ZM, Wang XR, et al. (2009). Mesothelioma in a worker who spun chrysotile asbestos at home during childhood. Am J Ind Med, 52, 282–7.

Yarborough CM. (2006). Chrysotile as a cause of mesothelioma: an assessment based on epidemiology. Crit Rev Toxicol, 36, 165–87.

Zamataro RSI, Franzini MJ. (2012). Verificar qualitativamente a tipologia das amostras de amianto extraídas na SAMA, por análise difratométrica de raios X [Relatório N° 021E-12]. São Paulo – SP, Brazil: Projecontrol Cons. Empresarial e Serviços Ltda.

Zielhuis RL, Versteeg JP, Planteijdt HT. (1975). Pleura mesothelioma and exposure to asbestos. A retrospective case-control study in the Netherlands. Int Arch Occup Environ Health, 36, 1–18.

DOI: 10.3109/10408444.2012.756454

## Appendix

Table A1. Chronic inhalation studies with chrysotile.

| Fiber type | ExposureTime (h/d, d/wk, total months) | Fiber concentration (f/cm$^3$) | Fiber mass concentration (mg/m$^3$) | Equivalent fiber concentration/ cm$^3$ TEM | Type, total number of rats | Number of pulmonary tumors | % of Pulmonary tumors | Number of mesotheliomas | References |
|---|---|---|---|---|---|---|---|---|---|
| Chrysotile Canadian (nickel, cobalt, chromium and lead contamination) | 6, 5, 14 | Nd | 86 mg/m$^3$ 42–106 mg/m$^3$ first year67–146 mg/m$^3$ second year | 8 600 000 | NS,41 | 10 | 24 | 1 | Gross et al. (1967) |
| Chrysotile UICC Canadian | 7, 5, 24 | Nd | 9.7 mg/m$^3$ | 970 000 | W, 21 | 10 | 48 | 1 | Wagner et al., (1974) |
| Chrysotile UICC Rhodesian | 7, 5, 24 | Nd | 14.7 mg/m$^3$ | 1 470 000 | W, 17 | 11 | 65 | 0 | Wagner et al., (1974) |
| Chrysotile Canadian 714-7D (friction linings) | 5, 5, 24 | $1.7 \times 10^5$ SEM 9978 > 5 μm | 15 mg/m$^3$ Total 5.2 mg/m$^3$ response | 1 500 000 | W, 45 | 9 | 20 | 0 | Le Bouffant et al. (1987) |
| SFA chrysotile | 7, 5, 24 | 430 > 5 μm PCOM 669 particles PCOM | 10.8 mg/m$^3$ | 1 080 000 | W, 22 | 8 | 36 | 1 | Wagner et al., (1980) |
| Grade 7 chrysotile | 7, 5, 24 | 1020 > 5 μm PCOM 745 particles PCOM | 10.8 mg/m$^3$ | 1 080 000 | W, 24 | 3 | 13 | 0 | Wagner et al., (1980) |
| UICC chrysotile | 7, 5, 24 | 3750 > 5 μm PCOM 338 particles PCOM | 10.8 mg/m$^3$ | 1 080 000 | W, 23 | 5 | 22 | 0 | Wagner et al., (1980) |
| Chrysotile Calidria | 5, 5, 12 | 241 by SEM 131>5 μm reported as ''thick bundles'' | 6 mg/m$^3$ | 600 000 | W, 50 | 0 | 0 | 0 | Muhle et al. (1987) |
| Chrysotile long | 7, 5, 12 | 1170 > 5 μm PCOM 33 > 20 μm PCOM 12% > 5 μm SEM | 10 mg/m$^3$ 0.5% > 20 μm SEM | 1 000 000 | W, 40 | 20 | 50 | 2 | Davis et al. (1988) |
| Chrysotile short | 7, 5, 12 | 5510 > 5 μm PCOM 670 > 20 μm PCOM 7% > 5 μm SEM | 10 mg/m$^3$ 0.2% > 20 μm SEM | 1 000 000 | W, 40 | 7 | 17 | 1 | Davis et al. (1988) |
| Chrysotile UICC A | 7, 5, 12 | 2560 > 5 μm PCOM | 10 mg/m$^3$ | 1 000 000 | Included for comparative fiber numbers without animal exposure | | | | Davis et al. (1988) |
| Chrysotile NIEHS | 6, 5, 24 | $1.02 \times 10^5$ SEM $1.06 \times 10^4 > 5$ μm | 10 mg/m$^3$ | 1 000 000 | F, 69 | 13 | 18 | 1 | Mast et al. (1995) and Hesterberg et al. (1993) |
| Chrysotile | 7, 5, 12 | 1950 > 5 μm PCOM 360 > 20 μm PCOM | 10 mg/m$^3$ | 1 000 000 | W, 40 | 15 | 38 | 0 | Davis et al. (1978) |
| Chrysotile | 7, 5, 12 | 390 > 5 μm PCOM 72 > 20 μm PCOM | 2 mg/m$^3$ | 200 000 | W, 42 | 8 | 19 | 1 | Davis et al. (1978) |
| *Author's reported ''With a 2 mg/m$^3$ cloud the percentage retention of chrysotile is almost double that for a 10 mg/m$^3$ cloud''.* | | | | | | | | | |
| Chrysotile Calidria | 7, 5, 12 | Nd | 7.78 mg/m$^3$ | 778 000 | F, 51 | 2 | 4 | 0 | Ilgren & Chatfield (1997, 1998) and Pinkerton et al. (1983) |
| Chrysotile Jeffrey | 7, 5, 12 | Nd | 11.36 mg/m$^3$ | 1 136 000 | F, 49 | 11 | 22 | 0 | Ilgren & Chatfield (1997, 1998) and Pinkerton et al. (1983) |
| Chrysotile UICC/B | 7, 5, 12 | Nd | 10.99 mg/m$^3$ | 1 099 000 | F, 54 | 13 | 24 | 0 | Ilgren & Chatfield (1997, 1998) and Pinkerton et al. (1983) |

STUDY No. 3

# AIRBORNE ASBESTOS EXPOSURES ASSOCIATED WITH THE INSTALLATION AND REMOVAL OF ROOFING PRODUCTS

**STUDY No. 3**

# AIRBORNE ASBESTOS EXPOSURES ASSOCIATED WITH THE INSTALLATION AND REMOVAL OF ROOFING PRODUCTS

**J Occup Environ (2016) Vol 13, Issue 8, 121-131**
**DOI:10.1080/15459624.2016.1183010**

**Lotter J. T., Ben Roberts, John L. Henshaw, and Jennifer S. Pierce**

Asbestos-containing roofing products were widely used throughout the 20th century, and certain products are still used in limited quantities today. Roofing products are generally considered non-friable and are not expected to release appreciable amounts of airborne asbestos fibers; however, despite the variety of roofing products that have contained asbestos over time, there are no comprehensive analyses of the exposure data associated with these products in the published literature. The objective of this study was to analyze the available data and characterize asbestos exposures associated with the installation, removal, and replacement of built-up roofing (BUR), felts, flashings, shingles, coatings, cements, and mastics under a variety of work practices. Published and unpublished literature that contained the following information was included in the analysis: (1) airborne fiber concentrations determined by PCM; (2) a description of the product(s) used; and (3) a description of the task(s) performed. More than 800 personal air samples from 12 studies performed between 1982 and 2010 were identified which fit the inclusion criteria. The findings indicate that short-term and full-shift exposures from the use of asbestos-containing roofing products were typically well below applicable occupational exposure limits. Additionally, the cumulative exposures associated with roofing work would be well below published chrysotile no-observed-adverse-effect levels (NOAELs) for asbestos-related diseases.

JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HYGIENE
2016, VOL. 13, NO. 8, D121–D131
http://dx.doi.org/10.1080/15459624.2016.1183010

Taylor & Francis
Taylor & Francis Group

## Case Study
## Column Editor: Charlie Blake

# Airborne asbestos exposures associated with the installation and removal of roofing products

Reported By
Jason T. Lotter[a], Ben Roberts[a], John L. Henshaw[b], and Jennifer S. Pierce[a]

[a]Cardno ChemRisk, Chicago, Illinois; [b]Cardno ChemRisk, Sanibel, Florida

**ABSTRACT**

Asbestos-containing roofing products were widely used throughout the 20th century, and certain products are still used in limited quantities today. Roofing products are generally considered non-friable and are not expected to release appreciable amounts of airborne asbestos fibers; however, despite the variety of roofing products that have contained asbestos over time, there are no comprehensive analyses of the exposure data associated with these products in the published literature. The objective of this study was to analyze the available data and characterize asbestos exposures associated with the installation, removal, and replacement of built-up roofing (BUR), felts, flashings, shingles, coatings, cements, and mastics under a variety of work practices. Published and unpublished literature that contained the following information was included in the analysis: (1) airborne fiber concentrations determined by PCM; (2) a description of the product(s) used; and (3) a description of the task(s) performed. More than 800 personal air samples from 12 studies performed between 1982 and 2010 were identified which fit the inclusion criteria. The findings indicate that short-term and full-shift exposures from the use of asbestos-containing roofing products were typically well below applicable occupational exposure limits. Additionally, the cumulative exposures associated with roofing work would be well below published chrysotile no-observed-adverse-effect-levels (NOAELs) for asbestos-related diseases.

**KEYWORDS**
Asbestos; chrysotile; exposure assessment; industrial hygiene; occupational exposure; roofing

## Introduction

Asbestos was first incorporated into roofing products sold in the U.S. in the late 1860s.[1] Asbestos-containing roofing products were preferred over non-asbestos materials due to their fire resistance and were adopted into many municipal building codes.[2] In the U.S., the use of asbestos in roofing products peaked in the 1970s at over 230,000 metric tons;[3] however, by the early 1980s asbestos in roofing products was largely discontinued, with the exception of certain roof coatings, cements, and mastics.[4,5] The estimated annual use of asbestos in roofing products in the U.S. as of 2003 was only 3000 metric tons.[3] The asbestos fibers in current and historical roofing products have been and are encapsulated or bound in cementitious, and bituminous or resinous matrices, such as asphalt or coal tar, all of which act to restrict or prevent the release of fibers, unless the product is subjected to significant wear, degradation, or abuse.[4,6,7] It has been reported that weathering can erode cementitious roofing products resulting in potential asbestos emissions.[8] However, because roofing products are necessary to prevent water from entering a building, roofs are typically repaired or replaced before such deterioration of the matrices has occurred.[4,5]

Chrysotile has been the predominant fiber type used in asbestos-containing roofing products in the U.S.[1,3,9–12] According to the Bureau of Mines, greater than 99% of the asbestos used in roofing products in the U.S. between 1974 and 1991 was chrysotile, principally Grade 7; however, small amounts of amosite and crocidolite have been used.[13] No data was found regarding the percentage of asbestos fiber types used specifically in roofing materials prior to 1974.

Several studies have reported the use of amphibole fibers in roofing products manufactured abroad,

**CONTACT** Jason T. Lotter Jason.Lotter@cardno.com Cardno ChemRisk, 30 N. LaSalle St., Suite 3910, Chicago, IL 60602.

including the use of amosite and/or crocidolite in asbestos cement roofing products in Australia, Italy, Germany, and the United Kingdom.[8,14–16] In addition, Anttila et al.[17] noted the use of talc in roof coatings in Finland which reportedly contained small amounts of tremolite asbestos. According to Virta,[18] talc was also used in roofing products in the U.S.; however, no data were located that indicate that the talc used in U.S. roofing products was contaminated with tremolite asbestos.

In the U.S., asbestos-containing shingles were manufactured using either asbestos cement, referred to as A/C roofing shingles, or felts coated in asphalt, referred to as asphalt shingles.[4] Asphalt shingles contained 35–50% asbestos (predominately chrysotile), while A/C roofing shingles, which were produced from at least 1907–1976, contained between 10 and 40% asbestos.[3,9,10,12,13,19,20] Chrysotile was the predominant asbestos fiber type used in A/C roofing shingles, although small amounts of amosite and crocidolite asbestos were also used in A/C sheets which may have been used in the production of A/C roofing shingles.[3,13] The National Roofing Contractors Association (NRCA)[4] reported that asphalt shingles were only produced with asbestos for a short period of time in the 1970s; however, according to the U.S. Environmental Protection Agency (U.S. EPA),[19] asbestos-containing asphalt shingles were manufactured from at least 1907–1979. Both A/C roofing shingles and asbestos-containing asphalt roofing shingles were historically nailed in place during installation, and were generally manually pried off during removal and/or broken up with hammers to facilitate removal, rather than dismantled intact. Additionally, A/C roofing shingles may have been cut on-site during installation using power tools, in order to create a straight clean edge.

Built-up roofing (BUR) consisted of alternating layers of bituminous or resinous compounds and roofing felts.[4,9,10,21] Asbestos was first used in roofing felts in the U.S. in the 1860s, and roofing felts have historically contained greater than 80% asbestos (predominately chrysotile).[1,3,4,9,10,22] BUR was typically installed by cutting the felt to proper lengths and rolling the felt over the roof area in layers, interspersed with the application of hot asphalt; this felt and hot asphalt layering process was repeated for a total or three to five layers, or plies. Removal of BUR involved the use of power roof cutters to cut the layers of felt into sections which could then be removed using prying tools or power tear-off machines. During minor roof repairs or other small jobs, BUR may have been removed by manual methods using hand tools to cut and pry off sections of material. Asbestos-containing asphalt felt was also used in the U.S. under roof shingles, where the felt was laid down under the shingles and installed with nails. The asphalt felt can be removed by cutting and rolling up the felt after shingles are removed.

Asbestos-containing roofing felts coated with a bituminous or resinous binder were also used as base flashings that sealed the edges of a roofing system at walls, perimeters, drains, chimneys, and other locations to prevent water penetration.[4] Base flashings were installed using flashing cements or adhesive coatings to hold the material in place, and were similar to a single ply of BUR except that the roofing felts contained far less asbestos (typically <15%) than those used as BUR, and the base flashings were always manually removed by cutting and prying off.[4,23]

Asbestos-containing roof coatings, cements, and mastics, typically contained five to 45% chrysotile, and are the only category of roofing products that are still manufactured in the U.S. using asbestos.[3–5,9,10,20] Roof coatings consist of solvent-thinned asphalts mixed with reinforcing mineral filler, which is often asbestos.[3,9,10] The coatings are applied in liquid form by spraying, mopping, brushing, or with a trowel, and are used to weatherproof new roofs and repair existing roofs.[4] These materials are often applied as a coating over BUR, and are removed by hand along with other roofing components to which they are attached.

Despite the variety of asbestos-containing roofing products that have been used and are still being used or in service, no systematic analyses of the exposure data associated with the use of these products was found in the published literature. The objective of this study was to assemble and summarize all published and unpublished literature that could be found that describes airborne asbestos fiber concentrations associated with the installation, removal, and replacement of asbestos-containing BUR, felts, flashings, shingles, coatings, cements, and mastics using a variety of work practices.

## Methods

A search was performed to identify published and unpublished literature that reported airborne fiber concentrations associated with the removal, installation, and/or replacement of asbestos-containing roofing products. Studies and reports that provided the following information were included in the analysis.:

1. airborne fiber concentrations of personal samples analyzed by phase contrast microscopy (PCM);
2. a description of the roofing product(s) used; and
3. a description of the task(s) performed and work practices used during sampling.

When available, the following information was extracted from each report or study: product type and composition, task description including tools and

techniques used, number of samples, sampling duration, and airborne fiber concentration. All sampling results were grouped according to sampling duration into the following categories: short-term (≤ 30 min), task-based (31–359 min), and full-shift (≥ 360 min). Exceptions were made for samples with durations of 31–359 min but which were described by the original authors as representative of either full-shift or 30 min short-term exposures; such samples were categorized according to the original authors' classifications. Samples greater than or equal to 360 min were determined not be indicative of individual tasks, but rather more representative of full-shift exposures. Measurements that lacked sampling duration information were assumed to be collected for the duration of the task being evaluated (i.e., task-based). Due to the similarities between BUR, flashings, and felts, and the fact that many of the identified samples included removal of a combination of these materials, these products were grouped together in the analysis.

Descriptive statistics were calculated for each study, including the range, weighted arithmetic mean, and the standard deviation of the fiber concentrations. The concentrations for samples that were below the limit of detection (LOD) were assigned a value of one-half the LOD; or one-half the lowest measured concentration when no LOD was reported. Additionally, data from the individual reports or studies were aggregated and analyzed by product category, task, and sampling duration.

## Results

A total of 12 evaluations performed between 1982 and 2010 met the inclusion criteria.[4,9,15,17,21,24–30] Over 800 personal air sample results, analyzed by PCM, were extracted from the literature and are summarized in Table 1.

### *Anderson et al. (1982)*

Anderson and colleagues[9] presented data on roofing products as part of a larger report submitted to the U.S. EPA in which the potential fiber releases while handling various asbestos-containing products were analyzed. The data pertaining to roofing products as reported by Anderson et al.[9] were collected by Johns-Manville and GCA Corp. in the 1970s and provided limited information regarding sampling duration and the tasks performed. However, SRI International in 1990[21] reported on the same BUR data set and included sample times from the GCA surveys. Anderson et al.[9] reported that task-based (durations and sample numbers not reported [NR]) samples collected during removal, installation, and replacement of spray-applied roof coatings ranged from 0.1–0.4 f/cc, 0.0–0.6 f/cc, and 0.0–0.3 f/cc, respectively. Short-term (14–28 min; n = NR) samples during installation of spray-applied roof coatings had a mean concentration of 0.0 f/cc, and full-shift (342–432 min; n = NR) samples during installation ranged from 0.003–0.15 f/cc. In addition, task-based (60–96 min and NR durations; n = 9) samples during removal of BUR had a mean concentration of 0.1 f/cc (range: 0.0–0.3 f/cc); task-based (60–180 min and NR durations; n = 21) samples during installation of BUR had a mean concentration of 0.2 f/cc (range: 0.0–0.6 f/cc).[9,21]

### *NIOSH (1985)*

In 1985, the National Institute for Occupational Safety and Health (NIOSH) published a Health Hazard Evaluation involving the manual removal of asbestos shingles and simultaneous installation of asbestos asphalt shingles on a residential building in Rockford, Illinois.[24] Workers loosened the shingles using "long-handled wedges" and then shoveled the loosened material from the roof and into a wheelbarrow.[24] Transmission electron microscopy (TEM) analysis of a bulk sample of the asbestos shingle indicated that the material contained 30% chrysotile. The mean task-based (61–101 min; n = 10) concentration for the removal of the asbestos shingles was 0.08 f/cc (range: <0.04–0.16 f/cc). For samples collected during installation of asbestos-containing asphalt shingles, the mean task-based (89–115 min; n = 5) concentration was 0.05 f/cc (range: 0.03–0.08 f/cc). However, the authors noted that due to the accumulation of fibers on the "insides of the cassettes", the measured concentrations were likely lower than the actual airborne fiber concentrations to which roofers are exposed.[24] Although the mean concentration of the task-based samples was below 0.1 f/cc, 3 of the 10 PCM samples collected during removal were above 0.1 f/cc and the authors recommended controls to reduce exposures during roofing work.

### *Brown (1987)*

Brown[15] evaluated exposures during various work activities involving A/C roof sheets on commercial and residential buildings in Australia. The roofs evaluated were 29–40 years old, and many were classified as "severe[ly]" weathered.[15] During standard roof replacement activities, A/C sheets were removed and carried to the edge of the roof where they were stacked on the roof, dropped into a bin, or stacked on a truck; the A/C sheets were replaced by steel roofing. During a building demolition activity, the building was demolished with asbestos roofing sheets in

**Table 1.** Airborne fiber concentrations measured during roofing removal, installation, and replacement activities.

| Reference | Study Type | Sample Duration (min) | Sample Type | Task Category | Number of Samples | Airborne Fiber Concentration (f/cc) Min | Max | Weighted Arithmetic Mean | Standard Deviation |
|---|---|---|---|---|---|---|---|---|---|
| *Shingles and A/C roofing* | | | | | | | | | |
| NIOSH, 1985 | Workplace | 61–101 | Task-based | Removal | 10 | <0.04 | 0.16 | 0.08 | 0.05 |
| | | 89–115 | Task-based | Installation | 5 | 0.03 | 0.08 | 0.05 | 0.02 |
| Brown, 1987 | Simulation | 120–360; NR | Task-based | Removal | 81 | <0.03 | 1.1 | 0.23 | 0.2[a] |
| NRCA, 1994 | Workplace (some simulation) | NR | Task-based | Removal | 32 | <0.0021 | 0.07 | 0.02 | 0.02 |
| | | 480 | Full-shift | Removal | 7 | 0.0013 | 0.0298 | 0.013 | 0.010 |
| | | 360 | Full-shift | Replacement | 3 | <0.01 | 0.03 | 0.02 | 0.01 |
| Anttila et al., 2009 (Riala et al., 1993) | Workplace | ≤44 | Task-based | Removal | 2 | NR | NR | 0.09 | NA[b] |
| *Coatings, cements, and mastics* | | | | | | | | | |
| Anderson et al., 1982 | Workplace | NR | Task-based | Removal | NR | 0.1 | 0.4 | NA[b] | NA[b] |
| | | 14–28 | Short-term | Installation | NR | NR | NR | 0.0 | NA[b] |
| | | NR | Task-based | Installation | NR | 0.0 | 0.6 | NA[b] | NA[b] |
| | | 342–432[c] | Full-shift | Installation | NR | 0.003 | 0.15 | NA[b] | NA[b] |
| | | NR | Task-based | Replacement | NR | 0.0 | 0.3 | NA[b] | NAb |
| Peterson and Franke, 1997 | Simulation (Glove box) | 15 | Short-term | Removal | 2 | <0.013 | 0.019 | 0.013 | NA[b] |
| | | 45 | Task-based | Removal | 2 | <0.004 | 0.006 | 0.004 | NA[b] |
| | | 45 | Task-based | Installation | 4 | <0.004 | <0.004 | 0.002 | 0.003 |
| Mowat et al., 2007 | Simulation | 30 | Short-term | Removal | 36 | <0.006 | 0.027 | 0.01 | 0.01 |
| | | 30 | Short-term | Installation | 12 | <0.006 | 0.009 | 0.007 | 0.001 |
| Sheehan et al., 2010 | Simulation | 30 | Short-term | Removal | 8 | 0.06 | 0.17 | 0.10 | 0.04 |
| *Built-up roofing (BUR), felts, and flashings* | | | | | | | | | |
| Anderson et al., 1982 | Workplace | 60–96; NR | Task-based | Removal | 9 | 0.0 | 0.3 | 0.1 | 0.1 |
| | | 60–180; NR | Task-based | Installation | 21 | 0.0 | 0.6 | 0.2 | 0.2 |
| Hays and Ainslie, 1990 | Workplace | NR | Task-based | Removal | 5 | 0.019 | 0.028 | 0.024 | 0.004 |
| SRI International, 1990 | Workplace | 22–30 | Short-term | Removal | 16 | <0.010 | 0.248 | 0.05 | 0.06 |
| | | 32–357; NR | Task-based | Removal | 275 | 0.001 | 0.132 | 0.01 | 0.02 |
| | | 368–1475 | Full-shift | Removal | 69 | <0.00031 | 0.046 | 0.01 | 0.01 |
| | | 50–210 | Task-based | Replacement | 3 | 0.03 | 0.05 | 0.04 | 0.01 |
| NRCA, 1995 | Workplace | 17–30 | Short-term | Removal | 17 | 0.06 | 0.115 | 0.06 | 0.02 |
| | | 31–135; NR | Task-based | Removal | 104 | 0.000 | 0.063 | 0.01 | 0.01 |
| | | 480 | Full-shift | Removal | 46 | 0.0007 | 0.027 | 0.01 | 0.01 |
| Recon Environmental Corp., 1995 | Workplace | 28–35[d] | Short-term | Replacement | 28 | 0.031 | 0.397 | 0.065 | 0.068 |
| | | 70–480[e] | Full-shift | Replacement | 37 | <0.002 | 0.056 | 0.017 | 0.014 |
| Lange and Thomulka, 2000 | Workplace | NR | Task-based | Removal | 13 | 0.0047 | 0.0752 | 0.020 | 0.020 |

[a]Individual sample results not reported; standard deviation calculated from weighted mean concentrations
[b]Unable to calculate based on available data
[c]Data were categorized as "full-shift" because individual samples and durations were not presented separately
[d]Described as representative of "short-term" exposures
[e]Described as representative of "full-shift" exposures

place; workers then removed the sheets intact and stacked them on a platform. In other building demolition activities, workers removed all claddings from the standing structures. Roof removal during building demolition took place at a faster pace (100 $m^2$ of sheeting/man-hour) than standard roof removal (5–10 $m^2$ of sheeting/man-hour), and the author noted that "[b]uilding demolition appeared to create considerably more visible dust emission."[15] The personal task-based (120–360 min and NR durations; n = 81) sample mean concentration during A/C roof sheet removal for both operations was 0.23 f/cc (range: <0.03–1.1 f/cc).

### *Hays and Ainslie (1990)*

Hays and Ainslie[25] collected fiber measurements during the removal of several non-friable asbestos-containing materials, including roofing materials. Approximately 790 $m^2$ of roofing felt and 152 linear meters of flashing were removed from two buildings by wet prying and peeling. Bulk analysis by polarized light microscopy (PLM) of the roofing materials indicated that the flashing contained approximately 30% chrysotile and the roofing felt contained approximately 20% chrysotile. The mean concentration associated with the five personal task-based samples of unreported duration collected during the removal of the roofing materials was 0.024 f/cc (range: 0.019–0.028 f/cc).

### *SRI International (1990)*

SRI International was contracted by the NRCA to evaluate data compiled by the NRCA for the purpose of obtaining an exemption from the Occupational Safety and Health Administration (OSHA) regulations regarding the monitoring of asbestos during roof removal jobs.[21] Of the 79 air monitoring reports that were evaluated, 47 were determined to have used monitoring methods consistent with the OSHA Reference Method. Therefore, only data from these 47 reports were used in this analysis. The mean concentration for short-term (22–30 min; n = 16) samples collected during removal of BUR was 0.05 f/cc (range: <0.010–0.248 f/cc). The mean concentration associated with task-based (32–357 min and NR durations; n = 275) and full-shift (368–1475 min; n = 69) personal samples were 0.01 f/cc (range: 0.001–0.132 f/cc) and 0.01 f/cc (range: <0.0003–0.046 f/cc), respectively. Two of the full-shift personal samples were reported to have been collected for 1470 and 1475 min. It is unclear if these samples indeed represent roofing work performed by an individual for greater than 24 hr. Additionally, three samples were collected during replacement of BUR; these task-based (50–210 min) samples ranged in concentration from 0.03–0.05 f/cc, with a mean concentration of 0.04 f/cc. The authors noted that there was no "solid evidence" that exposures resultant from removal of asbestos-containing roofing materials were at or above the OSHA regulatory limits.[21]

### *National Roofing Contractors Association (NRCA) (1994)*

The NRCA submitted a report to OSHA in 1994 in an attempt to provide the agency with "objective data" demonstrating that roofing products under normal foreseeable use would not produce asbestos fiber concentrations in excess of the new OSHA Permissible Exposure Limit (PEL) and excursion limit.[4] The report contained several hundred personal air measurements collected at various jobsites between 1986 and 1991 during the removal and replacement of BUR, felts, flashings, and shingles. Several of these data points were previously reported in the SRI International report, and thus are excluded from the analysis of the NRCA report.[21] The mean concentrations associated with task-based (NR durations; n = 32) and full-shift (480 min; n = 7) samples during removal of shingles and A/C roofing were 0.02 f/cc (range: 0.002–0.07 f/cc) and 0.013 f/cc (range: 0.0013–0.030 f/cc), respectively. Full-shift (360 min; n = 3) samples during replacement of such roofing products resulted in a mean concentration of 0.02 f/cc (range: <0.01–0.03 f/cc). During removal of BUR, felts, and flashings, short-term (17–30 min; n = 17), task-based (31–135 and NR durations; n = 104) and full-shift (480 min; n = 46) samples were represented by mean concentrations of 0.06 f/cc (range: 0.06–0.115 f/cc), 0.01 f/cc (range: 0.000–0.063 f/cc), and 0.007 f/cc (range: 0.0007–0.027 f/cc), respectively. Although sample durations were not reported for all task-based measurements, it was noted that sample durations were typically less than 180 min.

### *Recon Environmental Corp. (1995)*

In 1995, Recon Environmental Corp. collected airborne fiber samples from personnel involved in the removal and replacement of approximately 734 $m^2$ of roofing material from a gymnasium in East Orange, NJ.[30] Bulk analysis by PLM and TEM showed that the felt and tar paper contained 3–22% chrysotile and the flashing contained 14–17% chrysotile. It was noted that during removal of the roofing material, the first 518 $m^2$ of roofing material was removed using a "rotating blade cutter" and the remaining 216 $m^2$ was removed using a manual "slicing/chopping" technique.[30] Short-term samples (28–35 min; n = 28) during removal were noted to be representative of 30-min short-term exposures and resulted in a mean concentration of 0.065 f/cc (range: 0.031–0.397 f/cc). The authors noted that these short-term samples were associated with operations which were "most likely to produce exposures above the excursion limit."[30] Samples that the original authors deemed representative of full-shift exposures (70–480 min; n = 37) were reported to have a mean concentration of 0.017 f/cc (range: <0.002–0.056 f/cc). Additionally, asbestos structures were identified in only 4 of the 65 personal samples analyzed by TEM. Although bulk analysis of the roofing material identified the presence of only chrysotile fibers, TEM analysis of one of the personal samples identified the presence of a single actinolite fiber and a single tremolite fiber; in the other three

samples only chrysotile fibers were identified. Thus, is it unclear whether the source of the amphibole fibers was indeed from the roofing material.

### *Peterson and Franke (1997)*

Peterson and Franke[26] designed an experiment to determine fiber release associated with the application and removal of asbestos-containing asphalt roofing cement. In a glove box the authors simulated the application and removal of two roofing cements containing 8–12% and 8–15% chrysotile, respectively. Four simulations, two for each of the two products, were undertaken. The roofing cements were first applied to sheet metal within the glove box. All four of the task-based (45 min) samples collected during the application of the cements were below the LOD (<0.004 f/cc). The treated sheet metal was then removed from the glove box and allowed to remain in the sun for 56 and 68 days. The treated sheet metal was then placed back in the glove box and the cements were removed by hand scraping. Of the two short-term (15 min) samples collected during removal, one indicated a concentration below the LOD (<0.013 f/cc), while the other a concentration of 0.019 f/cc. Two task-based (45 min) samples were also collected during hand scraping; one sample was below the LOD (<0.004 f/cc) and the other sample had a concentration of 0.006 f/cc. The authors noted that differences in cement consistency may account for differences in fiber release, with heavier and stickier cements releasing fewer fibers.

### *Lange and Thomulka (2000)*

Lange and Thomulka[27] measured airborne asbestos concentrations during the abatement of 4645 $m^2$ of roofing materials from a building in Pennsylvania. The roof reportedly was composed of asbestos-containing felt, tar, and flashing which were 20–30 years old; based on PLM analysis, the asbestos content of these materials was 5–10% chrysotile. Workers used wet removal methods and a power saw to cut out sections of the roofing and then placed the sections into a chute connected to a dumpster. The authors reported that the arithmetic mean concentration, based on a total of 13 personal samples, was 0.020 f/cc (range: 0.0047–0.0752 f/cc); sampling durations were not reported.

### *Mowat et al. (2007)*

In 2007, Mowat and colleagues published results of a study in which they simulated the application and removal of five asbestos-containing "fibered" roof coatings and plastic roof cements in a test chamber (3.7×4.9×2.4 m).[28] These products, which were no longer manufactured at the time of the study, were reformulated to the specifications of the historical products; the asbestos content of the reformulated and historical products was between 3.04 and 15.5% chrysotile. Personal samples were collected during the application of these products to roof substrates, removal by hand sanding and hand scraping from the roof substrates, and removal from clothing and tools. The roof substrates measured 1.2 m × 2.4 m and consisted of either non-asbestos asphalt shingles, BUR, or modified bitumen roofing. All samples were analyzed using PCM and TEM, the results of which were used to derive phase contrast microscopy equivalent (PCM-E) 8-hr time-weighted average (TWA) values. Personal short-term (30 min) samples resulted in a mean concentration of 0.009 f/cc (range: <0.006–0.027 f/cc) during removal activities (n = 36) and 0.007 f/cc (range: <0.006–0.009 f/cc) during application (n = 12). For samples with detectable fiber concentrations based on PCM analysis, concentrations were converted to PCM-E concentrations based on the asbestos-to-total fiber ratio obtained by TEM and 8-hr TWA concentrations were calculated assuming 30 min of scraping or sanding and a background fiber concentration of 0.0002 f/cc for the remainder of the 8-hr work day; TWA concentrations ranged from 0.0003–0.002 f/cc.

### *Anttila et al. (2009)*

Anttila and colleagues[17] published in the peer reviewed literature the results of previously published data on exposure to carcinogenic agents in the bitumen waterproofing industry in Finland and Denmark. Included in the Anttila et al.[17] study were the results of a Finnish study by Riala et al.[31] in which samples were collected during the demolition of A/C roof slates on a detached house. Personal task-based (≤44 min; n = 2) samples during removal had a mean concentration of 0.09 f/cc (Piia Taxell, personal communication, May, 2014 and January, 2015).

### *Sheehan et al. (2010)*

In a follow-up to the Mowat et al.[28] study, Sheehan et al.[29] performed a simulation study to evaluate exposures associated with the removal of two asbestos-containing roofing products: an asphalt-based plastic roof cement and a fibered roof coating. These two products were reformulated to the same specifications as the historical roofing products and contained 15.5 and 3.04% chrysotile, respectively. The roof cement and fibered roof coating were applied to asphalt shingle and BUR roof substrates (1.2 m × 2.4 m) which were constructed in a similar manner to the 2007 study, except that the panels underwent artificially enhanced weathering prior to the

simulated exposure activities. Using a putty knife, hand scraping of the weathered substrates was performed continuously for 30 min inside a test chamber (3.7×4.9×2.4 m) to simulate the "worst case" exposure scenario; the authors noted that typically only a few minutes of scraping is required.[29] In addition, a hammer was reportedly used in some tests to "aggressively" remove material from the surfaces.[29] Personal short-term (30 min; n = 8) samples analyzed by PCM had a mean concentration of 0.10 f/cc (range: 0.06–0.17 f/cc). For short-term samples where fibers were detected using the PCM method, 8-hr TWA concentrations were calculated assuming 30 min of scraping and sanding and a background concentration of 0.0008 f/cc for the remainder of the 8-hr work day; 8-hr TWA concentrations were based on PCM-E concentrations calculated using the asbestos-to-total fiber ratio obtained by TEM, and ranged from 0.005–0.011 f/cc.

## Results by product type

A summary of the sampling data by product type, sampling duration, and work activity can be found in Table 2.

Four studies were identified which reported the results of samples (n = 140) collected during the removal, installation, or replacement of shingles or other asbestos cement (A/C) roofing materials.[4,15,17,24] For removal activities, task-based (≤44–360 min and NR durations; n = 125) concentrations ranged from <0.0021–1.1 f/cc, with a mean of 0.11 f/cc. For full-shift (480 min; n = 7) samples collected during removal of these products, the concentrations ranged from 0.0013–0.0298 f/cc, with a mean of 0.013 f/cc. Concentrations for samples collected during the installation of asbestos shingles (89–115 min; n = 5) ranged from 0.03–0.08 f/cc, with a mean of 0.05 f/cc. During replacement of A/C roofing materials, full-shift (360 min; n = 3) samples ranged from <0.01–0.03 f/cc, with a mean concentration of 0.02 f/cc.

Four studies were identified which provided exposure levels measured during the removal and installation of various roof cements and coatings (n > 64).[9,26,28,29] Short-term (15–30 min; n = 46) samples collected during removal tasks had a mean concentration of 0.04 f/cc (range: <0.006–0.17 f/cc). Concentrations for task-based (45 min and unreported durations; n = 2 and unreported sample numbers) samples collected during removal ranged from <0.004–0.4 f/cc with a mean of 0.1 f/cc. Short-term (14–30 min; n = 12 and unreported sample numbers) concentrations during application or installation of such products ranged as reported from 0.0–0.009 f/cc (mean: 0.004 f/cc). Results of task-based (45 min and NR durations; n > 4) and full-shift (342–432 min; n = NR) samples collected during application of roof coatings ranged from 0.0–0.6 f/cc and from 0.003–0.15 f/cc, respectively. During tear-off and replacement of spray-applied roof coatings, the asbestos concentrations based on task-based (NR durations; n = NR) samples ranged as reported from 0.0–0.3 f/cc.

Six studies were identified which reported the results of samples (n = 643) collected during the removal, installation, or replacement of BUR, flashings, and/or roofing felts.[4,9,21,25,27,30] Concentrations based on short-term (17–30 min; n = 33) samples collected during removal activities ranged from <0.010–0.248 f/cc, with a mean concentration of 0.06 f/cc. Reported task-based (31–357 min and unreported durations; n = 406) concentrations measured during removal activities ranged

**Table 2.** Airborne fiber concentrations measured during roofing work by product type, work practice, and sample duration.

| Product Type | Sample Duration (min) | Sample Type | Task Category | Number of Samples | Airborne Fiber Concentration (f/cc) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Min | Max | Arithmetic Mean |
| Shingles and A/C roofing | ≤44–360; NR | Task-based | Removal | 125 | <0.0021 | 1.1 | 0.11 |
| | 480 | Full-shift | Removal | 7 | 0.0013 | 0.0298 | 0.013 |
| | 89–115 | Task-based | Installation | 5 | 0.03 | 0.08 | 0.05 |
| | 360 | Full-shift | Replacement | 3 | <0.01 | 0.03 | 0.02 |
| Coatings, cements, and mastics | 15–30 | Short-term | Removal | 46 | <0.006 | 0.17 | 0.04 |
| | 45; NR | Task-based | Removal | > 2 | <0.004 | 0.4 | 0.1[a] |
| | 14 – 30 | Short-term | Installation | > 12 | 0.0 | 0.009 | 0.004 |
| | 45; NR | Task-based | Installation | > 4 | 0.0 | 0.6 | 0.2[a] |
| | 342 – 432 | Full-shift | Installation | NR | 0.003 | 0.15 | 0.07[a] |
| | NR | Task-based | Replacement | NR | 0.0 | 0.3 | 0.2[a] |
| Built-up roofing (BUR), felts, and flashings | 17–30 | Short-term | Removal | 33 | <0.010 | 0.248 | 0.06 |
| | 31–357; NR | Task-based | Removal | 406 | 0.000 | 0.3 | 0.03 |
| | 368–1475 | Full-shift | Removal | 115 | <0.0003 | 0.046 | 0.01 |
| | 60–180; NR | Task-based | Installation | 21 | 0.0 | 0.6 | 0.02 |
| | 28–35 | Short-term | Replacement | 28 | 0.031 | 0.397 | 0.065 |
| | 50–210 | Task-based | Replacement | 3 | 0.03 | 0.05 | 0.04 |
| | 70–480 | Full-shift | Replacement | 37 | <0.002 | 0.056 | 0.017 |

[a]In instances where the mean concentration was not provided for a study, the median of the range was used for calculation

from 0.000–0.3 f/cc, with a mean concentration of 0.03 f/cc. Fiber concentrations based on full-shift samples (368–1475 min; n = 115) during BUR removal were found to range from <0.0003–0.046 f/cc, with a mean concentration of 0.01 f/cc. No short-term or full-shift samples collected during installation were identified; however, task-based samples during installation (60–180 min and durations not reported; n = 21) resulted in a mean concentration of 0.02 f/cc (range: 0.0–0.6 f/cc). During replacement activities, short-term (28–35 min; n = 28), task-based (50–210 min; n = 3), and full-shift (70–480 min; n = 37) samples were found to have mean concentrations of 0.065 f/cc (range: 0.031–0.297 f/cc), 0.04 f/cc (range: 0.03–0.05 f/cc), and 0.017 f/cc (range: <0.002–0.056 f/cc), respectively.

## Discussion

### *Comparison to regulatory standards*

In this analysis, the mean concentrations for short-term samples were all well below the current OSHA excursion limit of 1.0 f/cc for all product categories. In fact, the maximum concentration reported for all short-term samples was 0.248 f/cc, more than three-fold below the excursion limit. Similarly, the mean fiber concentrations based on full-shift samples (≥360 min) were all below the current OSHA 8-hr TWA PEL of 0.1 f/cc. The mean concentrations for certain task-based samples, collected during specific activities, such as removal of asbestos-containing shingles and A/C roofing, and removal, installation, and replacement of coatings, cements, and mastics, were at or slightly above the current OSHA 8-hr TWA PEL. However, the sampling durations for many of these samples were not reported, and while these were task-based, it is unclear how long some of these tasks were performed in a given day.

Specifically, in regards to the elevated concentrations measured during the removal of shingles and A/C roofing, these data were largely influenced by data collected by Brown,[15] an Australian study which reported concentrations during both standard roof removal and demolition of buildings with asbestos cement claddings. It is unclear whether the fibers measured in this study were from asbestos roofing products or other fiber sources created during building demolition. In addition, according to the NRCA,[4] the dropping and stacking methods used in the Brown[15] study were inconsistent with typical work practices conducted in the U.S. Similarly, task-based results collected during removal, installation, and replacement of coatings, cements, and mastics were largely influenced by Anderson et al.[9] However, SRI International[21] noted that Johns-Manville personnel reported that the maximum concentration of these samples calculated as an 8-hr TWA and assuming no exposure during the unsampled period was 0.06 f/cc.

Additionally, OSHA has noted that, in "usual circumstances" when roof cements, coatings, mastics, and flashings are removed intact, "the potential for exposure is low."[5] According to OSHA, the asbestos fibers in such products are "tightly encapsulated" by adhesive bituminous and resinous compounds which "effectively prevent the fibers from being released."[5] The lack of exposure potential from work with such products is further evidenced by the fact that OSHA has exempted asbestos-containing roof coatings, cements, and mastics from the various work practice controls designed to reduce worker exposure, set forth in the agency's asbestos standard for the shipyard and construction industries.[32]

### *Comparison to NOAEL estimates for asbestos-related diseases*

Mean full-shift fiber concentrations obtained during removal, installation, and replacement activities for all products ranged from 0.01–0.07 f/cc. This corresponds to calculated working lifetime cumulative exposures for roofing workers ranging from 0.45–3.15 f/cc-years, assuming a 45-year work history. The estimated cumulative exposure for roofing workers is well below the no-observed-adverse-effect-levels (NOAELs) for lung cancer and mesothelioma as reported by Pierce and colleagues for predominately chrysotile exposed cohorts.[33] As noted above, the asbestos content of roofing products was greater than 99% chrysotile. Thus, roofers' exposures are consistent with the cohorts from which the NOAELs were derived, which were defined as having less than 10% of their potential asbestos exposure to amphiboles.

### *Epidemiological data*

The extent of epidemiologic literature which has evaluated asbestos-related diseases among roofing workers is limited; no cohort or case-control studies were identified which specifically examined mesothelioma rates in roofers, and evaluations of lung cancer risk are confounded by co-exposures to other chemicals and cigarette smoking. However, Christiani and Greene[34] performed a study in which chest X-rays were obtained from 69 union roofers. Forty-six participants, or 67% of the study population, were found to have radiologic evidence of pleural disease, and a significant relationship was found between years as a roofer and prevalence of pleural disease. The authors concluded that roofers were at an increased risk of developing asbestos-related disease. However, this study was limited by the relatively small

number of voluntary participants. Further, only 3% of the study population was found to have radiologic evidence of fibrosis.[34] Stocks and colleagues evaluated the incidence of work-related ill-health in roofers, as reported by clinical specialists and physicians in the UK between 2002 and 2008.[35] The authors reported a statistically significant increase in "long latency respiratory disease" among roofers, which was defined as pneumoconiosis, mesothelioma, lung cancer, or non-malignant pleural disease (combined); however, smoking was not controlled for, and it was unclear what proportion of the 33 estimated cases were asbestos-related diseases.[35]

NIOSH maintains the National Occupational Mortality Surveillance (NOMS) database, which reports cause-specific proportionate mortality ratios (PMRs) by occupation and/or industry for two time periods: 1985–1998 and 1999, 2003–2004, 2007. The PMRs for roofers due to malignant neoplasms of the peritoneum and pleura and malignant mesothelioma were not elevated in either time period.[36] Similarly, the majority of the studies which evaluated lung cancer rates found no statistically significant increased risk in roofers.[37–42] In the studies which reported a significant increase in lung cancer risk, other potentially confounding factors, such as smoking and exposure to coal tar pitch volatiles, were not controlled for.[43–45] Partanen and Boffetta[46] performed a meta-analysis of the epidemiological studies on cancer risk in roofers, and concluded that roofers are at a statistically significant increased risk of lung cancer. However, the authors concluded that the major contributor of the excess lung cancers was likely polycyclic aromatic hydrocarbon (PAH) exposure from coal-tar products, rather than asbestos exposure.[46]

### *TEM exposure data*

In order to permit comparisons of the asbestos concentrations associated with work with roofing materials to the OSHA standards, only measurements reported or assumed to be analyzed by PCM were included in this exposure analysis. The PCM method assesses total airborne fiber concentrations and is not specific to asbestos fibers. TEM, on the other hand, is capable of differentiating between asbestos fibers and non-asbestos fibers, and therefore may result in lower fiber counts if non-asbestos fibers are present in a sample. According to the NRCA, many of the fibers counted using PCM are actually non-asbestos from thermal insulation under roof systems.[4] Christiani and Greene[34] noted that during removal of roofing materials, roofers may cut into insulation layers. It should be noted that fiber counts using TEM may also yield higher results as it is approximately 100 times more sensitive than PCM analysis in identifying smaller fibers outside the PCM fiber range. A limited number of studies analyzed samples by both PCM and TEM; these studies support that PCM fiber concentrations exceed TEM asbestos concentrations for fibers > 5 μm in length.

### *Limitations*

The main limitation to this study was the inconsistency and incompleteness of the available exposure data and limited reporting regarding sampling and analytical conditions. As such, broadly defined search criteria were used to ensure the capture of all relevant data. Many studies included limited details on the data reported, and lacked even basic information (e.g., sample duration), making it difficult to compare to regulatory standards and standard industrial hygiene exposure parameters. As noted above, multiple studies have been previously found to contain major shortcomings which may account for the variability and inconsistencies in concentrations found in these studies. This is particularly problematic when such studies provide the only data for a task category, as these cannot be compared to other data collected in a more appropriate manner.

## Conclusions

To the best of our knowledge, this study represents the most comprehensive review of airborne asbestos exposures associated with the removal and installation of asbestos-containing roofing products conducted to date. The findings indicate that exposures from the installation, removal, and replacement of various asbestos-containing roofing products are typically well below applicable occupational exposure limits, and also well below published NOAELs for asbestos-related diseases.

## Disclaimer

All the authors are employed by Cardno ChemRisk, a consulting firm that provides scientific advice to the government, corporations, law firms, and various scientific/professional organizations. The authors designed and executed the study and have sole responsibility for the writing and content of the article. Cardno ChemRisk has been engaged by users, and manufacturers and suppliers of asbestos-containing products in litigation matters. It is likely that this work will be relied upon in scientific and medical research and litigation. Two of the authors (JSP and JLH) have served as expert witnesses in asbestos litigation, and they, along with others, may be called upon in the future to serve as expert witnesses in asbestos litigation.

## Funding

Funding for this study and manuscript preparation was provided by Cardno ChemRisk.

## References

[1] **Selikoff, I.J., and D.H.K. Lee:** *Asbestos and Disease.* New York: Academic Press, 1978.

[2] **Maines, R.:** *Asbestos and Fire: Technological Tradeoffs and the Body at Risk.* New Brunswick, NJ: Rutgers University Press, 2005.

[3] **Virta, R.L.:** Mineral commodity profiles: Asbestos. USGS Circular 1255-KK. US Geological Survey (USGS), 2005.

[4] **NRCA:** "Objective data" demonstration for certain roofing materials and operations under OSHA's 1994 asbestos standard. Rosemont, IL: National Roofing Contractors Association. 1994.

[5] "Occupational Exposure to Asbestos; Corrections to Final Rule," *Federal Register 60*(125):33973–34002 (29 June 1995).

[6] **U.S. EPA:** *Standards Support Document: Promulgated Amendments to the National Emission Standard for Asbestos,* D.R. Goodwin (Report #EPA-450/2-77-030). U.S. Environmental Protection Agency, Office of Air and Waste Management, Office of Air Quality Planning and Standards, June 1978.

[7] **Dotson, K.B.:** "Encapsulated Asbestos - Human Exposure, if any, from 'Locked-in' Fiber." *Asbestos medicine seminar;* Chicago, IL: dri; 2012. 477–498.

[8] **Spurny, K.R.:** On the release of asbestos fibers from weathered and corroded asbestos cement products. *Environ. Res. 48*:100–116 (1989).

[9] **Anderson, P.H., M.A. Grant, R.G. McInnes, and W.J. Farino:** *Analysis of Fiber Relase from Certain Asbestos Products.* Prepared for the U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances: Chemical Control Division, GCA Corporation, 1982.

[10] **Krusell, N., and D. Cogley:** *Asbestos Substitute Performance Analysis.* Prepared for the U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, GCA Corporation 1982.

[11] **Rice, C., and E.F. Heineman:** An asbestos job exposure matrix to characterize fiber type, length, and relative exposure intensity. *Appl. Occup. Environ. Hyg. 18*(7):506–512 (2003).

[12] **Townsend, T., J.J. Powell, and C. Xu:** *Environmental Issues Associated with Asphalt Shingle Recycling.* Prepared for Construction Materials Recycling Association, Asphalt Shingle Recycling Project. U.S. EPA Innovations Workgroup, Innovative Waste Consulting Services, LLC, 2007.

[13] **U.S. Bureau of Mines:** *Minerals Yearbook.* Washington, DC: United States Department of the Interior, Bureau of Mines, 1974–1991.

[14] **Campopiano, A., S. Casciardi, F. Fioravanti, and D. Ramires:** Airborne asbestos levels in school buildings in Italy. *J. Occup. Environ. Hyg. 1*(4):256–261 (2004).

[15] **Brown, S.K.:** Asbestos exposure during renovation and demolition of asbestos-cement clad buildings. *Am. Ind. Hyg. Assoc. J. 48*(5):478–486 (1987).

[16] **MRC Institute for Environment and Health:** *Asbestos and Man-Made Mineral Fibres in Buildings: Practical Guidance.* Leicester: MRC Institute for Environment and Health, 1999.

[17] **Anttila, P., P. Heikkila, M. Makela, V. Schlunssen, and E. Priha:** Retrospective exposure assessment for carcinogenic agents in bitumen waterproofing industry in Finland and Denmark. *Ann. Occup. Hyg. 53*(2):139–151 (2009).

[18] **Virta, R.L.:** Talc and pyrophyllite. *US Geological Survey Minerals Yearbook 2000.* Reston, VA: U.S. Geological Survey for Mineral Industry Surveys Annual Reviews. U.S. Government Printing Office for Minerals Yearbook.

[19] "Asbestos; Publication of Identifying Information," *Federal Register 55*(30):5144–5162 (13 February 1990).

[20] **ICF Incorporated:** *Asbestos Exposure Assessment.* Prepared for Dr. Kim Wong, Chemical Engineering Branch, Office of Pesticides and Toxic Substances, Washington, D.C.: U.S. Environmental Protection Agency, 1988.

[21] **SRI International:** *Exposure to Asbestos during Roofing Removal.* Prepared for National Roofing Contractors Association. SRI Project PYC-8654, 1990.

[22] "Occupational Exposure to Asbestos, Tremolite, Anthophyllite, and Actinolite; Final Rules," *Federal Register 51*(119):22612–22790 (20 June 1986).

[23] **NYSDOT:** Asbestos Management. In *NYSDOT Environmental Procedures Manual,* Ch. 4.4.19, New York State Department of Transportation, 2013.

[24] **NIOSH:** *Health Hazard Evaluation Report HETA 84-321-1590 Asbestos Shingle Tear-Off,* Rockford, Illinois. May 1985. Cincinatti, OH: Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 1985.

[25] **Hays, S. M., and V.H. Ainslie:** *Airborne Levels during Non-friable Asbestos-containing Material (ACM) Removal.* Phoenix, AZ: National Asbestos Council, Inc. Fall Technical Conference and Exposition, 1990.

[26] **Peterson, J.E., and J.E. Franke:** *Experiment to Determine Release of Asbestos Fibers from Asphalt Roofing Cement.* Prepared for Baltimore-Ennis Land Company, Inc., 1997.

[27] **Lange, J.H., and K.W. Thomulka:** Area and personal airborne exposure during abatement of asbestos-containing roofing material. *Bull. Environ. Contam. Toxicol. 64*(5):673–678 (2000).

[28] **Mowat, F., R. Weidling, and P. Sheehan:** Simulation tests to assess occupational exposure to airborne asbestos from asphalt-based roofing products. *Ann. Occup. Hyg. 51*(5):451–462 (2007).

[29] **Sheehan, P., F. Mowat, R. Weidling, and M. Floyd:** Simulation tests to assess occupational exposure to airborne asbestos from artificially weathered asphalt-based roofing products. *Ann. Occup. Hyg. 54*(8):880–892 (2010).

[30] **Recon Environmental Corp:** *Air Sampling Summary Report for the YMCA, Turrell Gymnasium Roof Replacement,* East Orange, NJ: Recon Project #W50276, 1995.

[31] **Riala, R., P. Pirhonen, and P. Heikkila:** *Asbesti purkuja huol- totoissa* [Asbestos in maintenance and demolition work]. Helsinki, Finland: Finnish Institute of Occupational Health, 1993 (in Finnish).

[32] "Occupational Exposure to Asbestos; Final Rule," *Federal Register 63*(124):35137–35138 (29 June 1998).

[33] **Pierce, J.S., M.A. McKinley, D.J. Paustenbach, and B.L. Finley:** An evaluation of reported no-effect chrysotile asbestos exposures for lung cancer and mesothelioma. *Crit. Rev. Tox. 38*(3):191–214 (2008).

[34] **Christiani, D.C., and R. Greene:** Asbestos disease in commercial roofers: radiologic signs. In *Proceedings of the VIIth International Pneumoconioses Conference*, Pittsburgh, Pennsylvania, August 23–26, 1988 Part II DHHS (NIOSH) Publication No 90-108 Part II November 1990. Washington, D.C.: U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, 1990. 1414–1317.

[35] **Stocks, S.J., S. Turner, R. McNamee, M. Cardner, L. Hussey, and R.M. Agius:** Occupation and work-related ill-health in UK construction workers. *Occ. Med. 61*(6):407–415 (2011).

[36] **NIOSH:** *NIOSH Safety and Health Topic: National Occupational Mortality Surveillance (NOMS).* Cincinnati, OH: National Institute for Occupational Safety and Health (NIOSH). Updated July 9, 2014; Available at http://www.cdc.gov/niosh/topics/noms/.

[37] **Schoenberg, J.B., A. Stemhagen, T.J. Mason, J. Patterson, J. Bill, and R. Altman:** Occupation and lung cancer risk among New Jersey white males. *J. Natl. Cancer Inst. 79*(1):13–21 (1987).

[38] **Lerchen, M.L., C.L. Wiggins, and J.M. Samet:** Lung cancer and occupation in New Mexico. *J. Natl. Cancer Inst. 79*(4):639–645 (1987).

[39] **Vineis, P., T. Thomas, R.B. Hayes, et al.:** Proportion of lung cancers in males, due to occupation, in different areas of the USA. *Int. J. Cancer. 42*(6):851–856 (1988).

[40] **Zahm, S.H., R.C. Brownson, J.C. Chang, and J.R. Davis:** Study of lung cancer histologic types, occupation, and smoking in Missouri. *Am. J. Ind. Med. 15*(5):565–578 (1989).

[41] **Morabia, A., S. Markowitz, K. Garibaldi, and E.L. Wynder:** Lung cancer and occupation: Results of a multicentre case-control study. *Br. J. Ind. Med. 49*(10):721–727 (1992).

[42] **De Stefani, E., M. Kogevinas, P. Boffetta, A. Ronco, and M. Mendilaharsu:** Occupation and the risk of lung cancer in Uruguay. *Scand. J. Work. Environ. Health 22*(5):346–352 (1996).

[43] **Hammond, E.C., I.J. Selikoff, P.L. Lawther, and H. Seidman:** Inhalation of benzpyrene and cancer in man. *Ann. NY Acad. Sci. 271*:116–124 (1976).

[44] **Menck, H.R., and B.E. Henderson:** Occupational differences in rates of lung cancer. *J. Occup. Med. 18*(12):797–801 (1976).

[45] **Stern, F.B., A.M. Ruder, and G. Chen:** Proportionate mortality among unionized roofers and waterproofers. *Am. J. Ind. Med. 37*(5):478–492 (2000).

[46] **Partanen, T., and P. Boffetta:** Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. *Am. J. Ind. Med. 26*(6):721–740 (1994).

STUDY No. 4

# PLEURAL MESOTHELIOMA AND LUNG CANCER RISKS IN RELATION TO OCCUPATIONAL HISTORY AND ASBESTOS LUNG BURDEN

**STUDY No. 4**

# PLEURAL MESOTHELIOMA AND LUNG CANCER RISKS IN RELATION TO OCCUPATIONAL HISTORY AND ASBESTOS LUNG BURDEN

---

**Occup Environ Med 2015, 0: 1-10**

**Clare Gilham, Christine Rake, Garry Burdett, Andrew G. Nicholson, Leslie Davison, Angelo Franchini, James Carpenter, John Hodgson, Andrew Darnton, Julian Peto**

---

Abstract:

This is a population-based study of pleural mesothelioma patients with occupational histories and measured asbestos lung burdens in occupationally exposed workers and in the general population.

The authors indicate that the relationship between lung burden and risk, particularly at environmental exposure levels, will enable future mesothelioma rates in people born after 1965 who never installed asbestos to be predicted from their asbestos lung burdens.

They indicate that the lifetime mesothelioma risk is approximately 0.02% per 1000 amphibole fibers per gram of dry lung tissue over a more than 100-fold range, from 1 to 4 in the most heavily exposed building workers to less than 1 in 500 in most of the population.

The measured lung burdens indicated that the asbestos fibers counted were amosite (75%), crocidolite (18%), other amphiboles (5%) and chrysotile (2%). It confirms the major contribution of amosite to UK mesothelioma incidence.

The authors conclude that the approximate linearity of the dose-response together with lung burden measurements in younger people will provide reasonably reliable predictions of future mesothelioma rates in those born since 1965.

OEM Online First, published on December 29, 2015 as 10.1136/oemed-2015-103074



OPEN ACCESS

ORIGINAL ARTICLE

# Pleural mesothelioma and lung cancer risks in relation to occupational history and asbestos lung burden

Clare Gilham,[1] Christine Rake,[1] Garry Burdett,[2] Andrew G Nicholson,[3] Leslie Davison,[4] Angelo Franchini,[1] James Carpenter,[1,5] John Hodgson,[6] Andrew Darnton,[6] Julian Peto[1]

▸ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/oemed-2015-103074)

[1]London School of Hygiene and Tropical Medicine, London, UK
[2]Health and Safety Laboratory, Buxton, UK
[3]Department of Histopathology, Royal Brompton and Harefield Hospitals NHS Foundation Trust, and National Heart and Lung Institute, Imperial College, London, UK
[4]Department of Cellular Pathology, Leeds Teaching Hospitals NHS Trust, Leeds, UK
[5]Medical Research Council Clinical Trials Unit, Kingsway, London, UK
[6]Health and Safety Executive, Bootle, UK

**Correspondence to**
Clare Gilham, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK; clare.gilham@lshtm.ac.uk

Received 16 May 2015
Revised 20 October 2015
Accepted 11 November 2015

**To cite:** Gilham C, Rake C, Burdett G, *et al*. *Occup Environ Med* Published Online First: [*please include* Day Month Year] doi:10.1136/oemed-2015-103074

## ABSTRACT

**Background** We have conducted a population-based study of pleural mesothelioma patients with occupational histories and measured asbestos lung burdens in occupationally exposed workers and in the general population. The relationship between lung burden and risk, particularly at environmental exposure levels, will enable future mesothelioma rates in people born after 1965 who never installed asbestos to be predicted from their asbestos lung burdens.

**Methods** Following personal interview asbestos fibres longer than 5 μm were counted by transmission electron microscopy in lung samples obtained from 133 patients with mesothelioma and 262 patients with lung cancer. ORs for mesothelioma were converted to lifetime risks.

**Results** Lifetime mesothelioma risk is approximately 0.02% per 1000 amphibole fibres per gram of dry lung tissue over a more than 100-fold range, from 1 to 4 in the most heavily exposed building workers to less than 1 in 500 in most of the population. The asbestos fibres counted were amosite (75%), crocidolite (18%), other amphiboles (5%) and chrysotile (2%).

**Conclusions** The approximate linearity of the dose–response together with lung burden measurements in younger people will provide reasonably reliable predictions of future mesothelioma rates in those born since 1965 whose risks cannot yet be seen in national rates. Burdens in those born more recently will indicate the continuing occupational and environmental hazards under current asbestos control regulations. Our results confirm the major contribution of amosite to UK mesothelioma incidence and the substantial contribution of non-occupational exposure, particularly in women.

## What this paper adds

- Britons born before the 1960s have the highest mesothelioma death-rate worldwide, reflecting high occupational asbestos exposure in men and widespread environmental exposure in both sexes before 1980, when asbestos use virtually ceased in Britain.
- The risk to younger people from asbestos still present in many buildings is not known but could be substantial.
- We have shown that lifetime mesothelioma risk is approximately 0.020% per 1000 asbestos fibres per gram of dry lung tissue over a more than 100-fold range, from 1 to 4 in the most heavily exposed building workers to less than 1 in 500 in most of the population.
- This will enable the risk from current asbestos exposure to be estimated in people born since the 1970s for whom lung samples are available (eg, resected lung cancer or pneumothorax patients), both in occupations at potential risk such as builders and teachers and in the general population.
- Such data will provide a rational basis for regulations on worker protection and asbestos monitoring and abatement, and for predicting UK mesothelioma rates over the next 50 years.

## BACKGROUND AND AIMS

A large amount of asbestos remains in many older buildings and there is continuing concern about environmental exposure to occupants and occupational exposure during maintenance, renovation and demolition in homes, schools and workplaces. The resulting mesothelioma risks cannot be calculated by extrapolation from historical occupational cohort studies because lifetime average airborne exposure levels in the breathing zone cannot be estimated even approximately either for the general public or for plumbers, electricians and other building or demolition workers. Asbestos lung burden is the only indicator of cumulative lifetime exposure that can be measured reliably in a population-based study. We have therefore developed a dose–response model in a population-based series of mesothelioma and resected lung cancer patients with occupational histories obtained by personal interview and measured lung burdens. This will enable future mesothelioma rates to be predicted from lung burdens in occupational groups and in the general population for people born after 1965 who began work after 1980 when asbestos use had virtually ceased in Britain (figure 1).

## METHODS

### Source of samples

The methods and results of the MALCS case–control study have been described elsewhere.[1] Telephone interviews on lifetime occupational[2]

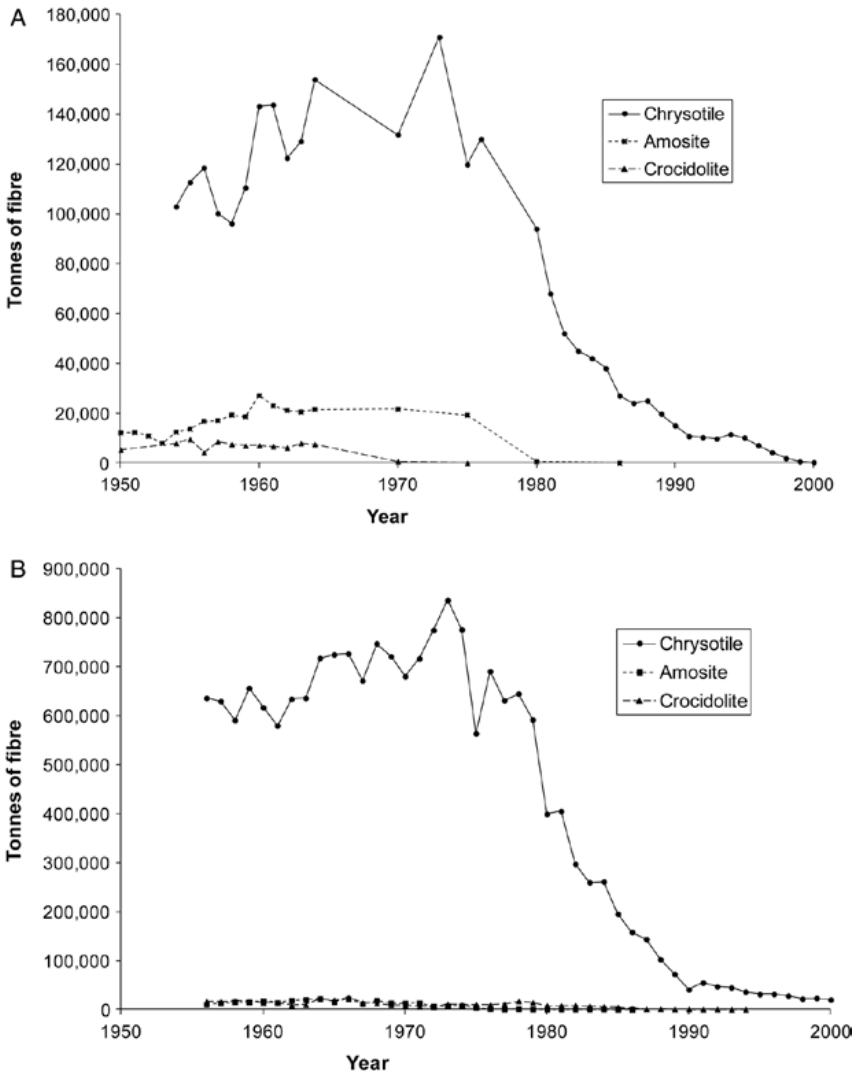



**Figure 1** (A) UK Asbestos imports from 1950 to 2000.[27–30] (B) US Asbestos imports from 1956 to 2000.[30]

history were conducted between 2001 and 2006 on 622 patients with mesothelioma and 1420 population controls. We also interviewed 420 patients with resected lung cancer born since 1940 for whom lung samples could be obtained as a control group for lung burden analyses. Patients with lung cancer and mesothelioma identified through chest physicians, lung cancer nurse specialists and Hospital Episode Statistics (HES) notifications were recruited from 170 hospitals throughout Britain.[1 2] Resected lung cancers provide the only adequate national source of lung samples in people who can be identified systematically, are available for interview and have an age distribution similar to mesothelioma. Only a small proportion of all lung cancers are caused by asbestos, so the asbestos lung burdens of this national sample are reasonably representative of the general population except for a few per cent with very high burdens. Written informed consent was obtained from 346 (77%) patients with mesothelioma and their next of kin for postmortem samples to be analysed and from 406 (96%) patients with lung cancer for analysis of resected tissue. Transmission electron microscopy (TEM) analysis was performed on samples as they became available, and 133 mesothelioma samples and 262 lung cancer samples were analysed. All were born since 1940 except 11 female mesotheliomas born 1925–1939. The study was approved by South Thames Multicentre Research Ethics Committee.

### Occupational classification

Job titles were assigned to Standard Occupational Classification 1990 (SOC 90) and Standard Industrial Classification 1992 (SIC 92) codes and grouped into main job categories. Proportional mortality ratios based on all mesothelioma deaths in Britain aged 16–74 years between 1991 and 2000[3] provided the basis for this categorisation.[1 2] Subjects were assigned to the highest-ranking occupation they had worked in irrespective of duration. Previously reported ORs for these categories[1] are shown in table 3.

### Lung sample preparation and TEM

Lung samples were anonymised and sent to the Health and Safety Laboratory (HSL) for TEM counting of asbestos fibres longer than 5 μm (appendix 2). The target analytical sensitivity, 0.01 mf/g (million fibres per dry gram), was achieved in all but 2.8% of the samples (2/133 mesotheliomas, 9/262 lung cancers). Sensitivity was increased to 0.003 mf/g for a subset of samples in which five or fewer asbestos fibres were originally counted.

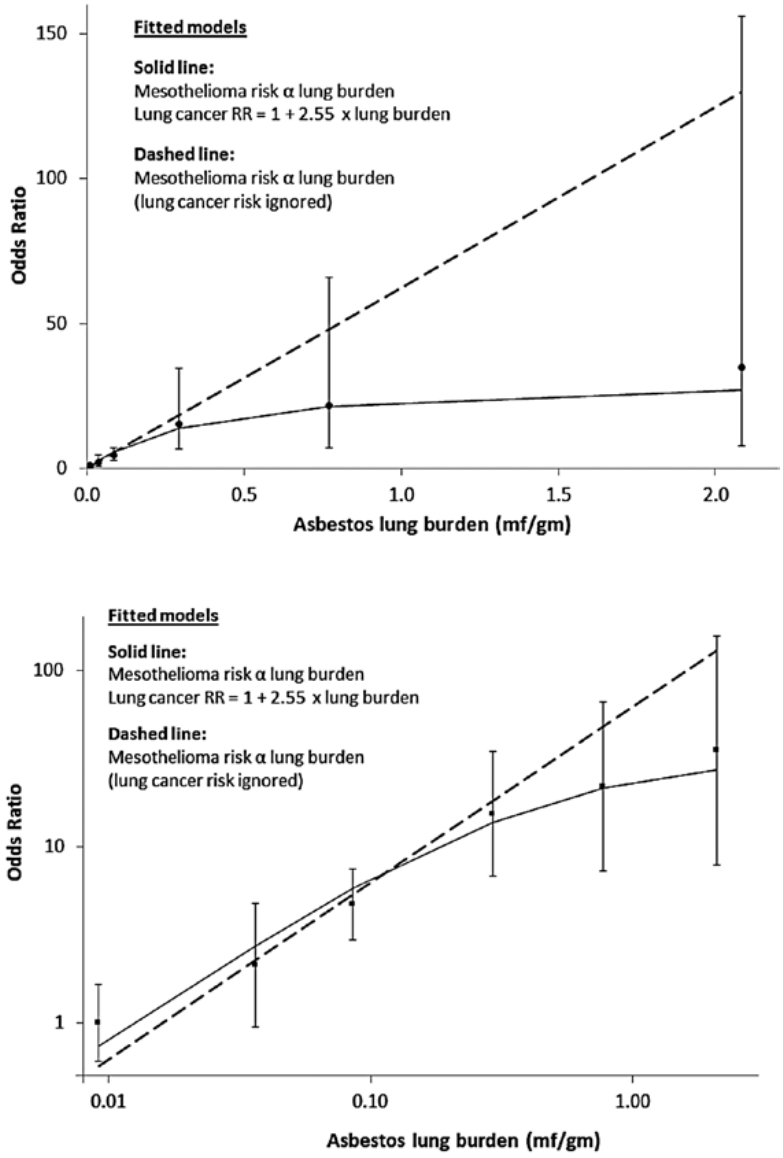



**Figure 2** Mesothelioma ORs (95% floating CIs) in men using resected lung cancers as controls, and asbestos lung burden: upper graph linear axes, lower graph logarithmic axes. When the lung cancer risk caused by asbestos is ignored the fit of the linear model is significantly worse (p=0.02; dashed line).

### Statistical methods

The analyses are described in appendix 1. The fitted model estimates and adjusts for the effect of using lung cancers as controls. At low doses the mesothelioma:lung cancer OR will reflect the true mesothelioma dose–response, but as lung burden increases there is increasing downward curvature (solid line in figure 2) due to the increasing proportion of lung cancers caused by asbestos. This model was used to estimate the distribution of lung burdens in British men born in 1945, and hence to calculate their lifetime risks for mesothelioma and lung cancer as a function of asbestos lung burden (see table 2 footnotes). Our mesothelioma cases are well represented by this birth cohort, as their median date of birth was September 1944. The 1945 birth cohort's future age-specific death-rates were estimated by unadjusted age and birth cohort analysis of British male mesothelioma and lung cancer death-rates in 5-year age-groups (35–39 to 85–89) and periods (1990–1994 to 2005–2009). Our dose–response model is linear, so predicted mesothelioma and excess lung cancer age-specific death rates are both proportional to mean lung burden in each lung burden category. The lifetime risk (probability of dying by age 90) was calculated actuarially in each lung burden category assuming current (2013) UK rates for all other causes of death. These lifetime risks were standardised to the projected probabilities of dying by age 90 for mesothelioma (0.86%) and lung cancer (4.67%) of all British men born in 1945.

The main results are based on total asbestos fibre burden irrespective of fibre type. The mesothelioma risk per fibre of crocidolite relative to amosite was estimated by logistic regression, fitting the weighted sum of the amosite and crocidolite lung burdens, ignoring other fibre types (which constituted only 7% of counted fibres) and adjusting the crocidolite:amosite weighting to give the best-fitting model.

## RESULTS

### Dose–response for mesothelioma and lung cancer

Table 1 shows the distribution of asbestos lung burdens in mesotheliomas and resected lung cancers. The estimated ORs

## Exposure assessment

**Table 1** Distribution of asbestos lung burdens (million fibres longer than 5 μm per dry gram) in men and women

| | TEM asbestos lung burden in million fibres per dry gram (Average lung burden for lung cancers in brackets)* | | | | | | |
|---|---|---|---|---|---|---|---|
| Source of sample | 0– (0.0092) | 0.025– (0.0364) | 0.05– (0.0854) | 0.2– (0.2930) | 0.5– (0.7690) | ≥1.0 (2.0829) | Total |
| Males | | | | | | | |
| Mesothelioma | 18 (16.8%) | 8 (7.5%) | 33 (30.8%) | 21 (19.6%) | 15 (14.0%) | 12 (11.2%) | 107 (100%) |
| Lung cancer | 105 (57.7%) | 22 (12.1%) | 41 (22.5%) | 8 (4.4%) | 4 (2.2%) | 2 (1.1%) | 182 (100%) |
| OR (95% CI) | 1.00 (ref) | 2.12 (0.82 to 5.49) | 4.70 (2.38 to 9.25) | 15.31 (5.89 to 39.8) | 21.88 (6.52 to 73.4) | 35.00 (7.22 to 169.6) | |
| Females | | | | | | | |
| Mesothelioma | 13 (50.0%) | 2 (7.7%) | 7 (26.9%) | 4 (15.4%) | | | 26 (100%) |
| Lung cancer | 62 (77.5%) | 11 (13.8%) | 6 (7.5%) | 1 (1.3%) | | | 80 (100%) |
| OR (95% CI) | 1.00 (ref) | 0.87 (0.17 to 4.39) | 6.36 (1.89 to 21.44) | 19.08 (1.97 to 184.91) | | | |
| Both sexes† | | | | | | | |
| Mesothelioma | 26 | 8 | 38 | 23 | 15 | 12 | 122 |
| Lung cancer | 167 | 33 | 47 | 9 | 4 | 2 | 262 |
| Adjusted OR (95% CI) | 1.00 (ref) | 1.51 (0.62 to 3.65) | 4.81 (2.61,8.85) | 13.91 (5.69 to 34.0) | 21.52 (6.45 to 71.7) | 30.70 (6.38 to 147.7) | |

†Data for both sexes combined exclude 11 female mesothelioma cases born 1925–1939: 5 (TEM <0.025), 2 (TEM 0.025-), 2 (TEM 0.05-), 2 (TEM 0.2-). ORs for both sexes combined are adjusted for sex and year of birth (1940–1944, 1945–1949, 1950–1954).
*Mean lung burden of lung cancer samples in each category except the highest (≥1 mf/g). One lung cancer with 22.0 mf/g was recoded as 2.08 mf/g, the mean for the other lung cancer and the 12 mesotheliomas ≥1 mf/g. The mean for samples ≥1 mf/g was also set as 2.08 mf/g. Retaining the original value has little effect on the fitted model but distorts the lung burdens shown in table 3.
TEM, transmission electron microscopy.

for males and females combined (last row) are adjusted for period of birth (1940–1944, 1945–1949, 1950–1954 and 1955 +) and sex, although neither was significant (p=0.6 for sex, $p_{trend}$=0.5 for period of birth). There were too few women for separate analysis, and further model-fitting was confined to men. The reference group for the mesothelioma ORs in figure 2 and table 2 is the lowest lung burden category (<0.025 mf/g, average 0.0092 mf/g).

In the fitted model risks for both mesothelioma and excess lung cancer are proportional to lung burden. The estimated coefficients from the fitted model (solid line in figure 2) are 82.2 (95% CI 54.3 to 124.5) per mf/g for the OR for mesothelioma and 2.55 (95% CI 0.62 to 10.37) per mf/g for the increase in the lung cancer RR. The corresponding projected lifetime risks and SMRs in each lung burden category are shown in table 2 for the cohort of British men whose central date of

**Table 2** Observed distribution of asbestos lung burdens in male mesotheliomas and lung cancers, estimated distribution in the UK male population (central cohort born 1945), and predicted lifetime risks for mesothelioma and lung cancer

| | TEM asbestos burden (million fibres ≥5 μm in length per dry gram) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0– | 0.025– | 0.05– | 0.2– | 0.5– | ≥1.0 | All men |
| Mean lung burden (mf/g)* | 0.00918 | 0.0364 | 0.0854 | 0.293 | 0.769 | 2.08 | |
| | *Distribution of lung burdens in mesotheliomas and lung cancers (from table 1) and fitted OR model (solid line in figure 2)* | | | | | | |
| Mesotheliomas (mean lung burden 0.430 mf/g) | 16.8% | 7.5% | 30.8% | 19.6% | 14.0% | 11.2% | 100% |
| Lung cancers (mean lung burden 0.082 mf/g) | 57.7% | 12.1% | 22.5% | 4.4% | 2.2% | 1.1% | 100% |
| Mesothelioma/lung cancer OR | | | | | | | |
| Observed | 1.0 (ref) | 2.12 | 4.70 | 15.31 | 21.88 | 35.00 | |
| Fitted† | 0.74 | 2.74 | 5.76 | 13.79 | 21.35 | 27.13 | |
| | *Estimated distribution of lung burdens and resulting mesothelioma and lung cancer risks due to asbestos in the UK male population born in 1945* | | | | | | |
| Lifetime mesothelioma risk‡ | 0.18% | 0.72% | 1.66% | 5.45% | 12.91% | 26.99% | 0.86% |
| Mesothelioma SMR§ | 21 | 83 | 193 | 633 | 1501 | 3137 | 100 |
| Lifetime lung cancer risk‡ | 4.55% | 4.83% | 5.34% | 7.41% | 11.67% | 20.64% | 4.67% |
| Lifetime excess lung cancer risk‡ | 0.10% | 0.38% | 0.89% | 2.97% | 7.22% | 16.20% | 0.47% |
| Lung cancer SMR¶ | 97 | 103 | 114 | 159 | 250 | 442 | 100 |
| UK population (estimated mean lung burden 0.047 mf/g)** | 63.08% | 12.38% | 20.70% | 2.82% | 0.83% | 0.19% | 100% |

*Mean lung burden of lung cancer samples in each category except the highest (≥1 mf/g). One lung cancer with 22.0 mf/g was recoded as 2.08 mf/g, the mean for the other lung cancer and the 12 mesotheliomas ≥1 mf/g. The mean for samples ≥1 mf/g was also set as 2.08 mf/g. Retaining the original value has little effect on the fitted model but distorts the lung burdens shown in table 3.
†Solid line in figure 2.
‡Actuarial calculation of probability of dying by age 90 from projected mesothelioma and lung cancer rates assuming national rates for other causes of death.
§Proportional to mean lung burden.
¶Proportional to 1+2.55× (mean lung burden).
**Proportional to number of lung cancers divided by lung cancer SMR.
TEM, transmission electron microscopy, SMR, Standardised Mortality Ratio.

**Table 3** TEM asbestos lung burdens (million asbestos fibres ≥5 μm in length per dry gram) by most hazardous occupation

**Panel A: Males born since 1940**

| Highest occupational exposure category | Asbestos lung burden (million fibres per dry gram) | | | | | | | Meso OR vs population controls* | Mean asbestos lung burden (million fibres per dry gram) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0– | 0.025– | 0.05– | 0.2– | 0.5– | ≥1.0 | Total | | Amosite | Crocidolite | Other amphiboles | Chrysotile | All asbestos |
| Non-construction high-risk occupations | | | | | | | | | | | | | |
| Mesothelioma | 5 | 3 | 11 | 7 | 6 | 4 | 36 | 17.5 | 0.375 | 0.094 | 0.014 | 0.005 | 0.487 |
| Lung cancer | 15 | 1 | 9 | 1 | 3 | 0 | 29 | | 0.100 | 0.013 | 0.003 | 0.005 | 0.121 |
| Carpenters | | | | | | | | | | | | | |
| Mesothelioma | 1 | 2 | 5 | 3 | 7 | 5 | 23 | 34.2 | 0.811 | 0.021 | 0.016 | 0.003 | 0.852 |
| Lung cancer | 3 | 1 | 3 | 2 | 0 | 0 | 9 | | 0.088 | 0.002 | 0.005 | 0.000 | 0.095 |
| Plumbers, electricians and painter/decorators | | | | | | | | | | | | | |
| Mesothelioma | 5 | 3 | 10 | 8 | 2 | 2 | 30 | 15.9 | 0.148 | 0.074 | 0.004 | 0.002 | 0.228 |
| Lung cancer | 12 | 4 | 5 | 3 | 1 | 1 | 26 | | 0.095 | 0.040 | 0.006 | 0.001 | 0.143 |
| Other construction or other reported exposure | | | | | | | | | | | | | |
| Mesothelioma | 3 | 0 | 2 | 0 | 0 | 1 | 6 | 5.1 | 0.056 | 0.192 | 0.000 | 0.000 | 0.248 |
| Lung cancer | 28 | 3 | 13 | 1 | | | 45 | | 0.027 | 0.004 | 0.002 | 0.002 | 0.036 |
| Medium risk industrial | | | | | | | | | | | | | |
| Mesothelioma | 3 | 0 | 2 | 3 | 0 | | 8 | 4.1 | 0.069 | 0.057 | 0.010 | 0.001 | 0.137 |
| Lung cancer | 22 | 5 | 7 | 1 | 0 | 1† | 36 | | 0.078† | 0.015† | 0.005 | 0.001 | 0.098† |
| Domestic exposure | | | | | | | | | | | | | |
| Mesothelioma | 0 | 0 | 2 | | | | 2 | 2.1 | 0.035 | 0.060 | 0.000 | 0.000 | 0.094 |
| Lung cancer | 4 | 4 | 0 | | | | 8 | | 0.009 | 0.004 | 0.006 | 0.001 | 0.020 |
| Low-risk occupations | | | | | | | | | | | | | |
| Mesothelioma | 1 | 0 | 1 | | | | 2 | 1.0 (ref) | 0.015 | 0.018 | 0.005 | 0.000 | 0.038 |
| Lung cancer | 21 | 4 | 4 | | | | 29 | | 0.010 | 0.003 | 0.007 | 0.002 | 0.021 |
| Total | | | | | | | | | | | | | |
| Mesothelioma | 18 | 8 | 33 | 21 | 15 | 12 | 107 | | 0.351 | 0.073 | 0.010 | 0.003 | 0.438 |
| Lung cancer | 105 | 22 | 41 | 8 | 4 | 2 | 182 | | 0.058† | 0.012† | 0.004 | 0.002 | 0.077† |

*Male mesothelioma ORs from the original case–control study.[1]
†One lung cancer with 22.0 mf/g was recoded as 2.08 mf/g (see table 2 footnote *).
Retaining the original value increases the mean fibre count in lung cancers to 0.555 for amosite, 0.091 for crocidolite and 0.651 for all asbestos in the medium risk group, and to 0.153 for amosite, 0.027 for crocidolite and 0.186 for all asbestos in all male lung cancers.
TEM, transmission electron microscopy.

birth is the beginning of 1945. (The median date of birth of our mesothelioma cases was September 1944.) The predicted lifetime excess risk for lung cancer due to asbestos (0.47%) is 55% of that for mesothelioma (0.86%). Mesothelioma and excess lung cancer risks in each category and overall are proportional to mean lung burden under this linear model, which implies a lifetime mesothelioma risk of 0.020% per 1000 asbestos fibres/g. The proportion of men with lung burdens exceeding 1 mf/g is 11.2% (12/107) in mesotheliomas, 1.1% (2/182) in lung cancers and is estimated as 0.19% in the UK male population. The estimated mean lung burden for the 1945 male birth cohort is 0.047 mf/g.

## Occupation and lung burden

Amosite and crocidolite lung burdens among male mesotheliomas are shown in figure 3 by occupational category as previously defined[1] (highest lifetime category irrespective of duration). Concentrations are generally higher for amosite than crocidolite. The highest amosite levels are predominantly in carpenters, while four of the five men with the highest crocidolite levels reported exposure to sprayed crocidolite. Table 3A, B show TEM results for males and females respectively by occupational category. Mesothelioma ORs (from Rake *et al*[1]) and mean lung burdens for each type of asbestos are also shown. Mean lung burdens are higher for mesothelioma than for lung cancer within each occupational category and increase with increasing occupational OR. Only six (3.3%) of 182 lung cancers in men and none of the mesotheliomas or lung cancers in women had lung burdens above 0.5 mf/g. In contrast, 27 (25.2%) of the male mesotheliomas were above 0.5 mf/g. All 27 had a high-risk occupational history and 16 had worked as a carpenter, plumber, electrician or decorator. Construction and medium risk industrial workers with lung cancer had much lower lung burdens, with 50 (61.7%) below 0.025 mf/g and only three (3.7%) above 0.2 mf/g. No asbestos fibres were detected in 4 of the 14 male mesotheliomas with lung burdens <0.025 mf/g who worked in high risk or construction jobs. These four men all reported short or occasional asbestos exposure in their work. Levels are much lower in women, with the highest concentrations in those who reported domestic exposure (table 3B).

## Effects of fibre type and size

Figure 1 shows UK asbestos imports since 1954. Of the five million tonnes imported over this period 89% was chrysotile, 9% amosite and 2% crocidolite. Crocidolite use had ended by 1970 and amosite by 1980. Chrysotile imports had fallen by 90% by 1990 and ended by 2000. Few of the asbestos fibres detected were chrysotile, which disappears from the lung with a half-life of a few months.[4] The majority of counted asbestos fibres were amosite (75%) or crocidolite (18%), with much lower numbers for chrysotile (1.9%), tremolite (1%), anthophyllite (2%), actinolite (0.6%) and uncharacterised amphiboles (1.7%). Burdens of chrysotile and these other amphiboles were correlated with the total fibre burden and were too low for their

**Table 3** Continued

**Panel B: Females: lung cancer cases born since 1940 and mesothelioma cases born since 1925. (The 11 mesotheliomas in women born 1925–1939 are included and also shown in brackets.)**

| Highest occupational exposure category | Asbestos lung burden (million fibres per dry gram) | | | | | | | Meso OR vs population controls* | Mean asbestos lung burden (million fibres per dry gram) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0– | 0.025– | 0.05– | 0.2– | 0.5– | ≥1.0 | Total | | Amosite | Crocidolite | Other amphiboles | Chrysotile | All asbestos |
| High–risk occupations | | | | | | | | | | | | | |
| Mesothelioma | 1 (1) | 1 (1) | 0 | 0 | | | 2 (2) | 4.8 | 0.025 | 0.000 | 0.000 | 0.000 | 0.025 |
| Lung cancer | 3 | 1 | 0 | 0 | | | 4 | | 0.010 | 0.000 | 0.000 | 0.003 | 0.013 |
| Medium risk industrial | | | | | | | | | | | | | |
| Mesothelioma | 5 (1) | 1 (1) | 1 | 0 | | | 7 (2) | 2.4 | 0.004 | 0.027 | 0.003 | 0.000 | 0.034 |
| Lung cancer | 20 | 1 | 4 | 0 | | | 25 | | 0.012 | 0.002 | 0.003 | 0.002 | 0.019 |
| Domestic exposure | | | | | | | | | | | | | |
| Mesothelioma | 2 (2) | 0 | 2 (1) | 3 (1) | | | 7 (4) | 1.9 | 0.103 | 0.077 | 0.004 | 0.003 | 0.186 |
| Lung cancer | 13 | 6 | 0 | 1 | | | 20 | | 0.018 | 0.006 | 0.002 | 0.001 | 0.027 |
| Low-risk occupations | | | | | | | | | | | | | |
| Mesothelioma | 5 (1) | 0 | 4 (1) | 1 (1) | | | 10 (3) | 1.0 (ref) | 0.067 | 0.017 | 0.001 | 0.003 | 0.087 |
| Lung cancer | 26 | 3 | 2 | 0 | | | 31 | | 0.009 | 0.002 | 0.002 | 0.001 | 0.013 |
| Total | | | | | | | | | | | | | |
| Mesothelioma | 13 (5) | 2 (2) | 7 (2) | 4 (2) | | | 26 (11) | | 0.056 | 0.034 | 0.002 | 0.002 | 0.095 |
| Lung cancer | 62 | 11 | 6 | 1 | | | 80 | | 0.012 | 0.003 | 0.003 | 0.001 | 0.019 |

*Female mesothelioma ORs from the original case–control study.[1]

effect on risk to be estimated. Table 4 (see online supplementary material) shows mesotheliomas and lung cancers classified by amosite and crocidolite concentration, ignoring other fibres. A logistic model in which one crocidolite fibre is equivalent to 1.3 (95% CI 0.4 to 3.3) amosite fibres gave the best fit.

Distributions of fibre length were similar irrespective of disease status, fibre type or lung burden, the overall distribution being 76.1% 5–10 μm, 21.2% 10–20 μm and 4.5% >20 μm. Median widths were 0.09 μm (chrysotile), 0.17 μm (crocidolite), 0.30 μm (amosite), 0.49 μm (tremolite), 0.58 μm (anthophyllite) and 0.61 μm (actinolite). No significant association was seen between disease status and fibre dimension after stratifying by fibre type.

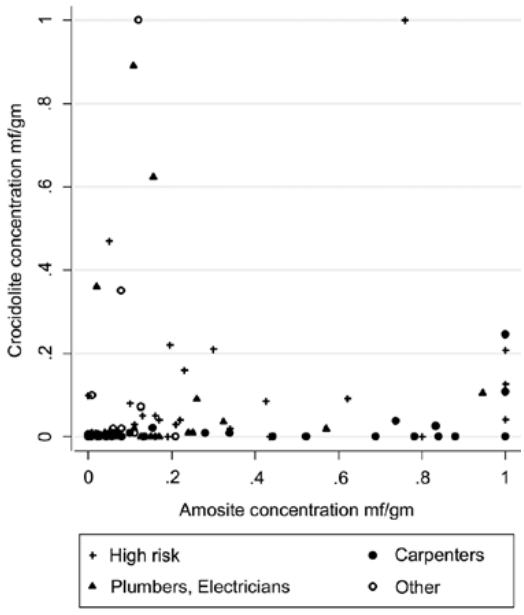



**Figure 3** Amosite and crocidolite lung burdens and occupational categories in male mesotheliomas. (Burdens >1 million fibres/g truncated to 1 mf/g).

## DISCUSSION

### Dose–response

This is the first study with occupational histories obtained by personal interview and asbestos lung burdens measured by TEM from a large population-based series of patients with mesothelioma. Our fitted model estimates and adjusts for the effect of using lung cancers as controls. Table 2 shows that the effect is small among the 96% of men whose lung burdens are less than 0.2 mf/g (lung cancer SMR <1.1) but implies that the majority of lung cancers are caused by asbestos in the 1% of men whose lung burdens are above 0.5 mf/g. As expected, mean lung burdens are consistently higher for mesothelioma than for lung cancer within each occupational category and increase with increasing occupational OR. Our results confirm that most mesotheliomas are caused by asbestos even in those who never worked in asbestos-related occupations. Larger numbers and more sensitive TEM would be required to estimate the incidence of spontaneous mesotheliomas unrelated to asbestos, which is ignored in our modelling. The ORs in figure 2 are scaled to make the observed OR unity in the lowest exposure group (<0.025 mf/g). The fitted value (solid line in figure 2) equals 0.74 in this group. This small and non-significant difference corresponds to a lifetime spontaneous mesothelioma risk of about 1 per 2000, almost an order of magnitude greater than early estimates of the spontaneous incidence in both sexes.[5]

Our estimates of lifetime excess risk due to asbestos in British men born in 1945 are 0.86% for mesothelioma and 0.47% for lung cancer, a ratio of excess lung cancer to mesothelioma of 0.55. Two independent sources also suggest that asbestos causes more mesotheliomas than lung cancers in British men. An analysis of proportional mortality ratios for different occupational groups concluded that the ratio of excess lung cancer to mesothelioma in British men is about 0.7.[6] The ratio was 1.3 (1795

deaths, 965 expected for lung cancer and 639 mesothelioma deaths) in men in the Great Britain Asbestos Workers prospective study, but adjustment for smoking reduced the estimate for the general population to 0.7.[7] The ratio would be substantially higher in the earlier birth cohorts included in that study due to their higher smoking-related lung cancer rates, so our estimate of the ratio for the 1945 birth cohort (0.55) may be approximately correct despite the imprecision of the estimated increase in lung cancer RR (2.55 per mf/g, 95% CI 0.62 to 10.37). The lifetime lung cancer risk at a given asbestos lung burden will continue to fall in later generations because they smoke less but the lifetime mesothelioma risk will be higher because they will live longer. The model used by Tan *et al*[8] implies that mesothelioma incidence in the 1945 male birth cohort will increase less steeply with age than in earlier generations. That analysis (updated to 2013) predicts that their lifetime mesothelioma risk will be 0.72%. If this proves more accurate than our simple projection (0.86%) the mesothelioma risk per 1000 fibre/g should be reduced from 0.020% to 0.017%. The lifetime risk per 1000 f/g in women will be slightly greater due to their longer life expectancy.

Linear dose–response is the most important assumption underlying the risk estimates for mesothelioma in table 2, which are constrained to match the predicted lifetime risk for the UK male population born in 1945. The relationship between mesothelioma risk and lung burden of asbestos measured by TEM is perhaps the only example of a major human carcinogen for which the data span such a wide range of measured dose and risk. Stomata in the parietal pleura where long fibres congregate may be the main site of carcinogenesis,[9] but for fibres of specified dimension it seems reasonable to assume a linear relationship between inhaled dose, fibre concentration in pleural stomata and our measurements in lung parenchyma. Linear dose–response might therefore be expected if mesothelioma were initiated by a single asbestos fibre in a single cell, but tumour progression may also involve dose-related local inflammatory processes.[9] Doll and Peto[10] observed a quadratic dose–response for cigarette smoking and lung cancer indicating both early and late effects in lung carcinogenesis and suggested that the linear relationship seen in other studies reflected inaccurate measurement of lifelong smoking rates. Our lifetime mesothelioma risk estimate of 0.020% per 1000 asbestos fibres/g provides a reasonably reliable basis for predicting future mesothelioma rates in birth cohorts born since 1965 from their average asbestos lung burdens. For estimating the exposure level or lung burden that would cause lifetime mesothelioma risks of the order of 1 in 100 000, an order of magnitude less than the estimated spontaneous rate[5] and two orders of magnitude below the range in our data, risk assessment conventions rather than epidemiology must determine the basis of the extrapolation. We did not include any peritoneal mesotheliomas, which are both under- and over-diagnosed due to confusion with cancers of the ovary and other abdominal sites.[11] Peritoneal mesotheliomas constitute less than 4% of all mesotheliomas in the UK, and if the dose-response with amphibole exposure is quadratic[13] the proportion will be even less at lower dose levels.

### Effects of amphiboles and chrysotile

Asbestos consumption per head since the 1950s was similar in Britain and the US for chrysotile and crocidolite but amosite consumption was about five times higher in Britain (figure 1). This seems likely to explain the fivefold greater mesothelioma death-rate in Britain than in the USA among men born around 1945.[1] UK crocidolite imports ceased by 1970, a decade earlier than amosite imports, but some exposure continued. The median concentration of crocidolite fibres was 0.009 mf/g in male mesothelioma cases born 1940–1949, and was still 0.004 mf/g in those born after 1950 who started work around or after the time that crocidolite use ended. Our model suggests that the mesothelioma risk per fibre is approximately 1.3 (95% CI 0.4 to 3.3) times higher for crocidolite than for amosite. The proportion of TEM fibres with width >0.2 μm and therefore observable by phase contrast optical microscopy (PCOM) was 38% for crocidolite and 75% for amosite. Our estimate of the risk per fibre of crocidolite relative to amosite is thus approximately doubled to give 2.6 (95% CI 0.8 to 6.6) for PCOM data, statistically consistent with the estimate of five based on cohort studies with PCOM fibre counting of air samples.[12]

Other evidence shows that chrysotile causes a much lower mesothelioma risk than amosite or crocidolite.[12 13] The rapid clearance of chrysotile from the lung with a half-life of a few months[3] explains its virtual absence in our samples, and implies that we cannot estimate its effects except by noting that amphibole lung burdens account very well for mesothelioma incidence. Rasmuson *et al*[14] reported a good correlation between lung burden and estimated cumulative exposure for amphiboles but not for chrysotile. The prolonged heavy chrysotile exposure that occurred in some British factories before the 1932 Asbestos Industry Regulations were introduced caused an Standardised Mortality Ratio (SMR) of more than 10 for lung cancer in chrysotile textile workers,[15] but the contribution of chrysotile to current UK lung cancer rates is not known and may be impossible to ascertain.

### Biopersistence of amphiboles

Earlier studies showing an approximately linear relationship between amphibole lung burden and mesothelioma risk reported higher lung burdens in cases and controls[16–18] than we observed. The half-life of amphiboles in the lung has been estimated as about 6–10 years[4 19–21] for crocidolite and perhaps 20 years for amosite.[4] Comparison of the distributions of lung burdens in this study and in an earlier study of 69 British men who died of mesothelioma[17] also suggests a longer half-life for amosite. These men, like our cases, were all born since 1940, and most died in 1994–1995, about 9 years before our cases. The respective proportions of male mesotheliomas exceeding 0.1 mf/g in our data and in the earlier series were 42.1% and 46.4% for amosite and 11.2% and 29.0% for crocidolite (fibres >6 μm; JC McDonald and B Armstrong, personal communication). However, we have not attempted to adjust our data for elimination, for two reasons. First, these men were born since 1940 and were exposed predominantly between 1960 and the late 1970s when amosite exposure ended, so their average intervals from exposure to lung sampling were similar. Second, our lung samples were obtained more than 20 years after substantial amphibole exposure had ceased. If as Tossavainen *et al*[22] suggest, further clearance of long amphibole fibres will be minimal, further studies over the next decade should show a similar dose–response. A higher proportion of inhaled fibres from more recent environmental exposure will be retained, however, somewhat exaggerating mesothelioma risks predicted from amphibole lung burdens in those born more recently.

Conversely, a residence time model in which earlier exposure causes a higher lifetime risk and later exposure is discounted[8] would imply higher dose-specific risks in younger people, particularly for environmental exposure which presumably began in childhood.

### Environmental asbestos exposure

Our case–control analysis suggested that 14% of male and 62% of female mesotheliomas were not attributable to occupational or domestic asbestos exposure.[1] Among men and women with only low-risk occupations 6 of 12 mesotheliomas and 6 of the 60 lung cancers had lung burdens exceeding 0.05 mf/g (table 3). Three of these six mesotheliomas and one of the six lung cancers mentioned potential asbestos exposure at work (one occasionally handling sealed asbestos waste, one using asbestos ironing boards at work and two office workers in companies handling building materials). These potential exposures, which had not been classified as substantial in coding these occupational histories, suggest that approximately 25% (3/12) of mesotheliomas in apparently low-risk occupations may be due to such work-related exposures.

## CONCLUSION

Our results confirm the major contribution of amosite to UK mesothelioma incidence and the substantial contribution of non-occupational asbestos exposure, particularly in women.[1] The overall distribution of asbestos lung burdens in British men born in the 1940s and their resulting mesothelioma and lung cancer risks are summarised in table 2. The lowest exposure category (<0.025 mf/g) includes 17% of mesotheliomas and 63% of the population, while 45% of mesotheliomas but only 4% of the population are above 0.2 mf/g. The approximate linearity of the dose–response together with lung burden measurements in younger people will provide reasonably reliable predictions of future mesothelioma rates in those born since 1965 whose risks cannot yet be seen in national rates. Burdens in those born more recently will indicate the continuing occupational and environmental hazards under current asbestos control regulations. Similar population-based studies with uniform TEM fibre counting methods in other countries, together with the international mesothelioma death-rates now available following the introduction of ICD-10 with a separate cause of death code for mesothelioma, would provide a worldwide perspective on future mesothelioma rates and more precise estimates of the risk per fibre for different amphiboles. Local studies are needed to estimate the risk per fibre, lung burdens in younger people, and hence the continuing environmental hazard from amphibole contamination in areas such as Libby, Montana[23] and from other naturally occurring asbestiform fibres such as erionite in Turkey and elsewhere.[24] The extent to which mesotheliomas in chrysotile workers are due to tremolite contamination or previous amphibole exposure could also be tested.[25] If most mesotheliomas are due to amphibole exposures, the quantitative relationship we have observed between amphibole lung burden and mesothelioma risk should also be seen in the USA and in Eastern European and South American countries where amphiboles are reported to have constituted a much lower proportion of asbestos consumption than in the UK.

**Acknowledgements** The authors thank Maria Kelly for the majority of the telephone interviews, Jane Hatch, Jayne Simpson, Samantha Warnakulasuriya, Laura Newmark, Bavithra Vijayakumar, Chris Heaton, Qichen Liu, Katie Field and Emma Afify for administrative work on the study, and Richard Houlihan, Catherine Taylor, Kirsty Dewberry and James Staff at HSL for TEM counting. The authors also thank Ben Armstrong and Corbett McDonald for providing further information regarding their study. Most importantly, the authors thank the patients with mesothelioma and lung cancer who agreed to participate.

**Contributors** CG performed the majority of the statistical analysis and contributed to the interpretation of data and writing the report. CR supervised the conduct of the case–control study and contributed to data collection. GB supervised the TEM, contributed to data collection and writing the report. AGN and LD provided advice on lung pathology and contributed to data collection. AF contributed to the statistical analysis and JC provided additional statistical advice. JH and AD contributed to interpretation of data and writing the report. JP designed the study and contributed to the statistical analysis, interpretation of data and writing the report. He is guarantor. All authors have contributed to the revision of the manuscript and have approved the final version.

**Funding** This research was supported by the Health & Safety Executive, the British Lung Foundation and Cancer Research UK. AGN was supported by the National Institute of Health Research Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** South Thames Multicentre Research Ethics Committee.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** The anonymised individual data on sensitivity, each fibre detected (size and type) and occupational history are available on application to the authors (CG or JP) for further analysis on a collaborative basis.



## REFERENCES

1 Rake C, Gilham C, Hatch J, *et al*. Occupational, domestic and environmental mesothelioma risks in the British population: a case-control study. *Br J Cancer* 2009;100:1175–83.
2 Peto J, Rake C, Gilham C, *et al*. *Occupational, domestic and environmental mesothelioma risks in Britain: a case-control study*. http://www.hse.gov.uk/research/rrhtm/rr696.htm:2009
3 McElvenny DM, Darnton AJ, Price MJ, *et al*. Mesothelioma mortality in Great Britain from 1968 to 2001. *Occup Med (Lond)* 2005;55:79–87.
4 Churg A, Wright JL. Persistence of natural mineral fibers in human lungs: an overview. *Environ Health Perspect* 1994;102(Suppl 5):229–33.
5 McDonald JC. Health implications of environmental exposure to asbestos. *Environ Health Perspect* 1985;62:319–28.
6 Darnton AJ, McElvenny DM, Hodgson JT. Estimating the number of asbestos-related lung cancer deaths in Great Britain from 1980 to 2000. *Ann Occup Hyg* 2006;50:29–38.
7 Frost G, Darnton A, Harding AH. The effect of smoking on the risk of lung cancer mortality for asbestos workers in Great Britain (1971–2005). *Ann Occup Hyg* 2011;55:239–47.
8 Tan E, Warren N, Darnton AJ, *et al*. Projection of mesothelioma mortality in Britain using Bayesian methods. *Br J Cancer* 2010;103:430–6.
9 Donaldson K, Murphy FA, Duffin R, *et al*. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part Fibre Toxicol* 2010;7:5.
10 Doll R, Peto R. Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers. *J Epidemiol Community Health* 1978;32:303–13.
11 Boffetta P. Epidemiology of peritoneal mesothelioma: a review. *Ann Oncol* 2007;18:985–90.
12 Hodgson JT, Darnton A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann Occup Hyg* 2000;44:565–601.
13 McDonald JC. Epidemiology of malignant mesothelioma—an outline. *Ann Occup Hyg* 2010;54:851–7.
14 Rasmuson JO, Roggli VL, Boelter FW, *et al*. Cumulative Retrospective Exposure Assessment (REA) as a predictor of amphibole asbestos lung burden: validation procedures and results for industrial hygiene and pathology estimates. *Inhal Toxicol* 2014;26:1–13.
15 Doll R. Mortality from lung cancer in asbestos workers. *Br J Ind Med* 1955;12:81–6.
16 Rogers AJ, Leigh J, Berry G, *et al*. Relationship between lung asbestos fiber type and concentration and relative risk of mesothelioma. A case-control study. *Cancer* 1991;67:1912–20.
17 McDonald JC, Armstrong BG, Edwards CW, *et al*. Case-referent survey of young adults with mesothelioma: I. Lung fibre analyses. *Ann Occup Hyg* 2001;45:513–18.
18 Rodelsperger K, Woitowitz HJ, Bruckel B, *et al*. Dose-response relationship between amphibole fiber lung burden and mesothelioma. *Cancer Detect Prev* 1999;23:183–93.
19 Berry G. Models for mesothelioma incidence following exposure to fibers in terms of timing and duration of exposure and the biopersistence of the fibers. *Inhal Toxicol* 1999;11:111–30.
20 de Klerk NH, Musk AW, Williams V, *et al*. Comparison of measures of exposure to asbestos in former crocidolite workers from Wittenoom Gorge, W. Australia. *Am J Ind Med* 1996;30:579–87.

21 Du Toit RS. An estimate of the rate at which crocidolite asbestos fibres are cleared from the lung. *Ann Occup Hyg* 1991;35:433–8.
22 Tossavainen A, Karjalainen A, Karhunen PJ. Retention of asbestos fibers in the human body. *Environ Health Perspect* 1994;102(Suppl 5):253–5.
23 Antao VC, Larson TC, Horton DK. Libby vermiculite exposure and risk of developing asbestos-related lung and pleural diseases. *Curr Opin Pulm Med* 2012;18:161–7.
24 Carbone M, Emri S, Dogan AU, *et al*. A mesothelioma epidemic in Cappadocia: scientific developments and unexpected social outcomes. *Nat Rev Cancer* 2007;7:147–54.
25 McDonald AD, Case BW, Churg A, *et al*. Mesothelioma in Quebec chrysotile miners and millers: epidemiology and aetiology. *Ann Occup Hyg* 1997;41:707–19.
26 Easton DF, Peto J, Babiker AG. Floating absolute risk: an alternative to relative risk in survival and case-control analysis avoiding an arbitrary reference group. *Stat Med* 1991;10:1025–35.
27 HSE 1977 Selected written evidence submitted to the Advisory Committee on Asbestos 1976–1977. HMSO - Appendix G.
28 HSE 1979 Asbestos. Volume 1: final report of the advisory committee. HMSO - Page 14 Table 3 and Page 16 Table 8.
29 Institute for Environment and Health 1997. Fibrous materials in the environment. Inst. For Environment and Health, Leicester - Table 3.1.3.
30 Virta RL. Worldwide asbestos supply and consumption trends from 1900 through 2003: US Geological Survey. *Circular* 2006;1298:80. http://pubs.usgs.gov/circ/2006/1298/c1298.pdf


## APPENDIX 1: STATISTICAL METHODS

### Linear dose–response model

In the ith lung burden category there are l(i) lung cancers with mean asbestos lung burden d(i) and m(i) mesotheliomas. If mesothelioma risk and excess lung cancer relative risk both increase linearly with increasing lung burden, with slope b for mesothelioma and k for lung cancer, the expected ratio of mesothelioma to lung cancer is proportional to [b·d(i)]/[1+k·d(i)].

The slopes b and k are estimated by maximum likelihood from the log(odds), which are treated as independent with normally distributed error variances v(i)=1/m(i)+1/l(i). Thus

$$\begin{aligned}\log(\text{odds}) &= \log[m(i)/l(i)] \\ &= \log(b \cdot d(i)) - \log(1 + k \cdot d(i)) + \text{constant} \\ &\quad + e\,(e \sim N(0, v(i)). \end{aligned} \quad (1)$$

The constant determines the scale of mesothelioma risk (odds, OR, lifetime risk or SMR). The constant was set as the observed value of log[m(1)/l(1)], the log(odds) in the lowest (reference) exposure category, giving the solid line in figure 2. ORs for each lung burden category, including the reference group, are shown in figure 2 with 'floating absolute risk' CIs corresponding to the log(odds) variances v(i).[25] Taking group 1 as the reference group, the usual definition of the OR in group i is (true odds in group i)/(true odds in groups 1). The definition of the floating OR is (true odds in group i)/(observed odds in group 1). The denominator, the observed odds in group 1, is a known constant with zero variance so the error variance of log(floating OR) in category i equals v(i), the variance of log(odds).

### Distribution of lung burdens in the general population and corresponding lifetime risks

In the ith lung burden category the number of lung cancers that were not caused by asbestos is l(i)/(1+k·d(i)). We assume that the general population have the same distribution of lung burdens as these non-asbestos lung cancers, so the proportion p(i) in the ith lung burden category among British men in the same birth cohort (ie, born around 1945) is estimated in table 2 as:

$$p(i) = [l(i)/(1 + k \cdot d(i))] / \sum [l(i)/(1 + k \cdot d(i))].$$

The mean lung burden of men in this birth cohort is thus $\sum p(i) \cdot d(i)$. Their projected death-rates at each age for mesothelioma and lung cancer were calculated by unadjusted age and birth cohort analysis of British male mesothelioma and lung cancer death-rates in 5-year age-groups (35–39 to 85–89) and periods (1990–1994 to 2005–2009). For men with average lung burden d(i) these projected rates were multiplied by M·d(i) for mesothelioma and L·(1+k·d(i)) for lung cancer. Their lifetime risks (respective probabilities of dying by age 90 of mesothelioma and lung cancer) were then calculated actuarially, assuming current (2013) rates for all other causes of death. The constants M and L were adjusted to make the population averages of these lifetime risks equal the overall population projections for lung cancer (4.67%) and mesothelioma (0.86%).

### Comparison of amosite and crocidolite

The effect of crocidolite relative to amosite was estimated in a logistic model fitting log(lung burden of amosite plus crocidolite) as a continuous variable in which crocidolite fibres were given a weighting w. We fixed the offset using the estimated lung burden coefficient k from the unweighted model to give nested models and a likelihood-based CI for w. For the jth individual with amosite lung burden $a_j$ and crocidolite lung burden $c_j$

$$\log(\text{odds}) = \log[b \cdot (a_j + w \cdot c_j)] + \text{offset}$$

where offset=log[m(1)/l(1)]−log(1+k·$d_j$).

## APPENDIX 2: LUNG SAMPLE PREPARATION AND TRANSMISSION ELECTRON MICROSCOPY

All lung tissue samples were sent to a pathology laboratory in Leeds for an initial assessment of their suitability. Thin tissue sections were microtomed from the waxed blocks for further assessment, before the blocks were de-waxed in xylene and washed in ether and microdissected to remove cancerous and fibrotic tissue. The samples were then anonymised and sent to the Health and Safety Laboratory (HSL) for quantitative transmission electron microscopy (TEM) analysis. At HSL a representative sample was taken from the tissue supplied and diced into cubes (approximately 3 mm sides) and dried overnight in a vacuum dessicator and then weighed to obtain the dry weight. The tissue was then digested in bleach and aliquots filtered onto membrane filters. The filter with the largest aliquot was ashed overnight under controlled conditions in a low temperature asher to further remove organic material. The ashed residual was resuspended in water and a range of aliquots filtered onto 0.2 μm pore size polycarbonate filters. When dry, strips of the final filters were carbon coated and sections cut out and transferred onto 200 mesh nickel index grids. Several grids from each filter were prepared by dissolving away the polycarbonate on a filter paper soaked with a mixture of 20% 1,2-diaminoethane and 80% 1-methyl-2 -pyrrolidone. This left a thin carbon film with the entrapped particles supported on the grid. New disposable containers and filtration equipment were used for each sample to avoid any cross-contamination and a process blank was run with each batch of analyses.

The prepared TEM grids were analysed in a FEI CM12 TEM equipped with an EDAX Inc beryllium window energy dispersive X-ray detector. Grid openings were step scanned on the fluorescent screen at 11k magnification to identify fibres (particles with parallel sides and >3:1 aspect ratio) over 5 μm in length. The length, width, type of diffraction pattern and quantitative weight percentage oxide composition from the energy dispersive X-ray analysis were recorded for each asbestos fibre

found. Counting continued until at least 30 asbestos fibres had been identified, or until 0.1 mg of defatted dry lung had been analysed, giving an analytical sensitivity of 0.01 mf/g (million fibres per dry gram). This sensitivity was not achieved in 2.8% of the samples (2/133 mesotheliomas, 9/262 lung cancers), due to samples with low fibre concentrations but high amounts of other inorganic particles that required lower filter loadings and hence large areas of the filter to be analysed by TEM. The sensitivity was increased threefold to 0.003 mf/g by later extending the TEM analysis on newer equipment for a selected subgroup of samples which included 25 of 26 male and 7 of 8 female samples from patients with mesothelioma born in 1940 or later in which 5 or fewer asbestos fibres were originally counted.

# Pleural mesothelioma and lung cancer risks in relation to occupational history and asbestos lung burden

Clare Gilham, Christine Rake, Garry Burdett, Andrew G Nicholson, Leslie Davison, Angelo Franchini, James Carpenter, John Hodgson, Andrew Darnton and Julian Peto

*Occup Environ Med* published online December 29, 2015

STUDY No. 5

# AN EVALUATION OF REPORTED NO-EFFECT CHRYSOTILE ASBESTOS EXPOSURES FOR LUNG CANCER AND MESOTHELIOMA

**STUDY No. 5**

# AN EVALUATION OF REPORTED NO-EFFECT CHRYSOTILE ASBESTOS EXPOSURES FOR LUNG CANCER AND MESOTHELIOMA

---

**Critical Reviews in Toxicology (2008) 38:191–214**

**Jennifer S. Pierce, Meg A. McKinley, Dennis J. Paustenbach, and Brent L. Finley, ChemRisk, Inc., San Francisco, California, USA**

---

Numerous investigators have suggested that there is likely to be a cumulative chrysotile exposure below which there is negligible risk of asbestos-related diseases. However, to date, little research has been conducted to identify an actual "no-observed-adverse-effect level" (NOAEL) for chrysotile-related lung cancer and mesothelioma.

The purpose of this analysis of several publications was to summarize and present all of the cumulative exposure-response data reported for predominantly chrysotile-exposed cohorts in the published literature. The review covered cohorts from A/C manufacturing, friction materials, textiles, mining and milling. A must read.

The authors conclude from their extensive review that "it does seem to indicate that low occupational exposures to chrysotile (e.g., exposures historically experienced by vehicle mechanics) are unlikely to cause mesothelioma."

# An Evaluation of Reported No-Effect Chrysotile Asbestos Exposures for Lung Cancer and Mesothelioma

**ARTICLE** *in* CRITICAL REVIEWS IN TOXICOLOGY · FEBRUARY 2008

Impact Factor: 5.1 · DOI: 10.1080/10408440701845609 · Source: PubMed

*Critical Reviews in Toxicology*, 38:191–214, 2008

ISSN: 1040-8444 print / 1547-6898 online
DOI: 10.1080/10408440701845609

informa
healthcare

# An Evaluation of Reported No-Effect Chrysotile Asbestos Exposures for Lung Cancer and Mesothelioma

**Jennifer S. Pierce, Meg A. McKinley, Dennis J. Paustenbach, and Brent L. Finley**
*ChemRisk, Inc., San Francisco, California, USA*

**Numerous investigators have suggested that there is likely to be a cumulative chrysotile exposure below which there is negligible risk of asbestos-related diseases. However, to date, little research has been conducted to identify an actual "no-effect" exposure level for chrysotile-related lung cancer and mesothelioma. The purpose of this analysis is to summarize and present all of the cumulative exposure-response data reported for predominantly chrysotile-exposed cohorts in the published literature. Criteria for consideration in this analysis included stratification of relative risk or mortality ratio estimates by cumulative chrysotile exposure. Over 350 studies were initially evaluated and subsequently excluded from the analysis due primarily to lack of cumulative exposure information, lack of information on fiber type, and/or evidence of significant exposures to amphiboles. Fourteen studies meeting the inclusion criteria were found where lung cancer risk was stratified by cumulative chrysotile exposure; four such studies were found for mesothelioma. All of the studies involved cohorts exposed to high levels of chrysotile in mining or manufacturing settings. The preponderance of the cumulative "no-effects" exposure levels for lung cancer and mesothelioma fall in a range of approximately 25–1000 fibers per cubic centimeter per year (f/cc-yr) and 15–500 f/cc-yr, respectively, and a majority of the studies did not report an increased risk at the highest estimated exposure. Sources of uncertainty in these values include errors in the cumulative exposure estimates, conversion of dust counts to fiber data, and use of national age-adjusted mortality rates. Numerous potential biases also exist. For example, smoking was rarely controlled for and amphibole exposure did in fact occur in a majority of the studies, which would bias many of the reported "no-effect" exposure levels towards lower values. However, many of the studies likely lack sufficient power (e.g., due to small cohort size) to assess whether there could have been a significant increase in risk at the reported no-observed-adverse-effects level (NOAEL); additional statistical analyses are required to address this source of bias and the attendant influence on these values. The chrysotile NOAELs appear to be consistent with exposure-response information for certain cohorts with well-established industrial hygiene and epidemiology data. Specifically, the range of chrysotile NOAELs were found to be consistently higher than upper-bound cumulative chrysotile exposure estimates that have been published for pre-1980s automobile mechanics (e.g., 95th percentile of 2.0 f/cc-yr), an occupation that historically worked with chrysotile-containing friction products yet has been shown to have no increased risk of asbestos-related diseases. While the debate regarding chrysotile as a risk factor for mesothelioma will likely continue for some time, future research into nonlinear, threshold cancer risk models for chrysotile-related respiratory diseases appears to be warranted.**

**Keywords** Asbestos, chrysotile, mechanics, threshold

## INTRODUCTION

Over the past 30 years, there has been an increasing amount of research devoted to understanding the relative carcinogenic potencies of the various asbestos fiber types (i.e., serpentine chrysotile versus amphibole forms, such as amosite, tremolite, and crocidolite). Wagner et al. (1965) were the first to note the apparent differences between crocidolite versus chrysotile potency when they reported that mesothelioma cases were quite common near crocidolite mines, but were absent in populations living and working near chrysotile mines. From the mid-1970s through the early 1990s, numerous epidemiology studies of asbestos-exposed cohorts described substantially higher disease rates in cohorts exposed to a mixture of fiber types (or predominantly amphiboles) versus those observed in cohorts

Address correspondence to Brent L. Finley, ChemRisk, Inc., 25 Jessie St. Suite 1800; San Francisco, CA 94105, USA. E-mail: bfinley@chemrisk.com

exposed to predominantly chrysotile (Enterline and Henderson, 1973; Meurman et al., 1974; McDonald and McDonald, 1977; Weiss, 1977; Acheson et al., 1981, 1982; Thomas et al.,1982; McDonald et al., 1983, 1984; Ohlson and Hogstedt, 1985; Gardner et al., 1986; Newhouse and Sullivan, 1989; Piolatto et al., 1990). In 1978 the American Conference of Governmental Industrial Hygienists (ACGIH) recommended threshold limit values of 0.2, 0.5, 0.5, 2 and 2 f/cc, for crocidolite, amosite, tremolite, chrysotile and "other forms" of asbestos, respectively (ACGIH, 1980). The more stringent recommendations for the amphiboles were "because of their greater potential for disease production" (p. 30). A U.S. Environmental Protection Agency (EPA) work group recently concluded that amphiboles are 4 times and 800 times as potent as chrysotile at inducing lung cancer and mesothelioma, respectively (Berman and Crump, 2003). Hodgson and Darnton (2000) of the United Kingdom (UK) Health and Safety Executive estimated that the risk of mesothelioma is in the ratio of 1:100:500 for chrysotile, amosite and crocidolite, respectively. In a more recent estimate of prospective mesothelioma incidence in the United Kingdom (based on import volumes of different asbestos fiber types), they assigned chrysotile a value of zero potency (Hodgson et al., 2005).

It has been suggested that differences in asbestos fiber type potency are due in part to differences in physicochemical properties that result in a much higher degree of biopersistence for amphibole fibers. Chrysotile fibers form large parallel sheets, while amphibole fibers are arranged in long linearly organized chains (Bernstein and Hoskins, 2006). The straight-chain structures are more biologically durable because they are more difficult to clear from the lung via macrophage engulfment or the mucociliary escalator. In addition, chrysotile fibers are easily depleted of critical components of their structure (e.g., magnesium and other cations) at low pH inside macrophages, thereby weakening the fibers, facilitating their destruction, and subsequently reducing their residence time in the lung (Jaurand et al., 1977; Roggli and Brody, 1984). Amphibole fibers are far more resistant to this type of leaching, and therefore have a much longer residence time (Jaurand et al., 1977; Roggli and Brody, 1984; Hesterberg et al., 1998). As such, the biological half-life of inhaled amphibole asbestos fiber types is in the range of years to decades, whereas the half-life of chrysotile is only days to weeks (de Klerk et al., 1996; Finklestein and Dufresne, 1999; Bernstein and Hoskins, 2006).

Chrysotile asbestos was historically used in hundreds of consumer products, including joint compound, floor tiles, brakes, manual clutches, automotive gaskets, mastic coatings, and welding rods. Although there are dozens of published epidemiological studies of asbestos-related diseases (i.e., lung cancer and mesothelioma) in occupational cohorts exposed to chrysotile during the manufacture or use of these products, to our knowledge there has been no systematic analysis of the available exposure-response information to identify a likely range of minimum cumulative chrysotile exposures necessary for increased risk. Browne (1986) provides the only quantitative estimate of a "threshold" cumulative exposure for asbestos-related diseases. He examined the relative risk of lung cancer stratified by cumulative exposure to asbestos of mixed fiber types (chrysotile and amphiboles) in 10 different cohorts and concluded that "the threshold for increased risk of lung cancer appears to be somewhere in the range of 25–100 f/cc-years" (p. 558).

However, this assessment was not specific to chrysotile because studies with probable or known significant amphibole exposure were included (Enterline and Henderson, 1973; Seidman et al., 1979). Also, the Browne (1986) review did not address mesothelioma. Dunnigan (1986) also reviewed the available epidemiological and experimental data and concluded that "for chrysotile only exposures (without amphiboles), there is a threshold, below which no adverse health effects can be detected," but did not offer a quantitative estimate of the threshold dose (p. 41). Several others have since suggested that a threshold dose for chrysotile-induced disease may indeed exist (Ilgren and Browne, 1991; Meldrum, 1996; Churg, 1988; Hodgson and Darnton, 2000) but, like Dunnigan (1986), they have not posited an actual value or range of values.

Approximation of the cumulative chrysotile exposures associated with increased lung cancer and mesothelioma disease would aid in the health risk assessments of chrysotile-exposed occupations in several ways. First, it would aid in the analysis of occupations with well-established epidemiological and industrial hygiene assessments. For example, we recently determined that vehicle mechanics working with chrysotile-containing automotive friction products in the 1970s experienced median cumulative chrysotile exposures ranging from 0.16 to 0.41 f/cc-yr (Finley et al., 2007). Since it has been established that vehicle mechanics are not at an increased risk of developing lung cancer or mesothelioma, this estimated range of exposures should be below the chrysotile exposures necessary to cause lung cancer or mesothelioma. Second, a more informed understanding of the available chrysotile exposure-response data would improve the health risk assessments for occupations where chrysotile exposure information is available, but for which relevant epidemiological analyses do not exist and/or are difficult to obtain due to confounding exposures. For example, up until the 1980s, welders often used welding rods that contained low levels (<1% by weight) of chrysotile asbestos in the flux. Some epidemiological studies report elevated rates of mesothelioma in welders, yet it is known that welders often experienced direct and indirect exposure to amphibole-containing insulation (Danielsen et al., 1996; Moulin et al., 1993; Newhouse et al., 1985; Teta and Ott, 1988). Hence, the potential contribution of chrysotile-flux exposures to these disease endpoints cannot be determined directly from the epidemiological data. However, it should be possible to characterize estimated chrysotile-flux exposures via comparisons to the cumulative chrysotile exposure-response data and thereby reach a risk assessment conclusion for welders. In addition to these and other retrospective analyses, as recently noted by Yarborough (2006), there are emerging

nanotechnology research techniques involving the use of synthetic chrysotile in microelectronics, and it would therefore be beneficial to be able to accurately predict the magnitude of any potential health risks associated with the manufacture and use of these materials.

In this article, we assemble and summarize the published lung cancer and mesothelioma information for all chrysotile-exposed cohorts for which exposure and response data are available. Emphasis is placed on those studies where amphibole exposures are relatively low and stratified exposure-response results are reported by the authors. A range of cumulative "no-effect" exposure levels (highest estimated cumulative exposure at which no increased risk was reported) is identified from all studies that meet the criteria for inclusion (as defined in this analysis). Uncertainties that are likely to introduce bias are described in detail, and the upper-bound estimates of cumulative chrysotile asbestos exposure for U.S. brake mechanics are compared to the putative no-effect exposure levels to assess consistency with the brake mechanic epidemiological literature. We also discuss applications of this analysis to prospective occupational and consumer settings that might involve chrysotile exposures in the future.

## METHODS

### Study Selection Criteria

We performed a literature search for asbestos-exposed cohorts in multiple databases using a variety of search strategies and keyword combinations. To locate additional studies we systematically searched the reference lists of all studies identified by our search, as well as key review papers. We incorporated into our analysis all of the studies on occupational cohorts that met the following criteria:

1. Outcomes of interest included lung cancer (variously identified as "lung cancer," "respiratory cancer," "malignant respiratory neoplasms" or "malignant neoplasms of the lung") and/or mesothelioma.
2. The cohort was predominantly exposed to chrysotile asbestos (less than 10% of the potential asbestos exposures involved amphiboles).
3. There were no other known occupational exposures to respiratory carcinogens.
4. Relative risk or relative mortality estimates were provided or could be calculated and stratified by cumulative chrysotile exposure.
5. Cumulative chrysotile exposures were stratified into two or more exposure levels by the authors.

If multiple studies existed on a single cohort, the study with the most power (i.e., longer follow-up period, larger study population) was selected for the analysis. Wherever possible, we identify the following study elements for each cohort:

- Workplace description, type of industry, and location.
- Cohort demographics (age, duration of employment, employment initiation date, smoking status, disease latency).
- Time period or decade(s) of exposure and follow-up.
- Diagnostic methods.
- Source of control population.
- Follow-up period.
- Quantity of chrysotile and amphiboles (if applicable) processed and best estimate of percent amphiboles used.
- Air sampling methods used and the method for calculating individual worker cumulative exposure.
- Stratified cumulative exposures for lung cancer and mesothelioma.

Due to the lack of available information regarding smoking habits and employment history in most studies, we were unable to control for smoking and previous occupational exposures to amphiboles (e.g., shipyard and insulation employment). We also did not attempt to differentially weight the studies, nor did we reinterpret any of the authors' findings.

No-observed-adverse-effect levels (NOAELs) were determined for each study as the highest exposure group at which there was no statistically significant increased risk for lung cancer and/or mesothelioma. If a risk metric (e.g., a mortality ratio or odds ratio) or confidence interval was not provided by the authors, when possible it and/or the 95% Fishers exact confidence interval was calculated based on the available data using OpenEpi software (available through Emory University School of Public Health; http://www.sph.emory.edu/~cdckms/exact-midP-SMR.html). To avoid confusion and for the sake of consistency, if the risk estimates were reported in the studies as a percentage, we reported the equivalent proportion in our analysis; this is noted in the text. If no increased incidence of cancer was reported in a cohort, the NOAEL was considered to be the highest exposure group in the study.

Cumulative exposure measurements reported in units other than fibers per cubic centimer per year (f/cc-yr; equivalent to f/ml-yr) were converted to f/cc-yr using the conversion factor provided by the individual study authors. If cumulative exposure was reported in millions of particles per cubic foot per year (mppcf-yr) and a conversion factor was not provided, a conversion factor was determined based on published factors for plants with similar operations.

Throughout this article we use the term "cumulative exposure" in lieu of "cumulative dose" because the degree to which the airborne asbestos levels measured in these studies actually resulted in an inhaled "dose" is unknown. Also, we use the term "NOAEL" instead of "threshold" to emphasize that the highest minimum cumulative exposures at which no effects were observed are simply that, exposures without observed effects; whether or not these exposures truly represent "thresholds" below which effects do not occur cannot necessarily be discerned due to study limitations (as described in the Discussion).

## RESULTS

### Cohort/Exposure Study Identification and Determination of No-Effect Exposures

During our review, over 350 studies were initially evaluated and subsequently excluded from the analysis. Reasons for study exclusion were primarily lack of cumulative exposure information, lack of information on fiber type, and/or evidence of significant exposures to amphiboles. The following studies met the inclusion criteria: Albin et al. (1990), Berry and Newhouse (1983), Brown et al. (1994), Dement and Brown (1994), Dement et al. (1994), Hughes et al. (1987), Lacquet et al. (1980), Liddell and Armstrong (2002), McDonald et al. (1983a, 1984, 1993), Neuberger and Kundi (1990), Peto et al. (1985), and Piolatto et al. (1990). These studies examined cohorts exposed to chrysotile asbestos during asbestos mining and milling or the manufacture of asbestos-containing cement, friction, and textile products.

No-effect cumulative exposure levels for lung cancer and mesothelioma for the studies just listed are presented in Tables 1 and 2. When possible, we provided best estimates of the fraction of amphiboles present, as reported in Berman and Crump (2003). In addition, if the NOAEL was in the highest or the lowest exposure group, and the NOAEL was reported as "$>$" or "$<$," respectively, the mean and median cumulative exposure of the NOAEL group was reported in Table 1 or 2 if this information was provided by the authors.

### Asbestos Cement Products Manufacturing

*Belgium*

Lacquet et al. (1980) is a follow-up to Van den Voorde (1967), and presents x-ray results and updated mortality data for workers in a Belgian cement factory. The factory processed about 39,000 tons of asbestos annually, consisting of 90% chrysotile, 8% crocidolite, and 2% amosite, which were used in the manufacture of building materials and pipes (Berman and Crump, 2003; p. A.29).

The cohort was comprised of male workers who worked in the factory for at least 12 months within the 15-yr period of 1963 through 1977 (the size of the cohort is not presented). Specific demographic information for each individual, such as employment duration, job classification, smoking history, average age at employment initiation, and latency were not provided. All causes of death were determined by family doctors and/or social workers who visited the relatives (Belgian authorities do not release individual information from death certificates). Expected mortalities by age group were calculated based on the yearly rates for Belgium for the years 1965 to 1975; rates for other years were estimated by the authors.

Fiber counts measured using the membrane filter method were available from 1970 through 1976; dust concentrations for the previous years were estimated by the authors using a logistic decay model with an inflection point at 1960. Fiber concentrations were estimated from 1928 onward, and were thought by the authors to be much higher than the actual levels measured post-1970. Lacquet et al. (1980) considered their estimates to be accurate to within one order of magnitude of the actual concentrations in the factory. Individual exposures were calculated based on the duration of time spent at each of the five general areas of the plant: Area 4 involved handling of raw asbestos fibers, milling, and mixing of asbestos cement; Area 3 involved the finishing of cement products by sawing, drilling, filing, etc.; Area 2, which was situated between the previous two areas, was where asbestos-cement pipes and sheets were molded, pressed, dried, and lifted off the mold; Area 1 represented nonmanufacturing locations with very low asbestos concentrations, such as offices; and Area 0 represented work outside the asbestos industry, with negligible dust levels. The asbestos concentrations in Areas 4, 3, 2, and 1 were estimated to be 100, 24, 16, and 0.4 fibers/cc, respectively. The authors did not present the individual time-weighted average concentrations or exposure estimates.

Lacquet et al. (1980) segregated the cohort into seven exposure groups, with a total of 29,366 man-years of observation, and stated that there were no statistically significant increases in respiratory cancer deaths in any exposure group, including those in the highest estimated cumulative exposures of 1600–3200 fiber/cc-yr. To address the possible influence of the "healthy-worker effect," an internal case-control study was also performed, in which 4 control subjects were selected at random per case. The authors reaffirmed that dust exposure did not significantly affect mortality due to respiratory cancer. Standardized mortality ratios (SMRs)* and confidence intervals for the different exposure groups were calculated by us for respiratory cancer, based on the number of observed and expected respiratory cancer deaths provided by the authors (see Table 8, p. 790). One death due to pleural mesothelioma was reported in the highest exposure group (1600–3200 fiber/cc-yr); however, the expected number of mesothelioma deaths based on the background incidence in Belgium was not provided. For the purposes of our evaluation, it was assumed that this single case represented a true increase in mesothelioma risk for that exposure group. The NOAELs for nonmesothelioma respiratory cancer and mesothelioma in this study therefore were 1600–3200 f/cc-yr and 800–1599 f/cc-yr, respectively.

*New Orleans*

A prospective cohort study was conducted among workers in two cement manufacturing plants in New Orleans that were in operation since the 1920s (Hughes et al., 1987; Weill et al., 1973, 1979). Chrysotile was the primary fiber type used in both plants. Plant 1 consisted of one building in which flat shingles and corrugated sheets were produced. Amosite was used in corrugated

*The standardized mortality ratio (SMR) is used to compare the mortality experience of a study population with a standard population, and is calculated as observed deaths divided by expected deaths. It is an estimate of the relative risk.

TABLE 1
Studies of Lung Cancer in Chrysotile Cohorts

| Authors | Year | Industry | Fraction Amphiboles[a] Best Estimate (%) (Range (%)) | Disease Classification | Minimum Latency (Years) | Total Number of Cases (# of Cases Associated with NOAEL) | Risk Estimate for Exposures at the NOAEL (95%CI) | NOAEL (f/cc-years) |
|---|---|---|---|---|---|---|---|---|
| Lacquet et al. | 1980 | Cement Manufacturing: (Belgium) | ~10 (NA) | Respiratory cancer | Not Specified | 21(0) | — | 1,600–3,200[d] |
| Berry & Newhouse | 1983 | Friction Materials Manufacturing: (United Kingdom) | 0.5 (0–2) | Lung cancer | 10 | 105(5) | OR = 0.88 (0.24–2.72) | 100–356[d] |
| McDonald et al. | 1983 | Textiles: (Pennsylvania) | 8(3–15) | Malignant neoplasms respiratory | 20 | 53(6) | SMR = 1. 60 (0.59–3.48) | 120–240[e] |
| McDonald et al. | 1984 | Friction Materials Manufacturing: (Connecticut) | 0.5 (0–2) | Malignant neoplasms respiratory | 20 | 73(1) | SMR = 0.55 (0.01–3.08) | ≥112[d,e,f] |
| Peto et al. | 1985 | Textiles: (Rochdale) | 5(2–15) | Lung cancer | 20 | 93(6) | SMR = 1.06 (0.39–2.31) | 85.7–114.3 |
| Hughes et al. | 1987 | Cement Manufacturing: Plant 1 (New Orleans) | 1(0–2) | Respiratory malignancies | 20 | 22(5) | SMR = 1.23 (0.40–2.85) | ≥140[d,e](mean = 256.2) |
| Hughes et al. | 1987 | Cement Manufacturing: Plant 2 (New Orleans) | 5(2–15) | Respiratory malignancies | 20 | 42(4) | SMR = 1.56 (0.42–3.94) | ≥70[d,e] |
| Albin et al. | 1990 | Cement Manufacturing: (Sweden) | 3 (0–6) | Malignant respiratory disease except mesothelioma | 20 | 27 (NA) | RR = 1.9 (0.5–7.1) | ≥40[d](mean = 67, median = 88.2) |

*(Continued on next page)*

TABLE 1
Studies of Lung Cancer in Chrysotile Cohorts *(Continued)*

| Authors | Year | Industry | Fraction Amphiboles[a] Best Estimate (%) (Range (%)) | Disease Classification | Minimum Latency (Years) | Total Number of Cases (# of Cases Associated with NOAEL) | Risk Estimate for Exposures at the NOAEL (95%CI) | NOAEL (f/cc-years) |
|---|---|---|---|---|---|---|---|---|
| Neuberger & Kundi | 1990 | Cement Manufacturing: (Austria) | NA (NA) | Lung cancer | Not Specified[b] | 49 (24) | SMR = 0.96 (0.64–1.43) | >25[d,g] |
| Piolatto et al. | 1990 | Mining and Milling: (Italy) | 0.3(0.1–0.5) | Lung cancer | Not Specified[c] | 22(10) | SMR = 1.1 (0.55–2.11) | >400[d] |
| McDonald et al. | 1993 | Mining and Milling: Asbestos Mine and Mill (Quebec) | 1(0–4) | Malignant neoplasms of the lung | 20 | 133(22) | SMR = 1.55 (0.97–2.35) | ≥942[d,e] |
| McDonald et al. | 1993 | Mining and Milling: Thetford Mines (Quebec) | 1(0–4) | Malignant neoplasms of the lung | 20 | 155(28) | SMR = 1.05 (0.70–1.52) | 314–942[e] |
| Liddell & Armstrong | 2002 | Mining and Milling: (Quebec) | 1(0–4) | Lung cancer | 20 | 44(8) | SMR = 1.12 (0.48–2.21) | ≥1,884[d,e,g,h] (mean = 3,832) |
| Brown et al. | 1994 | Textiles: (South Carolina) | 0.5 (0–2) | Lung cancer | 15 | 124(7) | SMR = 0.65 (0.28–1 .43) | 1.4–2.7[e] |

*a* = Source: Berman and Crump, 2003; *b* = In a further investigation on lung cancer mortality, the authors excluded all persons with less than 15 years latency. They reported that the results did not differ substantially from that provided for the whole cohort, however risk estimates stratified by cumulative exposure were not provided (p. 618); *c* = Although there was not a requisite minimum latency, Piolatto et al. reported that 3 deaths due to lung cancer occurred in individuals with less than 20 years from their first exposure to asbestos, 7 with between 20 and 30 years, and 12 with over 30 years since their first asbestos exposure, *d* = Indicates that this was the highest exposure group in the study; *e* = Converted units to f/cc-years; *f* = Lack of apparent dose-response; marginally significant increase observed in lowest exposure group, however, no statistically significant increase observed in higher exposure groups; *g* = Adjusted for smoking; *h* = Same principal cohort as McDonald et al. 1993.
NA = Not available.
− = Zero cases reported.

TABLE 2
Studies of Pleural Mesothelioma in Chrysotile Cohorts

| Authors | Year | Industry | Fraction Amphiboles[a] Best Estimate (%) (Range (%)) | Disease Classification | Minimum Latency (Years) | Total Number of Cases (# of Cases Associated with NOAEL) | Risk Estimate for Exposures at the NOAEL (95%CI) | NOAEL (f/cc-years) |
|---|---|---|---|---|---|---|---|---|
| Lacquet et al. | 1980 | Cement Manufacturing: (Belgium) | 10 (NA) | Mesothelioma | Not Specified | 1(0) | — | 800–1,599[d] |
| McDonald et al. | 1984 | Friction Materials Manufacturing: (Connecticut) | 0.5 (0–2) | Mesothelioma | 20 | 0(0) | — | ≥112[b,c] |
| Albin et al. | 1990 | Cement Manufacturing: (Sweden) | 3(0–6) | Mesothelioma | 20 | 12(NA) | RR = 1.9 (0.2–21.3) | <15 (mean = 3.1, median = 1.4) |
| Piolatto et al. | 1990 | Mining and Milling: (Italy) | 0.3 (0.1–0.5) | Cancer of the pleura | 20 | 2(1) | SMR = 10 (0.25–55.7) | >400[b] |

*a* = Source: Berman and Crump, 2003; *b* = Indicates that this was the highest exposure group in the study; *c* = Converted units to f/cc-years; *d* = Only one death due to mesothelioma was reported; however, authors did not indicate the expected number of deaths due to mesothelioma. Thus, we conservatively assumed that this case represented a statistically significant increase in mesothelioma.
NA = Not available.
– = Zero cases reported.

siding from the early 1940s through the late 1960s, and crocidolite was used occasionally for approximately 10 years beginning in 1962. Plant 2 consisted of 4 buildings, each manufacturing different products. Shingles were the first product manufactured by the plant, followed by roofing materials, pipes, and asphalt flooring products. Pipe production, which commenced in 1946, used crocidolite and chrysotile. All other areas used only chrysotile. Berman and Crump (2003) estimated, based on the plant history provided in Hughes et al. (1987), that amphiboles accounted for roughly 1% of the total asbestos used at plant 1 (range 0–2%) (see Table 7–16). For plant 2 the best estimate was 5%, with a range of 2–15%.

The cohort included all men who had been employed for at least one month before 1970, and for whom a valid Social Security number was available from company records. The total number of men in the study population was 6931, of whom 2565 were employed at plant 1 and 4366 at plant 2. Sixty-one percent of plant 1 workers initiated employment between 1942 and 1949, and 74% of plant 2 employees started working during the period 1937 through 1949 (Hughes et al., 1987; see Table 3, p. 163). The mean durations of employment for plants 1 and 2 were both less than 4 years, with median employments of less than one year. On average, age upon hiring was higher in plant 1 (31.7 years) than in plant 2 (26.8 years), and was particularly high in plant 1 during the Second World War (39.0 years). Although smoking was not controlled for, based on the results of a cross sectional study of over 95% of the workers employed in these plants in 1968, the authors indicated that there was a comparable smoking prevalence between the two plants (Weill et al., 1973, 1975). In addition, they reported that the smoking rates calculated for the two plants were only slightly less than the estimate for all United States men in 1969.

Follow-up continued until 1982 or to age 80, whichever was reached first, and over 96% of the population was traced. Of the deceased ($n = 2143$), death certificates were obtained for 2014 (94%). Deaths for which certificates were not acquired were assigned to categories of causes of death in the same proportion as those with certificates. The mortality experience of this cohort was compared to Louisiana rates obtained from the State of Louisiana Department of Health and from Marsh and Preininger (1980). The authors noted that age-adjusted lung cancer rates in Louisiana for the period of 1960–1979 were 29% higher for Caucasians and 9% higher for African Americans than rates reported for the country as a whole (Riggan et al., 1983).

Beginning in 1952, air sampling data were collected in both plants by industry, insurance companies, and government personnel using the midget impinger. A total of 100 samples were taken in plant 1 prior to 1970 and 1664 in plant 2. Membrane filter sampling began in 1969. The estimated exposure concentrations for the years prior to 1952 were based on both air sampling data and anecdotal information from company management and long-term employees. Individual exposures were estimated using the midget impinger data; the authors do not provide details, but this was presumably done by job classification and duration. The relatively recent exposures (up to 10–15 years previously) were not included in calculating the cumulative exposure for each worker.

Plant 1 employees were classified into the following cumulative exposure groups: <6, 6–24, 25–49, 50–99, and ≥100 mppcf-yr. The mean cumulative exposure for each of the exposure categories was 4, 13, 35, 74, and 183 mppcf-yr, respectively. Hughes et al. (1987) did not observe a statistically significant increase in deaths due to respiratory cancer in plant 1 employee, 20 years or more after their initial employment in any exposure category. For the highest cumulative exposure group (≥100 mppcf-yr), an SMR of 1.23 was reported (a confidence interval was not provided). Plant 2 employees with a lapse of 20 years or more since their initial employment were divided into two groups, one that included chrysotile-only exposed individuals, and the other with both chrysotile and crocidolite exposure. Plant 2 employees with chrysotile-only exposure were divided into the following cumulative exposure groups: <3, 3–5, 6–24, 25–49, and ≥50 mppcf-yr. No increase in death from lung cancer was reported in any of the exposure groups. An SMR of 1.56 (a confidence interval was not provided) was reported for the highest exposure category (≥50 mppcf-yr); the authors indicated that this was not significant at the 0.05 level. It is also important to note that the authors indicate that as a whole, there is an observed excess in lung cancer in Plant 2 workers. However, based on the results provided, this increased risk appears to be localized to employees with exposure to both crocidolite and chrysotile.

Hammad et al. (1979) developed a particle-to-fiber conversion factor based on comparative midget impinger and membrane filter samples collected in various areas of one of the plants. The authors approximated that 1.4 f/ml was roughly equivalent to 1 mppcf. We applied this conversion factor to the aforementioned exposures which yielded cumulative respiratory cancer NOAELs for plant 1 and plant 2 employees of ≥140 f/cc-yr (mean cumulative exposure for this group was 256.2 f/cc-yr) and ≥70 f/cc-yr (mean cumulative dose for this exposure group was not provided), respectively.

Nine pleural mesothelioma deaths occurred in this cohort. Seven of these deaths occurred in plant 2 workers, and six of these deaths occurred in workers who had previously been employed in the pipe production area where they had known exposure to crocidolite asbestos (Hughes et al., 1987; see Table 12, p. 169). Cumulative exposure levels for these workers were not provided, and therefore a mesothelioma NOAEL was not reported in this study.

*Sweden*

Albin and colleagues (1990, 1996) performed a cohort mortality study among Swedish cement factory workers, as well as a nested case control study of the workers with mesothelioma. The asbestos that was handled was mainly chrysotile (>95%), with smaller amounts of crocidolite or amosite. Crocidolite was used only in sheet production performed prior to 1966. The amounts used from 1953 were less than 1%, and purportedly did not

exceed 3 to 4% of the total amount of asbestos used. Amosite (maximum <18% total use) was used for a few years during the 1 950s. Extrapolating from the plant history, Berman and Crump (2003) estimated that the percentage of amphiboles used in this plant ranged from 0–6%; they reported a best estimate of 3% (see Table 7–16).

The exposed cohort consisted of all male employees registered in the company personnel records from 1907 through 1977 who were employed for at least 3 months ($n = 2898$). Follow up continued until December 31, 1986 (Albin et al., 1990). The referent cohort was comprised of 1552 men employed in five different industries in the region (fertilizer production, slaughter house, wool and polyester textile, sugar refinery, and metal industries) that were not known to have processed asbestos, and who fulfilled the same requirements as the asbestos workers. Additionally, the referents with suspected previous occupational exposure to asbestos were excluded from the analysis, resulting in a referent group comprised of 1233 subjects. Information regarding the demographics and smoking status of the exposed cohort and referent group was not provided.

Death certificates were obtained and recoded according to the International Classification of Diseases 8 (ICD-8) by the National Swedish Central Bureau of Statistics. Regional (1958–1986) and National (1958–1984) cancer registries were searched, and all available histopathological information was reviewed for cases of respiratory cancer. Mesothelioma cases were confirmed using light microscopy and immunohistochemical staining. A minimum latency of 20 years since start of employment was applied to both cohorts.

Dust measurements existed for the time period from 1956 through 1977; prior to 1969 impinger or gravimetric measurements were available, and after 1969 the membrane filter method was used. Albin et al. (1990) estimated average dust exposures for different jobs and periods using data on dust concentrations, production, and dust control measures. The estimates for the period 1947 to 1951 were used by the authors for the entire period before 1942 based on the assumption that the production process was mainly the same. The authors indicated that the actual exposure levels before 1942 may have been greatly underestimated for some tasks, but explained that workers engaged in these operations only accounted for 5–10% of the total cohort.

Individual exposures were calculated, presumably based on individual job classifications and work histories (details were not provided), for 1503 (78%) of the 1929 Swedish workers. Albin et al. (1990) developed three cumulative exposure groups, <15 f/cc-yr, 15–39 f/cc-yr, and ≥40 f/cc-yr, with a total of 17028 man-years of observation, and noted no statistically significant increase in death due to respiratory cancer (excluding mesothelioma) in any group, even those in the highest cumulative exposure group. The authors indicated that the relative risk estimate was adjusted for possible confounding by age and calendar year. A statistically significant increase in deaths ascribed to pleural mesothelioma was observed in the two highest exposure groups, 15–39 and ≥40 f/cc-yr, and the authors reported a relative risk of 21.2 (95% CI 2.5–178) and 22.8 (95% CI 2.4–212), for the groups, respectively. For the purposes of our analysis, the no-effect level identified for lung cancer was ≥40 f/cc-yr (mean cumulative exposure for this group was 67 f/cc-yr, median cumulative exposure was 88.2 f/cc-yr), and for mesothelioma was <15 f/cc-yr (mean cumulative exposure for this group was 3.1 f/cc-yr, median cumulative exposure was1.4 f/cc-yr).

*Vöcklabruck*

Neuberger and Kundi (1990) conducted a cohort study among workers of the oldest cement factory in the world, located in Vöcklabruck (upper Austria). From 1895 forward, chrysotile was the predominant fiber type used in the facility. From 1920 to 1977 crocidolite was also used in the pipe factory. Up to 33% of the asbestos used in pipe production was crocidolite, which amounted to roughly 4% of the total amount of asbestos used at the facility (Neuberger, 2006). Amosite (up to 3%) was also used in certain products from 1970 to 1986; however, according to the authors, this usage did not contribute to the overall exposure of this cohort.

The cohort included all persons employed for at least 3 years from 1950 to 1981. It was comprised of 2816 people, 82% of whom were employed before 1969, when the dust conditions had yet to significantly improve. Smoking information was obtained via interview for cohort members who had left the plant after 1950 and were still alive in 1982. Lung cancer deaths were initially determined by review of death certificates; a further analysis of the best available information was performed using results gathered from hospital, pathological institute and social insurance records. Information on mean age at initial employment, start date, duration of employment, and mean disease latency was not provided.

Individual cumulative exposures were estimated from personal records on duration of exposure at different workplaces, estimations of dust concentrations until 1965, and dust measurements (mainly by the conimeter method until 1975, and by personal air samples and membrane filter methods thereafter). The cohort was subsequently divided into two cumulative exposure groups, ≤25 f/cc-yr and >25 f/cc-yr.

The authors observed an overall increased risk for lung cancer (SMR = 1.7), when compared to the age- and sex-specific mortality rate for lung cancer in upper Austria. However, after controlling for smoking, the authors reported no increased risk in mortality from lung cancer in either of the two cumulative exposure groups. For the cumulative asbestos exposures of ≤25 f/cc-yr, an SMR of 1.26 was calculated (95% CI 0.83–1.95); and for >25 f/cc-yr cumulative exposure an SMR of 0.96 (95% CI 0.64–1.43) was calculated. The cumulative lung cancer NOAEL for this cohort was therefore >25 f/cc-yr.

Five mesothelioma cases were reported; however, the relative risk of mesothelioma stratified by cumulative exposure was not reported, and therefore a mesothelioma NOAEL could not

be determined from the information provided. It is worth noting that in a subsequent nested case-control study (Neuberger and Kundi, 1990), the authors found that that the mesothelioma cases had significantly higher crocidolite exposure than the controls.

### Friction Products Manufacturing

*United Kingdom*

A retrospective cohort mortality study was conducted on workers in a factory producing friction materials in the United Kingdom (Berry and Newhouse, 1983; Newhouse et al., 1982; Newhouse and Sullivan, 1989). The plant, founded in 1898, manufactured a variety of friction materials, such as brake blocks, and brake and clutch linings. Chrysotile was the only fiber type used in the facility, with the exception of brief periods from 1929 to 1933 and from 1939 to 1944 during which crocidolite was used to manufacture railway blocks. During both of these time periods, the blocks were made in a well-defined area of one of the workshops, and only a minority of the workforce was exposed. Small amounts of crocidolite were also used sporadically in an experimental workshop. Berman and Crump (2003) estimated that 0.5% (range 0–2) of the total asbestos used in this plant was amphiboles (see Table 7–16).

The initial study group consisted of individuals whose employment began in 1941 through 1979 who were identified by factory personnel files, resulting in 13460 subjects, of whom about two-thirds were men. Over two-thirds of the population began employment by 1960, and less than 6% of the cohort began work prior to 1941 (Berry and Newhouse, 1983). The follow up period was later extended to 1986 (Newhouse and Sullivan, 1989). The duration of employment ranged from less than 1 year to over 30 years. Approximately one-third of the men and women left before completing one year of service, but 27% of the men and 14% of the women remained at the factory for 10 years or more. Overall cohort mortality information was obtained from death certificates from the National Health Service Central Registrar and the Department of Health and Social Security, and was restricted to the period following 10 years after first employment in the factory.

Beginning in 1967, regular measurements of airborne dust levels were taken throughout the factory using the membrane filter method; personal sampling began in 1968. Airborne fiber concentrations in the earlier years were approximated by the authors by simulating earlier working conditions, using detailed knowledge of when processes were changed and exhaust ventilation introduced. Based on knowledge of the historical industrial hygiene practices and for purposes of quantifying asbestos concentrations, the authors divided the factory into four exposure periods: (1) pre-1931: before the Asbestos Regulations and when all operations were carried out in an open-plan area; (2) 1932–1950: when exhaust ventilation was implemented in most machining operations and there was increased separation between the stages of production; (3) 1951–1969: gradual improvement in air quality and application of exhaust ventilation to machines not included in the Asbestos Regulations; and (4) 1970–1979: following the introduction of the 2 f/ml threshold limit value (TLV) (Berry and Newhouse, 1983). In general, fiber concentrations in period 1 exceeded 20 f/ml. In period 2, most operations had exposures of under 5 f/ml with the exceptions of grinding (5–10 f/ml) and fiber preparation (10–20 f/ml). In period 3 all operations were below 5 f/ml, and in period 4 all exposures were generally in compliance with the TLV. The simulation studies employed the basic materials and original equipment operated in the appropriate work setting for the given time periods. Personal samples were collected in the workers' breathing zones for periods of 4 to 5 hours in order to calculate 8-h TWAs, and static area samplers were mounted nearby at head height to provide information on general atmospheric concentrations of asbestos fibers.

A case-control study nested within this cohort evaluated the association between asbestos exposure and lung cancer mortality (Berry and Newhouse, 1983). This study was restricted to men who entered the workforce between 1941 and 1960 and who had survived for at least 10 years after starting work at this factory. Although follow-up on this population continued until 1986 (Newhouse and Sullivan, 1989), risk estimates stratified by cumulative exposure were only available for a follow-up to 1979 (Berry and Newhouse, 1983). The mean year of initiation of employment for the lung cancer cases was mid-1949, and the mean year of death due to lung cancer among the cases was the end of 1970. Three controls were selected for each case, matched on (1) the year they started working in factory, (2) year of birth, and (3) survival up to the time of death of the case. The study population was divided into 4 exposure categories: 0–9, 10–49, 50–99, and 100–356 f/cc-yr. The authors observed no increased risk of lung cancer in any of the cumulative exposure groups with the exposure level-specific odds ratios of 1.0, 0.79, 0.86, and 0.88. Individual confidence intervals for the risk estimates were not presented by the authors. The NOAEL for lung cancer observed in this cohort was therefore taken to be 100–356 f/cc-yr.

Ten deaths due to mesothelioma were observed. The authors did not estimate the cumulative exposures for these cases, nor did they calculate a risk estimate for mesothelioma, and therefore a mesothelioma NOAEL could not be identified for this study group. It is worth noting that in a subsequent internal case-control study (Berry and Newhouse, 1983), the authors reported that 80% of the mesothelioma deaths occurred in people who worked on the crocidolite contract, compared with only 8% of the controls.

*Connecticut*

McDonald and colleagues (1984) studied a Connecticut friction products and packing manufacturing facility as part of their investigation into the effects of fiber type on asbestos-related disease. Until 1957, chrysotile, mainly from Canada, was the

only mineral type used in the plant; some anthophyllite was subsequently added in making paper discs and bands. In addition, between 1964 and 1972 approximately 400 lb crocidolite was handled experimentally in the company laboratory. Based on the data provided in McDonald et al. (1984), it was estimated that only 0.5% (range 0–2) of total asbestos used in this factory was amphiboles (see Table 7–16).

McDonald et al. (1984) analyzed the mortality of men who had worked for one calendar month or more before January 1, 1959 and who had a Social Security number and name matching the data in the U.S. Social Security Administration (SSA) records. Of the 3513 men who were traced until the end of the study period (December 31, 1977), 1267 (36%) had died, and death certificates were obtained for 1228 (96.9%). Cohort-specific information such as mean employment duration and age was not provided.

Information on exposure was available from surveys conducted by Metropolitan Life Insurance Company in 1930, 1935, 1936, and 1939. There was little additional information on exposure conditions until the 1970s. Estimates of exposure by process and period were made by an industrial hygienist who reviewed information related to process and jobs in the plant, as well as records on environmental conditions and dust control measures. Before 1970, measurements were made using the impinger method; in later years membrane filters were used. Individual work history records were obtained; these indicated the department in which the employee worked, but seldom specified a job description or the processes involved. Due to varying dust levels generated by tasks within a single department, all processes were taken into account when estimating airborne asbestos concentrations. The authors indicated that this strategy could lead to overestimation of exposure for many of the employees in these departments, and underestimation for a few. A conversion factor was not provided by the authors to convert mppcf-yr to f/cc-yr. For cement products manufacturing a factor of 1.4 (f/cc:mppcf) has been recommended (Hammad et al., 1979), and the U.S. EPA used a factor of 1.5 for asbestos products manufacturing (Nicholson, 1986); a conversion factor for friction products manufacturing was not found. For the purposes of our analysis, and for the sake of conservatism, we have used a factor of 1.4 (f/cc:mppcf) to convert dust measurements into fiber levels.

The authors classified male deaths 20 years after first employment into 5 cumulative exposure groups, the lowest being $<10$ mppcf-yr, and the highest being $\geq 80$ mppcf-yr. The authors observed a significant increase in respiratory cancer in the lowest exposure group (SMR = 1.67) in comparison to age-, sex-, race-, and year-specific death rates in Connecticut (CI 1.26–2.19;* not reported by authors). However, no statistically significant increase was observed in the four higher exposure categories. Likewise, there was an inverse relationship between duration of service and the calculated SMRs for respiratory cancer for exposures $<10$ mppcf-yr. The authors suggested that the lack of an apparent dose-response relationship could be explained by the selective employment of men in relatively poor health or with unhealthy habits, such as heavy smoking, in low-exposure jobs where they often remained for a fairly short time. They also considered the likelihood that the short-term employees had worked in other hazardous industries prior to or after their employment at the friction products plant. Following a further review of the occupational histories of low-exposure employees, the authors indicated that the increase in respiratory cancer was most likely the result of some form of selection bias. Due to the lack of increased risk in the four higher exposure groups, we have assumed that the highest exposure group represents the NOAEL for this cohort. No deaths due to mesothelioma were reported in any of the exposure groups in this study. Using the previously stated conversion factor of 1.4 (f/cc:mppcf), the NOAEL for this group was taken to be $\geq 112$ f/cc-yr for respiratory cancer and mesothelioma.

*The SMR in McDonald 1984 was reported as a percentage (167.4). For consistency with the other studies, we have converted it to a proportion.

### Asbestos Textile Manufacturing

*Pennsylvania*

A cohort mortality and subsequent case-referent study were conducted among workers at a Pennsylvania plant producing a variety of textiles and friction products (McDonald et al., 1983a). Chrysotile obtained primarily from Canada and Rhodesia was the predominant type of asbestos used at this facility, with between 3000 and 6000 tons processed annually. From 1924 on, both crocidolite and amosite were incorporated into insulation blankets for turbines, as well as equipment for chemical factories and paper mills. Based on the data provided in McDonald et al. (1983), it was estimated that the percentage of amphiboles used in this facility was 8% (see Table 7–16).

The cohort consisted of men ($n = 4137$) and women ($n = 998$) employed in the factory before January 1, 1959, for at least 1 month with a verified Social Security number. Survival status was determined through local inquiries and from information provided by the SSA as of December 31, 1977. Tracing was completed for 97% and 94% of the men and women, respectively, and of those traced, 35% of the men and 18% of the women had died. Death certificates were obtained for 97% ($n = 1354$) of the men who had died and 97% ($n = 165$) of the women. The authors chose to exclude females from further analysis, and noted that relevant information would be reported separately. The final study group consisted of 1392 male deaths. The process for determining the cause of death ($n = 38$) in those without death certificates was not disclosed. The average age at the start of employment was 28.92 and the average duration of service was 9.18 years. Roughly 31% of the study population was employed for less than 1 year, and slightly more than 25% of the population remained at the factory for 20 years or more. The authors indicated that of the men born between 1910 and 1919 included in this cohort, 75% smoked or had smoked cigarettes in their lifetime.

Air samples were taken in the factory by the Metropolitan Life Insurance Company from 1930 to 1939, by the U.S. Public Health Service in 1967 and 1970, and collected routinely by the company from 1956 onward. Until 1967, measurements were made by the midget impinger method. An industrial hygienist (A. J. Woolley) estimated dust levels for each department over time. The process used for estimating individual cumulative exposure was not discussed.

The authors classified male deaths 20 years after first employment into 5 cumulative exposure groups, the lowest being $<10$ mppcf-yr, and the highest being $\geq 80$ mppcf-yr. The authors observed a significant increase in respiratory cancer in only the highest exposure group (SMR = 4.16) in comparison to death rates in Pennsylvania prevalent at that time.* The authors did not provide a factor to convert particle counts to fiber counts; however, the ratios recommended for textile manufacturing have ranged from 1 mppcf = 3 f/cc to 1 mppcf = 6 f/cc (Ayer et al., 1965; Dement et al., 1982). In this analysis the conversion factor derived in the South Carolina textiles studies (1 mppcf-yr = 3 f/cc-yr) was applied to the results of McDonald et al. (1983). This factor was selected because (1) it is in the middle of the range of recommended factors in the published literature, and (2) there were some similarities in operations at the two plants. The respiratory cancer NOAEL for this cohort was therefore between 40 and 80 mppcf-yr (120–240 f/cc-yr).

Ten deaths due to pleural mesothelioma were identified from death certificates. Although specific exposure-response information was not provided for these cases (and hence, a mesothelioma NOAEL could not be identified), the authors indicated that they observed "the special risk of mesothelioma associated with exposure to even quite small proportions of amphibole," in this case predominantly amosite (McDonald et al., 1983a, p. 373).

*Rochdale*

Employees of a Rochdale asbestos textile factory were traced until June 30, 1983 (Peto et al., 1985). Chrysotile had always been the predominant fiber type used in the factory, although from 1932 to 1968 roughly 10322 tons of crocidolite was purchased, which accounted for approximately 2.6% of the total amount of asbestos purchased over that time period, and for roughly 5% of the amount used in the manufacture of textiles. Berman and Crump (2003) estimated that 5% (range 2–15%) of the asbestos processed was amphibole (see Table 7–16).

The cohort consisted of the following two subcohorts: (1) men first employed in 1933 or later, who had completed 5 years of total employment by the end of 1974, and who had ever worked in scheduled areas or in maintenance, and (2) a 1 in 10 sample of all male employees first employed between January 1, 1933, and December 31, 1974, irrespective of where or how long they had worked. Workers with Asian surnames (due to difficulty in tracing) and those with known previous occupational exposures were excluded, resulting in a principal cohort of 3211 men. Cohort demographics and ranges of occupational tenure were not specified. The company was aware of most suspected mesothelioma cases; however, a few additional cases were identified from the national mesothelioma register and from a review of death certificates. All obtainable diagnostic information was also reviewed by Sir Richard Doll, and each case was labeled as "established" on the basis of postmortem evidence with or without histological confirmation, "presumptive" on the basis of death certificate information alone, "uncertain" due to conflicting medical evidence, or "incorrect." Lung cancer deaths were obtained from company records. The mortality experience of the principal cohort was compared to the national mortality rates for selected causes in the United Kingdom, as well as those observed in Rochdale County Borough from 1969 to 1973.

Area dust measurements in particles per milliliter were taken in 23 locations with a thermal precipitator between 1951 and 1961 and later with static membrane filters. These exposure estimates were reevaluated and adjusted by industrial hygienists to account for advances in technology and knowledge regarding the conversion from particles/ml to fibers/cc; the authors suggested that 35 p/ml was equivalent to 1 f/cc. Dust levels prior to 1951 were assumed to be equivalent to those measured from 1951 to 1955 for departments in which no major changes had been made. For areas that underwent significant industrial hygiene improvements, higher values were assigned for the pre-1951 period. Cumulative exposure estimates were calculated for each of the subjects, allowing for a 5-year lag time between exposure to asbestos and any observed increase in mortality. Jobs were assigned average dust measurements for each 5-year period from 1951 onward. Although details are not provided, it appears that individual exposures were calculated based on the duration of time spent performing each job.

The authors classified men with 20 or more years since their first employment into 6 cumulative exposure groups that ranged from $<1000$ p/ml-yr ($<28.6$ f/cc-yr) to $\geq 5000$ p/ml-yr ($\geq 142.9$ f/cc-yr). The cohort was further segregated by year of first exposure (1933 and 1951). No increase in lung cancer risk was reported at exposures up to 3000–3999 p/ml-yr in either subcohort (risks were elevated at higher exposures for both subcohorts). Accordingly, for the purposes of this analysis, the NOAEL for lung cancer risk is 3000–3999 p/ml-yr (85.7–114.3 f/cc-yr).

Fourteen men in the principal (post-1933) cohort died of mesothelioma. The authors did not develop exposure-related estimates of mesothelioma risk, and therefore a mesothelioma NOAEL could not be identified from this study.

*South Carolina*

A retrospective cohort mortality study was conducted among workers at a South Carolina textiles plant (Brown, et al., 1994; Dement and Brown, 1994, 1998; Dement et al., 1982, 1983a,

*The SMR in McDonald 1983 was reported as a percentage (416.1). For consistency with the other studies, we have converted it to a proportion.

1983b, 1994; McDonald et al., 1983b). According to company personnel, 6 to 8 million pounds of chrysotile was processed annually. Small amounts of crocidolite yarn (less than 2000 pounds) were woven into tape or made into braided packing beginning in the 1950s until approximately 1975. The authors indicated that crocidolite processing was done using wet methods, resulting in very low exposures (Dement et al., 1983a). Berman and Crump (2003) provided a best estimate percentage of amphiboles as 0.5% (range 0–2), based on data reported in Sebastien et al. (1989) (see Table 7–16).

The cohort consisted of workers employed for at least 1 month between January 1, 1940, and December 31, 1965. In several analyses the cohort was limited to white male employees (Dement et al., 1982, 1983a, 1983b; Dement and Brown, 1998); however, parallel studies expanded the population to include white male ($n = 1247$) and female ($n = 1229$), as well as black male workers ($n = 549$) (Brown, et al., 1994; Dement et al., 1994). A nested case-control analysis was also undertaken on the expanded cohort to eliminate possible confounding effects due to mineral oil exposure in the authors' assessment of lung cancer risk (Dement et al., 1994).

Participants were initially followed until December 31, 1975, and subsequent tracing extended through December 31, 1990. Mortality information was based on SSA files for deaths occurring from 1976 to 1978, and records kept by the National Death Index (NDI) from 1979 to 1990. If a worker was known to be alive in 1975, he/she was assumed to be alive as of 1990 if his/her information could not be located in either the SSA or NDI files. The average number of years of observation was 35 for white females and black males, and 43 for white males. By December 31, 1990, 41.7% of the expanded cohort was known to be deceased; this was true for 48.7%, 29.5%, and 53.0% of white male, white female, and black male participants, respectively. For the overall cohort mortality study, age-, race-, sex-, and calendar-time specific death rates for the U.S. population were used to calculate expected deaths and SMRs.

The demographic variables for the entire cohort were not available; however, this information was provided for participants in the nested case-control analysis. Since age at death was the incidence density matching variable, cases and controls were nearly identical for this parameter. In addition, the authors reported that the mean year of birth (range cases: 1913–1917; controls: 1909–1911), mean employment initiation dates (range cases: 1941–1944; controls: 1941–1942), and mean time since first employed (range cases: 34.1–38.7; controls: 3 1.1–35.1) were similar for cases and controls (Dement et al., 1994, see Table 7). The mean and median exposure levels experienced by the cases and controls varied according to race and sex, and were thought to reflect the difference in job assignment patterns. Among lung cancer cases, the reported mean exposure level among black males was the highest (12.0 f/cc), followed by white males (5.5 f/cc) and white females (4.9 f/cc). A similar trend was observed for the controls. Mean cumulative exposures for black males, white males, and white females were 38400, 24500, and 13200 f/cc-day, respectively. Cases also experienced higher mean cumulative asbestos exposures than controls; mean cumulative exposures for black male, white male, and white female cases were 16400, 14600, and 11900 f/cc-day, respectively.

Linear statistical models were used for reconstructing historic exposure levels, taking into account textile processes, dust control measures, and job assignments, based on data from 5952 environmental samples that were collected from 1930 to 1975. Prior to 1965, all samples were taken using the impinger method, from 1965 to 1971 both impinger and membrane filter samples were collected, and from 1971 on, only the membrane filter method was used. Based on 120 side-by-side particle and fiber counts, a f/cc to mppcf ratio of 2.9 (95% CI 2.4–3.5) for all jobs except fiber preparation was derived (Dement, 1980). For fiber preparation, a conversion factor of 7.8 was calculated (95% CI 4.7–9.1). Unit conversions were previously made using a factor of 3 for all operations except fiber preparation, for which a factor of 8 was employed* (Dement et al., 1983a). Cumulative exposure estimates were made for each worker based on these estimated exposure levels in conjunction with detailed work histories. Notably, the cumulative exposures reported in this cohort were on the order of 10 to 10000 times lower than in any of the previously described studies.

The authors classified members of the cohort into exposure groups ranging from <500 to >100000 f/cc-day. With regards to the total cohort, lung cancer mortality, incorporating a 15 year latency period but not controlling for smoking, was significantly increased in the 1000–2500 f/cc-day (2.7–6.8 f/cc-yr) exposure group (SMR = 1.95, $p < .01$) and all higher exposure groups (Dement and Brown, 1994; Dement et al., 1994). There was no increase observed at exposures of 500–1000 f/cc-day (1.4–2.7 f/cc-yr) and lower. When examining the relationship between cumulative exposure and lung cancer mortality by race and sex, it is apparent that the white male and female populations were mostly responsible for the overall increased cohort risk estimates. White males showed a statistically significant increase in lung cancer at 1000–2500 f/cc-day (2.7–6.8 f/cc-yr)† and white females had increased deaths due to lung cancer in the lowest exposure group (<1000 f/cc-day; <2.7 f/cc-yr).‡ This increase was not observed for white females in the second

*It is important to note that in analyzing the same exposure data McDonald et al. (1983) reported a particle to fiber ratio that ranged from 1.3 to 10, with an average of roughly 6 f/cc per mppcf.

†A statistically significant increase in lung cancer is observed for white males in the 1000–2500 f/cc-day exposure group in Dement et al. (1994), Brown et al. (1994) and Dement and Brown (1998). However, the SMRs reported for lung cancer in this exposure group are inconsistent and are reported as 2.59 ($p < 0.01$) in Dement et al. (1994) and 2.42 ($p < .01$) in Brown et al. (1994) and Dement and Brown (1998), although the cohort composition and follow up periods are identical.

‡This increase for white females in the lowest exposure group was not reported in Brown et al. 1994, although the cohort and the duration of follow up appear to be identical.

lowest exposure group (1000–2500 f/cc-day, 2.7–6.8 f/cc-yr), but was present in all of the exposure groups at and above 2500–10000 f/cc-day (6.8–27.4 f/cc-yr). The authors indicated that the inconsistent exposure-response relationship among the females may be the result of the unequal distribution of those lost to follow up (Dement et al., 1994, p. 440). Further, among the 280 females lost to follow-up, 36% worked less than 3 months, 18% worked 3 to 6 months, and 17% worked 6 months to 1 year. The authors also reported that if it was assumed that all females lost to follow-up were alive at the end of the study, the dose-response would be altered for the white females, lowering the risks in the lowest exposure group, and resulting in a statistically significant increased risk due to lung cancer in only those groups with exposures above 2500 f/cc-day. Lastly, black males showed a statistically significant increase in lung cancer in only the highest cumulative exposure group (>40000 f/cc-day, >109.5 f/cc-yr). For the purposes of this analysis, the NOAEL for lung cancer for the expanded cohort is 500–1000 f/cc-day (1.4–2.7 f/cc-yr)(Brown et al., 1994).

Two deaths were attributed to mesothelioma, both of which had a latency of >30 years. Information on cumulative exposure was not provided; thus a NOAEL for mesothelioma could not be identified for this cohort.

## Asbestos Mining and Milling

*Balangero*

A cohort mortality study was conducted on miners in Balangero (northern Italy) (Piolatto et al., 1990; Rubino et al., 1979). Examination of the chrysotile from the mine did not detect measurable concentrations of amphiboles. However, a fibrous silicate (balangeroite) accounted for 0.2–0.5% of the total mass of the samples. A series of recent publications has indicated that based on its chemical composition, form, and durability, balangeroite is most similar to crocidolite (Gazzano et al., 2005, Groppo et al., 2005, Turci et al., 2005). A best estimate of the fraction of asbestos that consisted of amphiboles was 0.3% (range 0.1–0.5) (Berman and Crump, 2003; see Table 7–16).

The cohort consisted of males who had worked for at least 1 year at the factory between 1946 and 1975, and was later expanded to include employment through 1987 ($n = 1058$). Follow-up began on January 1, 1946, and ended on December 31, 1975, in the initial study, and was subsequently extended through December 31, 1987. Cohort-specific demographics were not provided. Vital statuses following termination of employment were ascertained through population registers, and death certificates were obtained from municipal registration offices.

Cumulative exposures were estimated from environmental measurements carried out from 1969 onward, and from simulated working conditions for earlier periods. The factory archives were examined for information on daily production, the equipment used, the nature of the job, and the historical numbers of hours worked per day. Additionally, four workers with continuous employment since 1935 helped to reconstruct the appropriate conditions, after which fiber counts were carried out by membrane filter collection and phase-contrast microscopy (Rubino et al., 1979).

Mortality from lung cancer and mesothelioma was reported for the following cumulative exposure groups: <100, 100–400, and >400 f/cc-yr. Lung cancer mortality was compared to age- and calendar-year-specific rates for Italian men. Statistically nonsignificant SMRs of 0.8, 1.3, and 1.1 were reported for lung cancer mortality for the three groups, respectively; confidence intervals were not provided.

No mesothelioma deaths were observed in the lowest exposure category, and one was noted in each of the higher categories. The expected number of deaths due to mesothelioma in the 100–400 and >400 f/cc-yr exposure groups was 0.1, yielding nonsignificant SMRs of 10.0 for both groups (95% CI 0.25–55.7). Both mesothelioma deaths occurred in individuals for whom at least 20 years had elapsed since their first asbestos exposure. For the purposes of this analysis the NOAEL was assumed to be >400 f/cc-yr for lung cancer and mesothelioma.

*Quebec*

Multiple analyses have been conducted on a cohort of Quebec chrysotile miners and millers (Liddell and Armstrong, 2002; McDonald et al., 1993; Liddell et al., 1977, 1997, 1998; McDonald et al., 1971, 1973, 1997, 1980). Males born between 1891 and 1920 who were employed in the Quebec chrysotile-producing industry for at least 1 month comprised the study population. Follow-up began for each individual after 20 years from first employment; 9780 men were traced to 1992 (Liddell et al., 1997). Death certificates were obtained for 98% of the cohort, and according to the authors, "adequate information was collected on most of the rest" (p. 16). For mesothelioma deaths, a "best diagnosis" was made after all available clinical, biopsy, and necropsy records were analyzed.

Members of the study population were described according to the location at which they were first employed; additional cohort specific information was not provided (e.g., mean start date and mean duration of employment). Although nine locations were identified, companies 5–9 were excluded from the analysis, leaving 9244 men in the cohort. Company 1 ($n = 4195$) was the mine and mill in the town of Asbestos. Company 2 ($n = 758$) was a factory in the town of Asbestos that in addition to processing chrysotile had also processed some crocidolite and amosite. The amount of amphiboles used at this facility was not included in any of the studies on this cohort. However, it appears that crocidolite was only used for a short duration in the 1940s (McDonald et al., 1973). The authors mention that some employees moved between the Asbestos mine and mill (company 1) and the Asbestos factory (company 2). Company 3 ($n = 4032$) was a large mining and milling complex (13 mines) near Thetford Mines, and company 4 ($n = 259$) consisted of a number of smaller mines and mills in the vicinity of Thetford. Based upon an extrapolation from the air data in Sebastien et al.

(1986), Berman and Crump (2003) reported a "best estimate" fraction of amphiboles of 1% (range 0–4) for companies 1, 3, and 4; an estimate was not provided for company 2 (see Table 7–16).

Estimates of dust concentrations at companies 1, 2, 3, and 4 have been made by year for each of the more than 5000 job classifications up to November 1966 by Gibbs and Lachance (1972) (McDonald et al., 1993). These estimates were based on roughly 4500 midget impinger dust counts from annual surveys conducted from 1948 to 1966. Estimates of the past and present dust conditions were made after interviews with employees of long service in collaboration with superintendents or others with special knowledge of past conditions. These estimates were later adjusted by Liddell et al. (1997, 1998) to account for new information on hours worked per week. The authors assumed that the dust level for 1967 was equal to that of 1966, and for each subsequent year calculated the annual dust concentration as proportion of that level in accordance with the average trend of fiber concentration for each worker's specific mine or mill. An average conversion factor of 3.14 (f/cc:mppcf) was calculated from side-by-side midget impinger and optical microscopy measurements (McDonald and McDonald, 1980). A subject's exposure for a particular year was calculated as the product of (the fraction of the year worked in a specific job) multiplied by (the dust level for the year for that job) times (an adjustment for the length of the working week).

Exposure accumulated to the age of 55 was determined for the entire cohort (companies 1, 2, 3, and 4), and each subject was subsequently grouped into one of the 10 following categories: <3, 3–<10, 10–<30, 30–<60, 60–<100, 100–<200, 200–<300, 300–<400, 400–<1000, and ≥1000 mppcf-yr. A statistically significant increase in deaths (after age 55) due to cancer of the trachea, bronchus, and lung was observed in the second to highest exposure group, compared to age-specific mortality rates for Quebec males (SMR = 1.84, 95%CI 1.48–2.27). The authors noted that at exposures over 300 mppcf-yr, the excess of lung cancer was 80.4 deaths, one-fifth of which was probably attributable to smoking. Consequently, Liddell and Armstrong (2002) analyzed the effects of smoking on lung cancer risks in this population. Of the initial 9780 men included in Liddell et al. (1997), 7279 met the follow-up study criteria. The SMRs for lung cancer for both nonsmokers and ex-smokers were not elevated even in the highest exposure group (≥600 mppcf-yr). Therefore, the lung cancer NOAEL for the entire cohort, when controlling for smoking, was ≥1884 f/cc-yr. The mean cumulative exposure for the entire ≥600-mppcf-yr exposure group, including nonsmokers, ex-smokers, and current smokers, was 1220.4 mppcf-yr (3832 f/cc-yr).

In a previous study with follow-up until 1988 (McDonald et al., 1993), standard mortality ratios were stratified by cumulative exposure (accumulated to age 55) for company 1, company 2, and companies 3 and 4 combined. There was no increase in lung cancer in the employees of the Asbestos Mine and Mill (company 1) even at the highest exposure category (≥300 mppcf-yr), while employees of the Thetford Mines (companies 3 and 4 combined) and the Asbestos Factory (company 2) demonstrated an elevated risk for lung cancer at this cumulative exposure level with SMRs of 1.89 and 7.00, respectively. The increased risk reported for these two subcohorts was likely the result of amphibole exposures (McDonald et al., 1997). Company 2 does not meet our selection criteria due to the failure to characterize amphibole contamination, and thus it is not considered further in this analysis. Cumulative NOAELs for lung cancer at the Asbestos Mine and Mill (company 1) and Thetford Mines (companies 3 and 4 combined), were ≥942 f/cc-yr and 314–942 f/cc-yr, respectively. Due to the difficulty in weighting one study more heavily than the other, values from both Liddell and Armstrong (2002) and McDonald et al. (1993) are included in Table 1.

Thirty-eight deaths due to mesothelioma (all companies combined) were classified into exposure groups and no clear exposure-response relationship was observed (Liddell et al., 1997). However, the authors did not provide the expected number of mesothelioma deaths, and therefore a mesothelioma NOAEL could not be derived from this study.

### Summary of Reported Chrysotile No-Effect Levels

Fourteen lung cancer NOAELs were taken from 12 published studies. The majority of the studies did not observe increased risk even at the highest chrysotile exposures; NOAELs in these studies ranged from >25 f/cc-yr (Neuberger and Kundi, 1990) to 1600–3200 f/cc-yr (Lacquet et al., 1980). NOAELs in those studies where increased lung cancer risks were reported ranged from 1.4–2.7 f/cc-yr (Brown et al., 1994) to 314–942 f/cc-yr (McDonald et al., 1993).

Four cohorts were identified in which pleural mesothelioma risk was stratified according to cumulative chrysotile exposure, two of which did not observe an increased risk at the highest cumulative exposures. NOAELs from these cohorts were >400 and ≥112 f/cc-yr for Piolatto et al. (1990) and McDonald et al. (1984), respectively. The mesothelioma NOAELs taken from Lacquet et al. (1980) and Albin et al. (1990) were 800–1599 f/cc-yr and <15 f/cc-yr, respectively.

## DISCUSSION

Identifying and cataloging the cumulative exposures at which no increased lung cancer or mesothelioma risk was reported in the studies considered here was a fairly straightforward exercise. Nonetheless, we are unaware of any other published paper that has attempted to summarize these data, even though the potential insight to be gained could be substantial. We recognize that none of the studies examined in this analysis were conducted for the strict purposes of identifying a NOAEL cumulative asbestos exposure. It is also understood that the studies cover a very broad range of industries and occupational practices, in addition to having large differences in air sampling methods and exposure estimation techniques. There are also known differences in latencies, cohort size, and percent amphibole exposure. As discussed next, where possible, we identify the limitations,

uncertainties and potential biases, and their influences on the reported NOAELs.

### Variability in Study Quality

We did not attempt to differentially weight the studies in this analysis; however, as would be expected, there is some degree of variability in the quality of the data collection and interpretation methods, particularly with respect to air sampling techniques, latency, use of an appropriate control population, cohort size, adjustment for smoking, length of follow-up, and loss during follow-up. Goodman et al. (2004) recently conducted a meta-analysis of 11 epidemiological studies concerning lung cancer and mesothelioma risk in vehicle mechanics. In that analysis, a scoring approach was used to classify the studies into three tiers based upon characteristics similar to those mentioned here. We did not employ a scoring system when evaluating the quality of the studies which met our inclusion criteria; however, it is apparent that some studies are indeed more informative than others. Perhaps, in a subsequent analysis, the technique used by Goodman et al. (2004) may be utilized to perform a similar analysis of the cohorts discussed in this study.

### Consistency of Findings

With respect to characterizing the exposure-response relationship for lung cancer or mesothelioma, some general observations can be made which apply to all the cohorts considered in this analysis: 1) all of the studies reported a NOAEL (i.e., none of the studies reported increased risk at all exposures), 2) the studies did not report an increased risk at an exposure below its respective NOAEL, and 3) all of the studies that reported a LOAEL (lowest exposure at which effects occurred) also observed significant risks at all exposures above the LOAEL. There are two exceptions to these general observations. McDonald et al. (1984) observed a significant increase in respiratory cancer in the lowest exposure group of $<14$ f/cc-yr (SMR = 1.67, 95% CI 1.26–2.18). This "effect level" of $<14$ f/cc-yr conflicts with the lung cancer NOAELs observed in the other studies (which range from $>25$ to 1600–3200 f/cc-yr) and, more importantly, no statistically significant increase in respiratory cancer was observed in the 4 higher exposure categories (up to $\geq 112$ f/cc-yr, which we took to be the NOAEL from this study) in McDonald et al. (1984). The authors suggested that this incongruity might be explained by the selective employment of men of relatively poor health or health habits (e.g., heavy smokers) into low-exposure jobs where they often remained for a fairly short time.

Similarly, Brown et al. (1994) observed significant increases in lung cancer risk at a cumulative exposure range of 2.7 to 6.8 f/cc-yr, but found no increase at a higher cumulative exposure range of 6.8 to 27.4 f/cc-yr (nonetheless, we took the NOAEL from this study to be 1.4–2.7 f/cc-yr). Therefore, like the McDonald et al. (1984) cohort, the low-exposure increased risk reported in Brown et al. (1994) (2.7–6.8 f/cc-yr) is internally inconsistent, and is also inconsistent with the lung cancer NOAELs reported in the other cohorts. It is not possible to determine from the information reported in Brown et al. (1994) whether the selective employment issues noted by McDonald et al. (1984) might also be applicable to or explain the incongruous low exposure effects that they reported. However, it is noteworthy that the methods used to estimate expected mortalities in this particular study have been previously criticized by other investigators. Expected mortalities in Brown et al. (1994) were developed from yearly mortality rates in the United States. Yet it was known at the time that the local, age-adjusted county rates were 75% higher than those reported for the United States as a whole (Mason and McKay, 1974). As noted by the U.S. EPA (Nicholson, 1986) and McDonald et al. (1983b), the increase in local rates was possibly the result of nearby shipyard employment (and perhaps by the study plant). It is unclear whether use of local lung cancer rates would yield a significant change in the findings of Brown et al. (1994). In short, the internal inconsistencies noted in McDonald et al. (1984) and Brown et al. (1994) are likely a result of study design issues, but a definitive conclusion cannot be reached from the available data.

The LOAEL for mesothelioma reported by Albin et al. (1990) (15–39 f/cc-yr) was not consistent with the findings of the other studies, which reported mesothelioma NOAELs ranging from $>400$ to 800–1599 f/cc-yr. This inconsistency may simply be a result of the inherent variability in the design and interpretation of the various cohort studies, but it may also be the result of significant amphibole exposure. Specifically, in Albin et al. (1990), the exposure-response relationship for pleural mesothelioma was evaluated in a nested case-referent study. For each of the cases ($n = 14$), 5 controls were selected based on the following factors: same nationality, alive at the time of the diagnosis of the case, and within 4 years of year of birth and first employment. The authors reported a significant relationship between cumulative exposure 40 years or more before diagnosis, and calculated a multiplicative risk of 1.9 for each f/cc-yr. Following an examination of lung tissue from seven of the mesothelioma cases, the authors found "much higher crocidolite and also higher total asbestos and tremolite counts when compared with matched nonexposure cases from the cohort" (p. 609). The authors suggested that exposure to amosite and crocidolite may have occurred in all of the mesothelioma cases. In short, it is difficult to determine whether the mesothelioma NOAEL of Albin et al. (1990) conflicts with (is lower than) the mesothelioma NOAELs from other studies due to methodological issues or uncertainties, or whether this simply reflects the inherent variability in these cohort studies.

The respiratory cancer NOAEL from Lacquet et al. (1980) also deserves mention. The authors reported no increased risk at estimated cumulative asbestos exposures of 1600–3200 f/cc-yr. Aside from the Brown et al. (1994) results discussed earlier, this is well beyond the cumulative exposures reported to be associated with the NOAELs reported in McDonald et al. (1983a) (120–240 f/cc-yr), Peto et al. (1985) (85.7–114.3 f/cc-yr), and McDonald et al. (1993) (314–942 f/cc-yr). This could very well be a result of an insufficient observation period (up to 15 years) accounting for the long latency for disease.

It is admittedly difficult to determine any degree of "consistency" when a majority of the studies reported no increased risk at any cumulative exposure. Further, most of the studies did not develop an estimate of a mean cumulative exposure that can be considered representative of the NOAEL [exceptions are Hughes et al. (1987), Albin et al. (1990), Liddell and Armstrong (2002); see Table 1]. Therefore, for example, the observation that the >25 f/cc-yr NOAEL from Neuberger and Kundi (1990) is "consistent with" the ≥140 f/cc-yr NOAEL from Hughes et al. (1987) is constrained by the fact that there is no information regarding the SMRs in the 25–140 f/cc-yr and ≥140 f/cc-yr exposure ranges from Neuberger and Kundi (1990). This also makes it difficult to identify a discrete exposure range from these studies that can be considered a NOAEL for chrysotile-related lung cancer or mesothelioma. At best, one can observe that in chrysotile-exposed cohorts where amphibole exposure was thought to be relatively low, the preponderance of the cumulative exposure NOAELs for lung cancer and mesothelioma fall in a range of approximately 25–1000 f/cc-yr and 15–500 f/cc-yr, respectively.

## Limitations, Uncertainties, and Potential Biases

*General Limitations and Uncertainties*

One of the greatest sources of uncertainty in the chrysotile studies is the potential misclassification in the cumulative exposure estimates. None of the studies provided exposure information specific to the individuals in the cohort, such as job classification, airborne asbestos concentration, duration, or cumulative exposure estimate. Therefore, it was not possible to evaluate the accuracy of the exposure estimates.

Also, in many cases the asbestos levels were derived from total dust measurements, not asbestos fiber counts (see Table 1). This uncertainty is minimized somewhat by the fact that in most studies the measured dust levels were compared to fiber levels based on side-by-side samples, which were then used to derive a plant- or operation-specific conversion factor. However, as noted by Berman and Crump (2003), the "correlation between fiber counts and total dust is sometimes poor within a plant and generally poor between plants" (p. 5.2). To date, no universal conversion has been established to compare earlier dust measurements and current fiber counts, although as described previously, several "manufacturing-specific" conversion factors have been reported in the literature. McDonald et al. (1983a, 1984) collected dust data without developing a specific conversion factor, so for these analyses we applied the conversion factor derived in the South Carolina textiles studies (1 mppcf-yr = 3 f/cc-yr) to the estimated cumulative exposures. If the lowest conversion factor reported in the literature (1.4 f/cc/mppcf) were used instead, the calculated no-effect exposure range would be reduced by a factor of 2 (60–120 f/cc-yr); if the highest conversion factor reported in the literature (6 f/cc/mppcf) were used, the calculated no-effect exposure range would increase by a factor of 2 (240–480 f/cc-yr).

We also recognize that the measured concentrations in some of these studies may not correlate well with specific work practices or even temporally with the cohort's tenure in the facility. In many cases, the air concentrations were measured years after the exposures began (Berry and Newhouse, 1983; Hughes et al., 1987; Lacquet et al., 1980; Neuberger and Kundi, 1990). In general, this measurement would likely result in an underestimate of exposure if the asbestos concentrations declined over time (e.g., due to changes in processes and/or hygiene controls and greater awareness of the asbestos hazard). In a majority of the studies, the investigators did, in fact, attempt to "correct" for possibly higher concentrations in previous years, although the accuracy of these corrections is difficult to determine. Also, many of the samples were "area" samples that may not represent exposures for specific occupations that might experience higher or lower exposures. Individual worker exposures were generally calculated using job descriptions described in factory records in conjunction with the duration of time spent in each job category. Within a company, specific jobs and processes were assigned expected asbestos concentrations over time, an approach that does not take into account variability in the ways that tasks were performed by different workers in different factories or locations within a single factory. Cumulative exposure estimates are therefore also dependent upon the accuracy of the work histories documented in the factory records.

Fiber length information was not reported in any of the studies evaluated in this paper, yet it is known that inhaled fiber size is directly related to respiratory disease potential (risk of disease generally increases with increasing fiber length). It is likely that the mining cohorts, and perhaps many of the manufacturing cohorts, were exposed to unprocessed fibers with average fiber lengths greater than those associated with handling finished end products that were made primarily from "short" (<5 $\mu$m) fiber chrysotile (e.g., most joint compound and friction products). Lack of fiber size information may therefore introduce a degree of uncertainty in the reported NOAELs, particularly if they are used to characterize exposure and risk associated with shorter fibers.

A majority of the studies utilized national or state age-adjusted mortality rates as reference values. While these rates are easily accessible and a more appropriate comparison may not be feasible, it is understood that such standard populations contain both unhealthy and healthy individuals, while working populations are generally comprised of those healthy enough to work. As a result, the calculated SMRs for total mortality are sometimes lower than expected (the so-called "healthy worker effect"). Although most of the studies considered here did use state or national mortality rates as a comparison group, in many cases a healthy worker effect was explicitly evaluated and determined to have no influence on the results (Brown et al., 1994; Lacquet et al., 1980; Liddell and Armstrong, 2002; McDonald et al., 1984; Neuberger and Kundi, 1990; Piolatto et al., 1990). Peto et al. (1985) is the only study to have concluded the likely presence of such an effect; the others did not evaluate the influence of a healthy worker effect (Berry and Newhouse, 1983; Hughes et al., 1987; McDonald et al., 1983a). Conversely, use

of national or state mortality rates for reference values can lead to an overestimate of worker risk if regional asbestos exposures contribute significantly to disease, vis-à-vis the aforementioned critique of Brown et al. (1994), Mason and McKay (1974), and Nicholson (1986).

Frequently, the diagnosis of lung cancer and/or mesothelioma in asbestos cohort studies is based primarily on death certificates. Information on the causes of death is then commonly supplemented with additional material from hospital records, pathology reports and autopsy data. Due to the regular discordance between death certificate diagnoses and diagnoses made after reviewing all relevant clinical and histopathological data, if the same diagnostic procedure is not adhered to for both the study and the reference populations (i.e., if the diagnoses were based solely on death certificate data for the control population), differential misclassification could result (Selikoff and Seidman, 1992). As described by Enterline (1976), "Supplementing death certificates with other information and, in effect, changing causes of death in the study populations (but not in the control populations) invalidates comparisons and the calculated relative risks" (p. 152). Differential classification likely did not have a large effect on the estimates reported in these cohorts because the investigators retrieved and relied upon death certificates for both the cases and noncases. An exception to this may have occurred in Albin et al. (1990) due to the rate of necropsies performed on the mesothelioma cases compared to those performed on referents. The percentage of necropsies in the referents was not reported due to the fact that they were found in regional and national cancer registry databases; however, it is very likely that necropsies were not performed for this group. Similarly, asbestos-related diseases may have been preferentially diagnosed in asbestos-exposed workers due to increased rates of necropsies as a result of worker's compensation packages.

Loss due to follow-up can also play a critical role in the uncertainty of epidemiology studies. When addressing this matter, the U.S. EPA has noted that "Generally, 10 percent to 30 percent of an observation cohort will be deceased (sometimes even less). If 10 percent of the group is untraced and most are deceased, very large errors in the determination of mortality could result, even if no person-years are attributed to the loss-to-follow-up group" (Nicholson, 1986, p. 46). The loss-to-follow-up was minimal in the studies included in this analysis. With few exceptions, the tracing was complete for upwards of 95% of the populations in each study.

Insufficient latency was a general limitation of many of the studies evaluated. The latency between first exposure and the development of disease is believed to be at least 30 years for mesothelioma, and at least 20 years for lung cancer (ATSDR, 2001; Lanphear and Buncher, 1992). Six of the 11 lung cancer cohorts evaluated in this analysis allowed for at least 20 years of latency (Table 1), while the others ranged from 10 to 15 years.*

*Three of the cohorts had unspecified latencies (Lacquet et al. 1980; Neuberger and Kundi 1990; Piolatto et al. 1990). See Table 1 for more details.

Three of the four mesothelioma cohorts had a minimum of a 20-year latency period (McDonald et al., 1984; Albin et al., 1990; Piolatto et al., 1990), while the other's was not specified (Lacquet et al., 1980). As with any epidemiology study involving a chronic disease with a long latency, it is possible that an asbestos-related disease was diagnosed in one or more individuals; however, their death occurred after the study was completed. Since the risk estimates were based on deaths within the cohort during a given follow up period, this may have resulted in an overestimation of the NOAEL. However, depending on the distribution of cases throughout the study period, insufficient latency may have underestimated the NOAEL. As seen in Tables 1 and 2, there was no clear relation between minimum disease latency and the risk for lung cancer or mesothelioma (i.e., the risk estimates reported do not increase with increasing minimum latency).

Lastly, as noted earlier, in many studies increased risks were not observed for any of the cumulative exposure groups, and thus the no effect level for these studies was defined as the highest cumulative exposure group in the study. For certain studies the highest exposure group was reported by the authors as greater than (">"), greater than or equal to ("≥"), or less than ("<") a certain cumulative exposure. In these instances, if reported by the authors, the mean or median of the NOAEL was included in Tables 1 and 2. This limits the ability to accurately quantify a NOAEL, as it could be slightly or substantially higher than the highest cumulative exposure group reported.

### *Potential Factors That Could Underestimate the Reported NOAELs*

There are several potential or known biases that could result in underestimation of a cumulative chrysotile NOAEL reported in these studies. For example, smoking is by far the leading cause of lung cancer in the world, yet smoking-adjusted risk estimates were only reported for 2 of the 11 lung cancer cohorts included in this analysis (the Austrian cement workers and the Quebec miners and millers). This lack of reporting is particularly important because the percentages of blue-collar workers and tradesmen who smoke exceed the national averages (Bang and Kim, 2001; Blair et al., 1985; Hall and Rosenman, 1991). Limited evidence suggests that smoking may in fact have contributed to elevated lung cancer rates in these studies. Specifically, Neuberger and Kundi (1990) calculated smoker-adjusted and nonadjusted SMRs for lung cancer, stratified by cumulative exposure for the Austrian cement worker cohort. The nonadjusted SMRs for lung cancer for both cumulative exposure groups (≤25 and >25 f/cc-yr) were significantly elevated; however, after adjusting for smoking, the SMRs for both groups were close to the null value (see Table 2, p. 617). In addition, when controlling for smoking, Liddell and Armstrong (2002) found no increased lung cancer risk at any dose in the Quebec millers and miners. When McDonald et al. (1993) examined subgroups of this cohort, they did not control for smoking and reported increased risk at exposures lower than the NOAEL reported in the Liddell and Armstrong (2002) study (>1884 f/cc-yr).

Exposure to amphiboles (amosite and crocidolite) is likely to have occurred to some degree in all cohorts. On average the cohorts experienced exposure to chrysotile asbestos that contained over 3% amphiboles (Berman and Crump, 2003). As noted earlier, amphiboles have been estimated to be 100–500 times as potent as chrysotile for producing mesothelioma (Hodgson and Darnton, 2000). Thus, in some cases amphibole exposures alone might have been sufficient to induce lung disease, particularly mesothelioma. Furthermore, because the previous exposure histories of the individuals are unknown, it is not possible to determine whether significant amphibole exposure may have occurred in workers prior to their employment at the facility studied (e.g., in shipyards).

The presence of other known or suspected respiratory carcinogens, such as crystalline silica, which was often used in cement production, and mineral oil, which was frequently used to suppress airborne asbestos during manufacturing processes, could also have biased the no-effect levels for lung cancer towards lower values. There is no clear consensus regarding the risks of lung cancer with respect to silica exposure in cement production workers (Jakobsson et al., 1993; McDowall, 1984; Smailyte et al., 2004; Vestbo et al., 1991), and none of the studies in our analysis examined this issue. It has been suggested that exposures to mineral oil are responsible for the elevated lung cancer risk seen in textile workers (but not in other similar chrysotile-exposed cohorts) (McDonald, 1998). However, a nested case-control study evaluating the potential effect of mineral oil exposure on lung cancer risk in the South Carolina textile workers concluded that "mineral oil exposure does not appear to be a significant confounder in the risk estimates associated with cumulative asbestos exposure (Dement et al., 1994, p. 442). Based on the results of this analysis, it appears that exposure to other carcinogens (besides possible amphibole exposure) did not contribute to the increased lung cancer risk observed in this cohort.

As noted earlier, in most cases the original investigators often attempted to account for higher airborne asbestos concentrations that likely existed prior to sampling events. However, in some instances it was not feasible to account for certain activities that were likely to generate very high concentrations. For example, according to McDonald et al. (1983b), high-exposure tasks that were performed at the South Carolina textiles facility were not considered in the exposure estimates. In particular, during the years 1937 through 1953, the facility's dust filtration system (receiving dust from ventilation inflow in the preparation and carding departments) consisted of burlap bags stretched across wooden frames. The baghouse operators would beat the burlap bags with whips on a daily basis to dislodge the accumulated dust, resulting in extremely high exposures. Tasks such as this were often carried out on weekends or as optional overtime, and were performed by anyone who volunteered. In addition, from 1945 to 1964 the mixing of fibers, which until that time was subject to varying degrees of control, was transferred to an alternate location in the plant (the mezzanine), where asbestos was moved around by men with pitch forks without any form of dust suppression. As noted by the authors, "these mezzanine and baghouse exposures, which could neither be assessed nor identified in any analysis, have not been included in any analysis" (p. 363). Clearly, failure to incorporate such high exposure tasks into the cumulative exposure estimates can lead to a significant underestimate of the NOAEL.

### *Potential Factors That Could Overestimate the Reported NOAELs*

Overestimation of worker exposure may have biased the NOAELs toward higher values in some cases. For example, such overestimation could occur if samples taken in high dust- or asbestos-producing operations were subsequently used to characterize exposures to workers involved in lower-exposure tasks. In addition, the NOAELs could have been overestimated if the workers with the highest exposure were lost to follow-up.

The primary factor that could bias the reported NOAEL in any given study toward an artificially high value would be lack of statistical power. Indeed, it is entirely possible that in many of these studies a power analysis would indicate that statistically significant risks could exist at (or below) the reported NOAEL, but that the increased risks were simply not measurable due to small cohort size, insufficient number of f/cc-years, or other factors (it is primarily for this reason that we have chosen not to refer to the NOAELs in this article as "thresholds," since that term often implies a known exposure or dose below which effects do not occur). While it is beyond the scope of this article to conduct a detailed power analysis of all of these studies, a preliminary review suggests that the confidence with which the NOAELs can truly be considered "maximum exposures at which no measurable effect was observed" varies considerably from study to study. For example, the McDonald et al. (1993) lung cancer cohorts (company 1 and companies 3 and 4) appear to be sufficiently powerful to detect an increased risk of disease. Specifically, at 95% confidence, the power is 100% for detecting a minimum SMR of 2.0 for company 1's 363,000 person-years and companies 3 and 4's 607,000 person-years at the NOAEL of 100–300 f/cc-years. However, for the Brown et al. 1994 lung cancer cohort, the power to detect a minimum SMR of 2.0 for the 21,901 person-years associated with the $<1000$ f/cc-day NOAEL is only 29.8%. The minimum SMR detectable for this study at the NOAEL with a power of 80% is 3.5.

Hence, we believe that the NOAELs summarized in this article cannot be taken as true "thresholds" unless and until a thorough statistical analysis supports such a conclusion. Along these lines, it is worth noting that Berman and Crump (2003) recently evaluated exposure-response data from several asbestos-exposed cohorts, including many of those summarized in this article. For both lung cancer and mesothelioma, they found that a nonthreshold, linear model provided an "adequate" description of the cumulative exposure–cancer response results. However, to our knowledge there has been little effort to determine whether one or more "threshold models" might also provide a

reasonable fit to the exposure-response data, and the use of such models warrants future research.

All of the studies considered here were cohort studies wherein relative risks were determined by comparing disease rates in an exposed versus nonexposed (or general) population. This study design is usually appropriate for diseases with fairly high incidence, such as lung cancer. However, a case-control study design is more appropriate for rare diseases such as mesothelioma, particularly if the size of the cohort is fairly small (Wong, 2001). Of the four cohorts in the mesothelioma analysis, three reported two or fewer cases of mesothelioma in total (Lacquet et al., 1980; McDonald et al., 1984; Piolatto et al., 1990) and one reported no cases (and therefore no risk at any dose) (McDonald et al., 1984). It is unknown whether a case-control study or an alternate study design, with a larger cohort, would have yielded a significantly different outcome. While only four of the mesothelioma studies considered in this analysis stratified risk by cumulative exposure, it is important to note that many of the other studies reported cases of mesothelioma in workers (Berry and Newhouse, 1983; Hughes et al., 1987; McDonald et al., 1983a, 1993; Neuberger and Kundi, 1990; Peto et al., 1985; Dement and Brown, 1998; Liddell et al., 1997). In most of these instances, the authors suggested that amphibole exposure was more likely responsible for the mesothelioma cases than chrysotile.

## Comparison of Chrysotile NOAELs to Vehicle Mechanic Cumulative Exposures

Finley et al. (2007) recently developed estimates of cumulative chrysotile exposures experienced by vehicle mechanics working with friction products in the 1970s. Automotive friction products (brakes and manual clutches) in this time frame typically contained chrysotile, and the numerous published industrial hygiene surveys of vehicle repair garages in the 1970s permit a fairly thorough analysis of these historical exposures. Finley et al. (2007) reported that the 95th percentile and 99th percentile cumulative exposures for vehicle mechanics in the 1970s were 2.0 and 5.7 f/cc-yr, respectively. As shown in Figure 1, with the exception of the studies of South Carolina textile workers (Brown et al., 1994), all of the reported cumulative chrysotile NOAELs reported for lung cancer were far above the 95th percentile and 99th percentile cumulative vehicle mechanic exposures. As shown in Figure 2, the cumulative chrysotile NOAELs reported for mesothelioma are all well above the 95th and 99th percentile cumulative asbestos exposure for vehicle mechanics. These results are consistent with the epidemiology literature showing that vehicle mechanics are not at an increased risk of developing asbestos-related diseases (e.g., Goodman et al. 2004).

## Recent Research on Chrysotile Exposure and Mesothelioma Risk

The question of whether or not chrysotile exposure is a risk factor for mesothelioma is a matter of ongoing debate, and there are some relatively recent published papers that have reviewed the epidemiological evidence and reached conclusions on this issue. Some researchers support the proposition that chrysotile exposures theoretically might cause mesothelioma, but that the epidemiological weight of evidence is

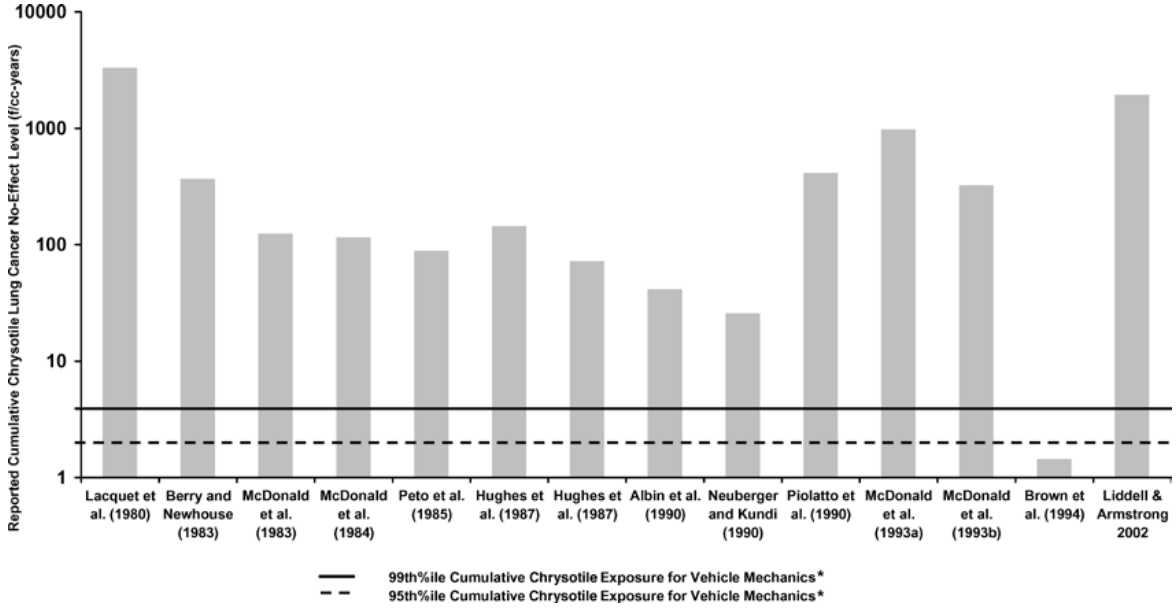



FIG. 1. Comparison of upper bound cumulative chrysotile exposures for vehicle mechanics to reported Lung Cancer No-Effect Levels.[1]

*Presented in Finley, 2007

[1]see Table 1 for the cumulative NOAEL presented in each study.

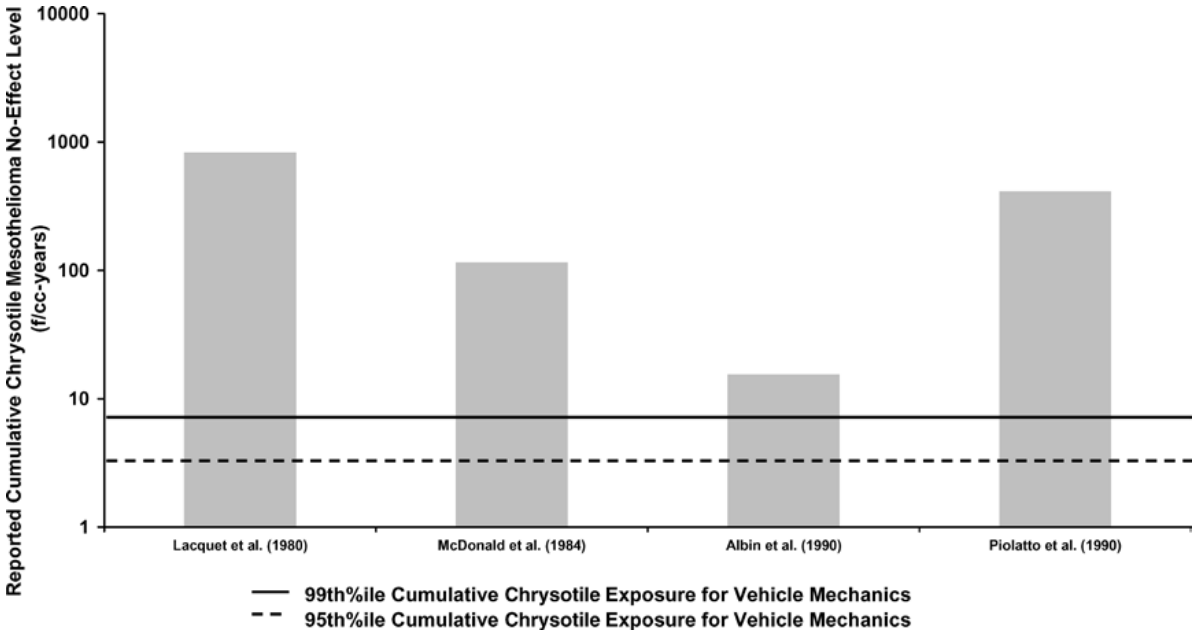



FIG. 2. Comparison of upper bound cumulative chrysotile exposures for vehicle mechanics to reported Mesothelioma No-Effect Levels.[1]

[1]see Table 2 for the cumulative NOAEL presented in each study.

lacking (Doll, 1989; McDonald and McDonald, 1991), while others believe the evidence clearly demonstrates that only amphiboles, not chrysotile, can induce mesothelioma (Ilgren and Chatfield, 1998; Yarborough, 2006; Dunnigan, 1988). For example, Yarborough (2006) recently analyzed the results of 71 asbestos-exposed cohorts studies, and concluded that "Epidemiological review of cohorts does not support the hypothesis that exposures to chrysotile fibers, uncontaminated by amphiboles, cause mesothelioma" (p. 180). It should be noted that the "chrysotile-only" cohorts considered by Yarborough suffer from the same study design limitations as those considered here (i.e., lack of case-control methodology for a relatively rare disease).

Conversely, others have concluded that the evidence is clear that chrysotile alone can cause mesothelioma. For example, in an analysis conducted by Smith and Wright (1996), 25 asbestos cohort studies were examined, and the authors stated that "Since asbestos is the major cause of mesothelioma, and because chrysotile constitutes 95% of all asbestos used worldwide, it can be concluded that chrysotile asbestos is the main cause of pleural mesothelioma in humans" (p. 252). In a more recent analysis, Li et al. (2004) reviewed the evidence from 26 different cohorts and concluded that chrysotile asbestos exposure alone can cause both mesothelioma and lung cancer. According to the authors, "Only cohort studies on cancer mortality among workers exposed to chrysotile alone were incorporated in to the meta-analysis" (Li et al., 2004, p. 460). However, at least half of the cohorts included in this analysis were known or suspected to have some degree of amphibole exposure (Dement et al., 1994; Hughes et al., 1987; McDonald et al., 1983b, 1984; Peto et al., 1985; Piolatto et al., 1990; Newhouse and Sullivan, 1989; Liddell et al., 1997; Germani et al., 1999; Raffn et al. 1996; Gardner et al., 1986; Thomas et al., 1982; Ohlson and Hogstedt, 1985).

While the exposure-response summary described in this article cannot directly address the general question "Is chrysotile a risk factor for mesothelioma under any circumstances?" due to the presence of amphiboles in most of the mesothelioma cohorts considered here, it does seem to indicate that low occupational exposures to chrysotile (e.g., exposures historically experienced by vehicle mechanics) are unlikely to cause mesothelioma. Our findings suggest that a thorough understanding of chrysotile exposures that might occur in a given setting (e.g., estimated exposures that might occur during manufacture or use of microelectronics with synthetic chrysotile fibers) will provide assistance in reaching conclusions regarding the relative safety of such activities.

## ACKNOWLEDGMENTS

The research supporting this analysis and the time needed to write the article were funded almost entirely by Chrysler Corporation, Ford Motor Company, and General Motors Corporation, which have been and are currently involved in litigation involving brake dust. These three funding sources (and their counsel) did not provide editorial comments or review

the manuscript prior to submission to the journal. Some of the authors have served and may continue to serve as expert witnesses regarding the historical exposures of mechanics to asbestos during brake repair.

## REFERENCES


Acheson, E.D., Gardner, M.J., Pippard, E.C., and Grime, L.P. (1982). Mortality of two groups of women who manufactured gas masks from chrysotile and crocidolite asbestos: A 40-year follow-up. *Br. J. Ind. Med.* **39**(4):344–348.

Agency for Toxic Substances and Disease Registry. (2001). *Toxicological profile for asbestos*. Atlanta, GA: U.S. Department of Health and Human Services (DHHS).

Albin, M., Horstmann, V., Jakobsson, K., and Welinder, H. (1996). Survival in cohorts of asbestos cement workers and controls. *Occup. Environ. Med.* **53**:87–93.

Albin, M., Jakobsson, K., Attewell, R., Johansson, L., and Welinder, H. (1990). Mortality and cancer morbidity in cohorts of asbestos cement workers and referents. *Br. J. Ind. Med.* **47**(9):602–610.

American Conference of Governmental Industrial Hygienists. (1980). *Documentation of the Threshold Limit Values*, 4th ed. Cincinnati, OH: ACGIH.

Ayer, H.E., Lynch, J.R., and Fanney, J.H. (1965). A comparison of impinger and membrane filter techniques for evaluating air samples in asbestos plants. *Ann. NY Acad. Sci.* **132**(1):274–287.

Bang, K.M., and Kim, J.H. (2001). Prevalence of cigarette smoking by occupation and industry in the United States. *Am. J. Ind. Med.* **40**(3):233–239.

Berman, D.W., and Crump, K.S. (2003). Final draft: Technical support document for a protocol to assess asbestos-related risk. U.S. Environmental Protection Agency (EPA), Office of Solid Waste and Emergency Response, Washington, D.C.

Berry, G., and Newhouse, M.L. (1983). Mortality of workers manufacturing friction materials using asbestos. *Br. J. Ind. Med.* **40**:1–7.

Blair, A., Hoar, S.K., and Walrath, J. (1985). Comparison of crude and smoking-adjusted standardized mortality ratios. *J. Occup. Med.* **27**(12):881–994.

Brown, D.P., Dement, J.M., and Okun, A. (1994). Mortality patterns among female and male chrysotile asbestos textile workers. *J. Occup. Med.* **36**(8):882–888.

Browne, K. (1986). A threshold for asbestos related lung cancer. *Br. J. Ind. Med.* **43**(8):556–558.

Churg, A. (1988), Neoplastic asbestos-induced diseases. In *Pathology of Occupational Lung Disease*. Igaku-Shoin Medical Publishers, New York. Pages 213–277.

Danielsen, T.E., Langard, S., and Andersen, A. (1996). Incidence of cancer among Norwegian boiler welders. *Occup. Environ. Med.* **53**(4):231–234.

Dement, J., and Brown, D. (1998). Cohort mortality and case-control studies of white male chrysotile asbestos textile workers. *J. Clean. Technol. Environ. Toxicol. Occup. Med.* **7**(4):413–419.

Dement, J.M., and Brown, D.P. (1994). Lung cancer mortality among asbestos textile workers: A review and update. *Ann. Occup. Hyg.* **38**(4):525–532.

Dement, J.M., Brown, D.P., and Okun, A. (1994). Follow-up study of chrysotile asbestos textile workers: Cohort mortality and case-control analyses. *Am. J. Ind. Med.* **26**(4):431–447.

Dement, J.M., Harris, R.L., Jr., Symons, M.J., and Shy, C.M. (1983a). Exposures and mortality among chrysotile asbestos workers. Part I: Exposure estimates. *Am. J. Ind. Med.* **4**(3):399–419.

Dement, J.M., Harris, R.L., Jr., Symons, M.J., and Shy, C.M. (1983b). Exposures and mortality among chrysotile asbestos workers. Part II: Mortality. *Am. J. Ind. Med.* **4**(3):421–433.

Dement, J.M., Harris, R.L., Jr., Symons, M.J., and Shy, C.M. (1982). Estimates of dose-response for respiratory cancer among chrysotile asbestos textile workers. *Ann. Occup. Hyg.* **26**(1–4):869–887.

Dement, J.M. (1980). Estimation of dose and evaluation of dose-response in a retrospective cohort mortality study of chrysotile asbestos textile workers. University of North Carolina at Chapel Hill.

Doll, R. (1989). Mineral fibres in the non-occupational environment: Concluding remarks. In *Non-occupational exposure to mineral fibres*, ed. J. Bignon et al., 511–518. IARC Scientific Publication No. 90. International Agency for Research on Cancer, Lyon, France.

Dunnigan, J. (1988). Linking chrysotile asbestos with mesothelioma. *Am. J. Ind. Med.* **14**(2):205–209.

Dunnigan, J. (1986). Threshold exposure level for chrysotile. *Can. J. Public Health.* **77**(1):41–43.

Enterline, P.E. (1976). Pitfalls in epidemiological research: An examination of the asbestos literature. *J. Occup. Med.* **18**(3):150–156.

Enterline, P.E., and Henderson, V. (1973). Type of asbestos and respiratory cancer in the asbestos industry. *Arch. Environ. Health.* **27**(5):312–317.

Finley, B.L., Richter, R.O., Mowat, F., Mlynarek, S., Paustenbach, D.J., Warmerdam, J., and Sheehan, P.J. (2007). Cumulative asbestos exposure for U.S. automobile mechanics involved in brake repair (circa 1950s–2000). *Journal of Exposure Science and Environmental Epidemiology* **17**(7):644–655.

Gardner, M.J., Winter, P.D., Pannett, B., and Powell, C.A. (1986). Follow up study of workers manufacturing chrysotile asbestos cement products. *Br. J. Ind. Med.* **43**(11):726–732.

Gazzano, E., Riganti, C., Tomatis, M., Turci, F., Bosia, A., Fubini, B., and Ghigo, D. (2005). Potential toxicity of nonregulated asbestiform minerals: Balangeroite from the western Alps. Part 3: Depletion of antioxidant defenses. *J. Toxicol Environ. Health A.* **68**(1):41–49.

Germani, D., Belli, S., Bruno, C., Grignoli, M., Nesti, M., Pirastu, R., and Comba, P. (1999). Cohort mortality study of women compensated for asbestosis in Italy. *Am. J. Ind. Med.* **36**(1):129–134.

Gibbs, G.W., and Lachance, M. (1972). Dust exposure in the chrysotile asbestos mines and mills of Quebec. *Arch. Environ. Health.* **24**(3):189–197.

Goodman, M., Teta, M.J., Hessel, P.A., Garabrant, D.H., Craven, V.A., Scrafford, C.G., and Kelsh, M.A. (2004). Mesothelioma and lung cancer among motor vehicle mechanics: A meta-analysis. *Ann. Occup. Hyg.* **48**(4):309–326.

Groppo, C., Tomatis, M., Turci, F., Gazzano, E., Ghigo, D., Compagnoni, R., and Fubini, B. (2005). Potential toxicity of nonregulated asbestiform minerals: Balangeroite from the western Alps. Part 1: Identification and characterization. *J. Toxicol. Environ. Health A.* **68**(1):1–19.

Hall, N.E., and Rosenman, K.D. (1991). Cancer by industry: Analysis of a population-based cancer registry with an emphasis on blue-collar workers. *Am. J. Ind. Med.* **19**(2):145–159.

Hammad, Y.Y., Diem, J., and Weill, H. (1979). Evaluation of dust exposure in asbestos cement manufacturing operations. *Am. Ind. Hyg. Assoc. J.* **40**(6):490–495.

Hodgson, J.T., and Darnton, A.J. (2000). The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann. Occup. Hyg.* **44**(8):565–601.

Hodgson, J.T., McElvenny, D.M., Darnton, A.J., Price, M.J., and Peto, J. (2005). The expected burden of mesothelioma mortality in Great Britain from 2002 to 2050. *Br. J. Cancer.* **92**(3):587–593.

Hughes, J.M., Weill, H., and Hammad, Y.Y. (1987). Mortality of workers employed in two asbestos cement manufacturing plants. *Br. J. Ind. Med.* **44**(3):161–174.

Ilgren, E.B., and Chatfield, E. (1998). Coalinga: A short, amphibole-free chrysotile. Part 2: Evidence for a lack of tumorigenic activity. *Indoor. Built Environ.* **7**:18–31.

Ilgren, E.B., and Browne, K. (1991). Asbestos-related mesothelioma: Evidence for a threshold in animals and humans. *Regul. Toxicol Pharmacol.* **13**(2):116–132.

Jakobsson, K., Horstmann, V., and Welinder, H. (1993). Mortality and cancer morbidity among cement workers. *Br. J. Ind. Med.* **50**(3):264–272.

Lacquet, L.M., van der Linden, L., and Lepoutre, J. (1980). Roentgenographic lung changes, asbestosis and mortality in a Belgian asbestos-cement factory. *IARC Sci. Publ.* **30**:783–793.

Lanphear, B.P., and Buncher, C.R. (1992). Latent period for malignant mesothelioma of occupational origin. *J. Occup. Med.* **34**(7):718–721.

Li, L., Sun, T.D., Zhang, X., Lai, R.N., Li, X.Y., Fan, X.J., and Morinaga, K. (2004). Cohort studies on cancer mortality among workers exposed only to chrysotile asbestos: A meta-analysis. *Biomed. Environ. Sci.* **17**(4):459–468.

Liddell, F.D., and Armstrong, B.G. (2002). The combination of effects on lung cancer of cigarette smoking and exposure in Quebec chrysotile miners and millers. *Ann. Occup.* Hyg. **46**(1):5–13.

Liddell, F.D., McDonald, A.D., and McDonald, J.C. (1998). Dust exposure and lung cancer in Quebec chrysotile miners and millers. *Ann. Occup. Hyg.* **42**(1):7–20.

Liddell, F.D., McDonald, A.D., and McDonald, J.C. (1997). The 1891–1920 birth cohort of Quebec chrysotile miners and millers: Development from 1904 and mortality to 1992. *Ann. Occup. Hyg.* **41**(1):13–36.

Liddell, F.D., McDonald, J.C., and Thomas, D. (1977). Methods of cohort analysis: Appraisal by application to asbestos mining. *J. R. Stat. Soc. A.* **140**:469–491.

Marsh, G.M., and Preininger, M. (1980). OCMAP: A user-oriented occupational cohort mortality analysis program. *Am. Statistician.* **34**:245–246.

Mason, T.J., and McKay, F.W. (1974). U.S. Cancer Mortality by County: 1950–1969. Pub No. 74-615. U.S. Department of Health Education and Welfare (DHEW) and National Cancer Institute, Washington, D.C.

McDonald, A.D., Case, B.W., Churg, A., Dufresne, A., Gibbs, G.W., Sebastien, P., and McDonald, J.C. (1997). Mesothelioma in Quebec chrysotile miners and millers: Epidemiology and aetiology. *Ann. Occup. Hyg.* **41**(6):707–719.

McDonald, A.D., Fry, J.S., Woolley, A.J., and McDonald, J.C. (1984). Dust exposure and mortality in an American chrysotile asbestos friction products plant. *Br. J. Ind. Med.* **41**(2):151–157.

McDonald, A.D., Fry, J.S., Woolley, A.J., and McDonald, J.C. (1983a). Dust exposure and mortality in an American chrysotile textile plant. *Br. J. Ind. Med.* **40**(4):361–367.

McDonald, A.D., Fry, J.S., Woolley, A.J., and McDonald, J.C. (1983b). Dust exposure and mortality in an American factory using chrysotile, amosite, and crocidolite in mainly textile manufacture. *Br. J. Ind. Med.* **40**(4):368–374.

McDonald, A.D., and McDonald, J.C. (1980). Malignant mesothelioma in North America. *Cancer.* **46**(7):1650–1656.

McDonald, J.C. (1998). Unfinished business: The asbestos textiles mystery. *Ann. Occup. Hyg.* **42**(1):3–5.

McDonald, J.C., Liddell, F.D., Dufresne, A., and McDonald, A.D. (1993). The 1891–1920 birth cohort of Quebec chrysotile miners and millers: Mortality 1976–88. *Br. J. Ind. Med.* **50**(12):1073–1081.

McDonald, J.C., and McDonald, A.D. (1991). Epidemiology of mesothelioma. In *Mineral Fibers and Health*, ed. D. Liddell and K. Miller, 147–168. CRC Press, Boca Raton, FL.

McDonald, J.C., Liddell, F.D., Gibbs, G.W., Eyssen, G.E., and McDonald, A.D. (1980). Dust exposure and mortality in chrysotile mining, 1910–75. *Br. J. Ind. Med.* **37**:11–24.

McDonald, J.C., Rossiter, C., Eyssen, G., and McDonald, A.D. (1973). Mortality in the chrysotile producing industry of Quebec: A progress report. *Proceedings of the IVth International Pneumoconiosis Conference*, Bucharest.

McDonald, J.C., McDonald, A.D., Gibbs, G., Siemiatycki, J., and Rossiter, C. (1971). Mortality in the chrysotile asbestos mines and mills of Quebec. *Arch. Environ. Health.* **22**(6):677–686.

McDowall, M.E. (1984). A mortality study of cement workers. *Br. J. Ind. Med.* **41**(2):179–182.

Meldrum, M. (1996). *Review of Fibre Toxicology*. HSE Books, HMSO, London.

Moulin, J.J., Wild, P., Haguenoer, J.M., Faucon, D., De Gaudemaris, R., Mur, J.M., Mereau, M., Gary, Y., Toamain, J.P., Birembaut, Y., Blanc, M., Debiolles, M.P., Jegaden, D., Laterriere, B., Leonard, M., Marini, F., Massardier, C., Moulin, M., Reure, M., Rigal, L., Robert, G., and Viossat, M. (1993). A mortality study among mild steel and stainless steel welders. *Br. J. Ind. Med.* **50**(3):234–243.

Neuberger, M. 2006. Personal communication, June 6.

Neuberger, M., and Kundi, M. (1990). Individual asbestos exposure: Smoking and mortality—A cohort study in the asbestos cement industry. *Br. J. Ind. Med.* **47**(9):615–620.

Newhouse, M., and Sullivan, K. (1989). A mortality study of workers manufacturing friction materials: 1941–86. *Br. J. Ind. Med.* **46**:176–179.

Newhouse, M.L., Berry, G., and Wagner, J.C. (1985). Mortality of factory workers in east London 1933–80. *Br. J. Ind. Med.* **42**(1): 4–11.

Newhouse, M., Berry, G., and Skidmore, J.W. (1982). A mortality study of workers manufacturing friction materials with chrysotile asbestos. *Ann. Occup. Hyg.* **26**(1–4):899–909.

Nicholson, W. (1986). Airborne asbestos health assessment update. U.S. Environmental Protection Agency (U.S. EPA) and Office of Health and Environmental Assessment. EPA 600/8–84/003F. June. Washington, D.C.

Ohlson, C.G., and Hogstedt, C. (1985). Lung cancer among asbestos cement workers. A Swedish cohort study and a review. *Br. J. Ind. Med.* **42**(6):397–402.

Peto, J., Doll, R., Hermon, C., Binns, W., Clayton, R., and Goffe, T. (1985). Relationship of mortality to measures of environmental asbestos pollution in an asbestos textile factory. *Ann. Occup. Hyg.* **29**(3):305–355.

Piolatto, G., Negri, E., La Vecchia, C., Pira, E., Decarli, A., and Peto, J. (1990). An update of cancer mortality among chrysotile asbestos miners in Balangero, northern Italy. *Br. J. Ind. Med.* **47**(12):810–814.

Raffn, E., Villadsen, E., Engholm, G., and Lynge, E. (1996). Lung cancer in asbestos cement workers in Denmark. *Occup. Environ. Med.* **53**(6):399–402.

Riggan, W., Van Bruggen, J., Acquavella, J., Beaubier, J., and Mason, T. (1983). *US Cancer Mortality Rates and Trends, 1950–1979*, Vol. 2. NCI/EPA Interagency Agreement on Environmental Carcinogenesis, U.S. Government Printing Office, Washington, DC.

Rubino, G.F., Piolatto, G., Newhouse, M.L., Scansetti, G., Aresini, G.A., and Murray, R. (1979). Mortality of chrysotile asbestos workers at the Balangero Mine, Northern Italy. *Br. J. Ind. Med.* **36**(3):187–194.

Sebastien, P., McDonald, J.C., McDonald, A.D., Case, B., and Harley, R. (1989). Respiratory cancer in chrysotile textile and mining industries: Exposure inferences from lung analysis. *Br. J. Ind. Med.* **46**(3):180–187.

Sebastien, P., Plourde, M., Robb, R., Ross, M., Nadon, B., and Wypruk, T. (1986). Ambient air asbestos survey in quebec mining towns. Part II: Main study, Environmental Protection Service, Environment Canada. EPS 5/AP/RQ/2E. July. Ottawa, Canada.

Seidman, H., Selikoff, I.J., and Hammond, E.C. (1979). Short-term asbestos work exposure and long-term observation. *Ann. NY. Acad. Sci.* **330**:61–89.

Selikoff, I.J., and Seidman, H. (1992). Use of death certificates in epidemiological studies, including occupational hazards: Variations in discordance of different asbestos-associated diseases on best evidence ascertainment. *Am. J. Ind. Med.* **22**(4):481–492.

Smailyte, G., Kurtinaitis, J., and Andersen, A. (2004). Mortality and cancer incidence among Lithuanian cement producing workers. *Occup. Environ. Med.* **61**(6):529–534.

Smith, A.H., and Wright, C.C. (1996). Chrysotile asbestos is the main cause of pleural mesothelioma. *Am. J. Ind. Med.* **30**(3):252–266.

Teta, M.J., and Ott, M.G. (1988). A mortality study of a research, engineering, and metal fabrication facility in western New York State. *Am. J. Epidemiol.* **127**(3):540–551.

Thomas, H.F., Benjamin, I.T., Elwood, P.C., and Sweetnam, P.M. (1982). Further follow-up study of workers from an asbestos cement factory. *Br. J. Ind. Med.* **39**(3):273–276.

Turci, F., Tomatis, M., Gazzano, E., Riganti, C., Martra, G., Bosia, A., Ghigo, D., and Fubini, B. (2005). Potential toxicity of nonregulated asbestiform minerals: Balangeroite from the western Alps. Part 2: Oxidant activity of the fibers. *J. Toxicol Environ. Health A.* **68**(1):21–39.

Van de Voorde, H., Meulepas, E., Gyselen, A., and Koppen, O. (1967). Doodsoorzaken bij de bij de arbeiders werkzaam in een asbestverwerkende nijverheid in het noorden van Brabant. *Acta. Tuberc. Pneumol. Belg.* **58**:924–942.

Vestbo, J., Knudsen, K.M., Raffn, E., Korsgaard, B., and Rasmussen, F.V. (1991). Exposure to cement dust at a Portland cement factory and the risk of cancer. *Br. J. Ind. Med.* **48**(12):803–807.

Weill, H., Hughes, J., and Waggenspack, C. (1979). Influence of dose and fiber type on respiratory malignancy risk in asbestos cement manufacturing. *Am. Rev. Respir. Dis.* **120**(2):345–354.

Weill, H., Waggenspack, C., Bailey, W., Ziskind, M., and Rossiter, C. (1973). Radiographic and physiologic patterns among workers engaged in manufacture of asbestos cement products. *J. Occup. Med.* **15**(3):248–252.

Weiss, W. (1977). Mortality of a cohort exposed to chrysotile asbestos. *J. Occup. Med.* **19**(11):737–740.

Wong, O. (2001). Malignant mesothelioma and asbestos exposure among auto mechanics: Appraisal of scientific evidence. *Regul. Toxicol Pharmacol.* **34**(2):170–177.

Yarborough, C.M. (2006). Chrysotile as a cause of mesothelioma: An assessment based on epidemiology. *Crit. Rev. Toxicol.* **36**(2):165–187.

STUDY No. 6

# EVALUATION OF THE DEPOSITION, TRANSLOCATION AND PATHOLOGICAL RESPONSE OF BRAKE DUST WITH AND WITHOUT ADDED CHRYSOTILE IN COMPARISON TO CROCIDOLITE ASBESTOS FOLLOWING SHORT-TERM INHALATION: INTERIM RESULTS

**STUDY No. 6**

# EVALUATION OF THE DEPOSITION, TRANSLOCATION AND PATHOLOGICAL RESPONSE OF BRAKE DUST WITH AND WITHOUT ADDED CHRYSOTILE IN COMPARISON TO CROCIDOLITE ASBESTOS FOLLOWING SHORT-TERM INHALATION: INTERIM RESULTS

**Toxicology and Applied Pharmacology 276 (2014) 28-46**

**David M. Bernstein, Rick Rogers, Rosalina Sepulveda, Peter Kunzendorf, Bernd Bellmann, Heinrich Ernst, James I. Phillips**

Chrysotile has been frequently used in the past in manufacturing brakes and continues to be used in brakes in many countries. This study was designed to provide an understanding of the biokinetics and potential toxicology following inhalation of brake dust following short term exposure in rats. The deposition, translocation and pathological response of brake dust derived from brake pads manufactured with chrysotile was evaluated in comparison to the amphibole, crocidolite asbestos. Rats were exposed by inhalation 6 h/day for 5 days to either brake dust obtained by sanding of brake-drums manufactured with chrysotile, a mixture of chrysotile and the brake dust or crocidolite asbestos. No significant pathological response was observed at any time point in either the brake dust or chrysotile/brake dust exposure groups. The long chrysotile fiber (>20 $\mu$m) cleared quickly with $T_{1/2}$ estimated as 30 and 33 days, respectively in the brake dust and the chrysotile/brake dust exposure groups. In contrast, the long crocidolite fibers had a $T_{1/2}$ >1000 days and initiated a rapid inflammatory response in the lung following exposure resulting in a 5-fold increase in fibrotic response within 91 days. These results provide support that brake dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung following short term inhalation.

Toxicology and Applied Pharmacology 276 (2014) 28–46

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

# Evaluation of the deposition, translocation and pathological response of brake dust with and without added chrysotile in comparison to crocidolite asbestos following short-term inhalation: Interim results

David M. Bernstein [a,*], Rick Rogers [b], Rosalina Sepulveda [b], Peter Kunzendorf [c], Bernd Bellmann [d,1], Heinrich Ernst [d], James I. Phillips [e,f]

[a] Consultant in Toxicology, 1208 Geneva, Switzerland
[b] Rogers Imaging, Needham, MA 02494, USA
[c] GSA Gesellschaft für Schadstoffanalytik mbH, D-40882 Ratingen, Germany
[d] Fraunhofer Institute for Toxicology and Experimental Medicine, D-30625 Hannover, Germany
[e] National Institute for Occupational Health, National Health Laboratory Service, South Africa
[f] Department of Biomedical Technology, Faculty of Health Sciences, University of Johannesburg, South Africa

ARTICLE INFO

*Article history:*
Received 14 November 2013
Revised 15 January 2014
Accepted 18 January 2014
Available online 28 January 2014

*Keywords:*
Brake dust
Chrysotile
Amphibole asbestos
Inhalation toxicology
Pathology
Lung/pleura

ABSTRACT

Chrysotile has been frequently used in the past in manufacturing brakes and continues to be used in brakes in many countries. This study was designed to provide an understanding of the biokinetics and potential toxicology following inhalation of brake dust following short term exposure in rats. The deposition, translocation and pathological response of brake dust derived from brake pads manufactured with chrysotile were evaluated in comparison to the amphibole, crocidolite asbestos. Rats were exposed by inhalation 6 h/day for 5 days to either brake dust obtained by sanding of brake-drums manufactured with chrysotile, a mixture of chrysotile and the brake dust or crocidolite asbestos. No significant pathological response was observed at any time point in either the brake dust or chrysotile/brake dust exposure groups. The long chrysotile fibers (>20 μm) cleared quickly with $T_{1/2}$ estimated as 30 and 33 days, respectively in the brake dust and the chrysotile/brake dust exposure groups. In contrast, the long crocidolite fibers had a $T_{1/2}$ > 1000 days and initiated a rapid inflammatory response in the lung following exposure resulting in a 5-fold increase in fibrotic response within 91 days. These results provide support that brake dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung following short term inhalation.



## Introduction

The use of braking systems for automobiles had evolved from the earliest automobiles. Initially, friction materials were used that consisted of materials like camel hair, cotton belting, elm wood and cotton based materials impregnated with different ingredients (Harper, 1998; Paustenbach et al., 2004). These initial materials, however, were limited in their ability to withstand heat and control speed. From the early 1900s chrysotile fibers were found to be an effective replacement for these earlier materials. The chrysotile fibers maintained their integrity under higher temperatures which allowed the driver to brake at increased vehicle speeds (Harper, 1998). Because of these unique characteristics, chrysotile became the material of choice for vehicle brakes.

With the use of chrysotile, researchers began to investigate the degree of exposure to the fibers experienced by mechanics servicing the brakes. Short duration activities, such as removal of brake-wear debris (e.g., brake dust) from brake assemblies (often using compressed air or a dry brush) and the machining of brake linings (often by grinding or bevelling the lining surfaces to provide a better fit with the drum) have been reported to produce occupational dust exposures (Richter et al., 2009).

While many publications have reported that brakes that have used chrysotile are not related to disease when taking into consideration con founders such as smoking and other exposures (Butnor et al., 2003; Finley et al., 2012; Laden et al., 2004; Marsh et al., 2011; Paustenbach et al., 2004); others continue to report a relationship with mesothelioma (Dodson and Hammer, 2012; Freeman and Kohles, 2012; Lemen, 2004). Although chrysotile containing brakes are not manufactured in the Unites States they can be imported, and they are still manufactured and used in many countries. The U.S. Census

* Corresponding author at: 40 chemin de la Petite-Boissière, 1208 Geneva, Switzerland. Fax: +41 22 735 1463.
*E-mail addresses:* davidb@itox.ch (D.M. Bernstein), rarogers5@yahoo.com (R. Rogers), Peter.Kunzendorf@GSA-Ratingen.de (P. Kunzendorf), Heinrich.ernst@item.fraunhofer.de (H. Ernst), jim.phillips@nioh.nhls.ac.za (J.I. Phillips).
[1] Deceased.

http://dx.doi.org/10.1016/j.taap.2014.01.016

Bureau indicated that companies in the United States imported asbestos-containing brake pads and linings from Brazil, China, Colombia, India, and Mexico in 2012 (Harmonized Tariff Schedule code 6813.20.00.10 and 6813.20.00.20, www.census.gov/foreign-trade/). In addition, the United Nations Commodity Trade Database indicates that Indonesia, Kazakhstan, Malaysia, Russia, and Ukraine also may have exported asbestos-containing brakes in 2012 (Harmonized Tariff Schedule code 681320, comtrade.un.org/db/). The potential for exposure to chrysotile containing brake dust remains.

This study was designed to evaluate the hypothesis of whether brake dust from chrysotile containing brake drums will produce a pathological response following short term exposure in rats. Brake dust has not been previously evaluated in animal studies.

The exposure design was based upon a frequently used protocol for the evaluation of fiber biopersistence and short term toxicity (EUR, 18748 EN, 1999; ILSI, 2005) which has been used for evaluating a wide variety of synthetic and natural mineral fibers. This design is based upon the evaluation of fiber lung clearance using lung digestion procedures. In addition to criteria specified in these protocols, the current study includes histopathological examination of the lungs, the evaluation of fiber localization and number in the lung and pleura using confocal microscopy, and the quantification of fibrosis (collagen) in the lung and pleura through confocal microscopy (Antonini et al., 1999). This is the first such study in which the fibrotic response following fiber inhalation has been quantified using confocal microscopy.

The interim results presented here on the lung provide a basis for evaluating the biopersistence and pulmonary response of both brake dust alone and brake dust combined with added chrysotile in comparison to crocidolite asbestos following short term exposure through 91 days post exposure. The procedures used for evaluation of the pleural space included examination of the diaphragm as a parietal pleural tissue and the *in situ* examination of the lungs and pleural space obtained from freeze-substituted tissue in deep frozen rats, however, due to the large amount of data these results will be published separately.

The brake dust used in this study was obtained from commercial brake pads that were produced using chrysotile as one of the components by sanding the surfaces of the brake pads using a commercial brake pad sanding machine with the dust collected on filters. The sanded brake dust consists largely of binders with some chrysotile. To achieve the recommended aerosol concentration referred to in the above protocol (>100 f/cm$^3$ longer than 20 μm; this length category being related to pathogenesis) two brake dust exposure groups were included, one with brake dust alone and the other with brake dust with added chrysotile. Also included in this study was a comparative positive control group using crocidolite asbestos exposed at a similar concentration of fibers longer than 20 μm. This group was unique in that the crocidolite asbestos was obtained directly from South Africa without prior selection or milling as has been performed for most previously studied samples.

## Methods

The aerosol generation/exposure, in-life and pathology phases of this study were performed by the Fraunhofer Institute for Toxicology and Experimental Medicine (Hannover, Germany) in compliance with the Principles of Good Laboratory Practice (German Chemicals Act §19a, Appendix 1, July 02, 2008, Federal Law Gazette I, No. 28, p. 1146) and the German animal protection law (Tierschutzgesetz of May 18, 2006, German Federal Law Gazette I, page 1206, 1313). The fiber counting and sizing was performed by Gesellschaft für Schadstoffanalytik mbH (Ratingen, Germany). The confocal microscopy was performed by Rogers Imaging (Needham, Massachusetts, USA).

### *Brake dust preparation*

The brake dust was produced directly from asbestos-containing friction products (automotive drum brake shoes) by the RJ LeeGroup Ltd. (Monroeville, PA, USA). The brake shoes were obtained from Davies McFarland & Carroll (Pittsburgh, PA). The shoes were designed to fit the drum brakes of mid-1960's Chevrolet Impala model cars. These shoes were labeled either "BX MN FF" or "BX MG FF" (manufactured by Bendix). Two of the shoes had never been installed in vehicles; the other shoes that were used in this project were installed and operated in vehicles for a two week period. The friction material was evaluated and found to contain approximately 30% (by area) chrysotile asbestos (analyzed in accordance with EPA 600/R-93/116). No amphibole asbestos minerals have been observed in any of the aerosol or lung samples from these brake shoes or in the added chrysotile used in this study.

The brake dust was produced by grinding the brake shoes using a commercial AMMCO arc grinder (Model 8000, S/N 24788) with a modified dust collection system. The arc grinder is a motorized sander that is swept across the surface of the brake shoe with the dust collected on an attached 8 × 10 inch quartz micro-fiber filter that was used in place of a collection bag. A Tisch high volume air sampler sampling pump (Tisch Environmental Inc., Ohio, USA) was used following the filter to provide uniform sampling suction over the course of the grinding operation. All brake dust preparation took place at the RJ LeeGroup facility in a room equipped with an Aramsco Comanche® HEPA ventilation unit (Model 55011) with a nominal flowrate of 1800 cfm (50 m$^3$/min).

The composition of the brake dust was determined quantitatively using inductively coupled plasma mass spectrometry (ICP-MS) following the German norm DIN EN ISO 17294-2 (INDIKATOR GmbH, Wuppertal, Germany). The results are presented in the Supplementary data, Tables S-1 and S-2.

### *Chrysotile*

The chrysotile fiber used in this study had the mineralogical grade of 5R04 according to the Canadian chrysotile asbestos classification (Cossette and Delvaux, 1979). The chrysotile sample was chosen based upon an evaluation of which chrysotile grade was ordered or supplied for use in brake manufacturing in a random search of 67 formulations dating from 1964 to 1986. Chrysotile grade 5R04 was used most frequently (25% of the samples) and was chosen for use in the study. All of the grade 5R04 chrysotile in these samples was supplied by Johns-Manville. The sample used in this study was obtained directly from Mine Jeffery Canada (formerly the Johns-Manville Mine).

The 5R04 sample received had some large bundles of fibers. To separate these bundles into respirable fibers without significantly reducing the fiber length, the bulk material was passed one time for 60 s through a table top rotating blade mill to break up the large bundles and then was passed once to separate the fibers through the Cyclotec Sample Mill (FOSS Tecator, Denmark) which rolls the sample against the inner circumference and then separates the fibrils through a fine mesh screen.

### *Crocidolite asbestos*

The crocidolite asbestos used previously in animal studies has been largely either the Union for International Cancer Control (UICC) or US National Institute of Environmental Health Sciences (NIEHS) prepared crocidolite. Both of these samples were ground extensively more than 30 years ago using large scale industrial mills resulting in size distribution not typical of the commercial product (Bernstein et al., 2013). In this study, a crocidolite asbestos sample from the Voorspoed mine in South Africa was obtained from the National Institute of Occupational Health — NIOH, South Africa. This mine is located in Limpopo Province which at the time when mining took place was called Transvaal Province.

The chemical compositions of chrysotile, a serpentine asbestos, and crocidolite, an amphibole asbestos, have been described previously (Shedd, 1985; Virta, 2002).

*Experimental design*

The experimental design of the study is illustrated in the flowchart in Fig. 1. All end points were analyzed for each group with the exception that lung digestion was not performed in the control group on days 1, 2 and 7 in order to limit animal use.

*Animal exposure*

Groups of laboratory rats (Groups 1, 2, 3 and 4) were exposed for 6 h per day for 5 days to:

Group 1: Filtered air (negative control Group)
Group 2: Brake dust powder mixed with chrysotile 5R04.
Group 3: Brake dust powder.
Group 4: Crocidolite asbestos.

For groups 2 and 4, the exposure concentrations were set based upon the number of fibers longer than 20 μm/cm$^3$. In group 2, the chrysotile concentration was increased over that recommended by the EC Biopersistence Protocol (Bernstein and Riego-Sintes, 1999) of 100 fibers L > 20 μm/cm$^3$ due to the tendency of chrysotile to clump (this was minimized through the use of the cyclone, see below). Group 3 was included as a comparative exposure of the brake dust particulate material (with a relatively low aerosol concentration of chrysotile fibers) using a similar gravimetric exposure concentration as the brake dust component of group 2. A negative control group 1 was exposed in a similar fashion to filtered air.

Weanling (8–10 weeks old at exposure) male Wistar rats (Crl: Wi(Han), Specific Pathogen Free from Charles River Deutschland, Sulzfeld, Germany) were used. The rats were exposed by flow-past nose-only exposure for 6 h/day for a period of 5 consecutive days.

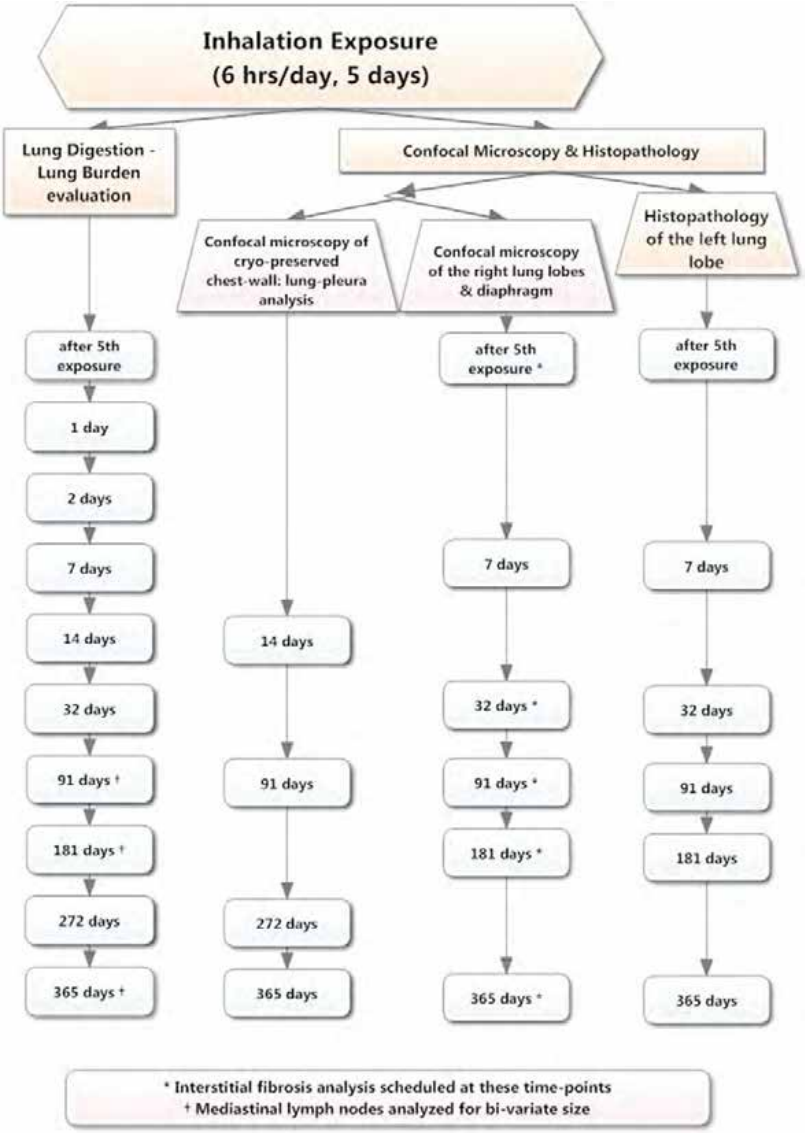



**Fig. 1.** Flowchart of the experimental design of the analyses in the study.

*Exposure system*

The fiber aerosol generation system (Model CR 3020, CR Equipments, Switzerland) was designed to loft the bulk fibers without breaking, grinding or contaminating the fibers (Bernstein et al., 1994). The animals were exposed using stainless steel flow-past nose/snout-only inhalation exposure systems with 16 animals per level. This system was derived from Cannon et al. (1983) and is different from conventional nose-only exposure systems in that fresh fiber aerosol is supplied to each animal individually and exhaled air is immediately exhausted. The exposure units were placed in separate ventilated chambers connected to the animal room to avoid cross contamination between the groups.

For group 2 (chrysotile fiber 5R04 mixed with brake dust powder), a fiber aerosol was generated from chrysotile fiber 5R04 and separately a dust aerosol from the brake dust using individual rotating brush aerosol generators (Fig. 2). The fiber aerosol generator used was followed by a 500 mL pyrex glass cyclone to assist in the elimination of any remaining fiber bundles from the aerosol. The brake dust aerosol generator was followed by a micronising jet mill to reduce the particle size to be rat respirable. Following each generator, in-line $^{63}$Ni charge neutralisers reduced the electrostatic charge from fibers and particulate material in the generated aerosols. Following the charge neutralizers, the fiber and powder aerosols were mixed through a Y-piece connection and then delivered directly into the nose-only flow-past exposure chamber.

The group 3 brake dust aerosol was generated using only the 'powder aerosol generator' as shown in bottom left of Fig. 2.

The group 4 crocidolite asbestos aerosol was generated using only the 'fiber aerosol generator' as shown in the top left of Fig. 2. A pre-study technical trial revealed stronger electrostatic properties of the crocidolite fiber aerosol which resulted in losses in the transfer tubing. To achieve a similar degree of neutralization with similar fiber transfer efficiency as with the chrysotile an electronic charge neutraliser at the brush head of the aerosol generator (WEKO Model AP230, Weitmann & Konrad GmbH, Germany) was used in addition to the $^{63}$Ni charge neutraliser.

*Exposure system monitoring*

The aerosol concentration was monitored continuously using an aerosol photometer developed by Fraunhofer ITEM. Actual concentrations were measured in the breathing zone of the animals as described below. The temperature and the relative humidity of the exposure atmosphere were monitored continuously with data on temperature, relative humidity, and air flow rate collected by the Fraunhofer ITEM animal exposure facility computer system.

Gravimetric Determination of Aerosol Concentrations: Gravimetric determinations of aerosol concentration were performed at least once daily for each group with samples collected on a Millipore® glass fiber filter (Type 13400-25-J) for approximately 4–6 h per day.

Fiber number and size distribution of Aerosol Concentrations: Aerosol samples for bivariate analysis of fiber size distribution and counting were collected onto NUCLEPORE® filters (PC membrane, 25 mm, pore size 0.8 μm – SN 110.609, Whatman Ltd.) for approximately 2 h successively during each exposure period in parallel with the gravimetric sampling. For group 1 (air control) one sample per treatment day was collected over approximately 5 h per day. These samples were analyzed for bivariate fiber size distribution and counting (# fibers/cm$^3$ aerosol) using analytical Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray analysis (EDAX).

Counting rules for the evaluation of air and lung samples by scanning electron microscopy (Fiber/Particle Analysis and Lung Digestion): Unless otherwise specified, the basis for the evaluation using the scanning electron microscope (SEM) was WHO-Reference Methods

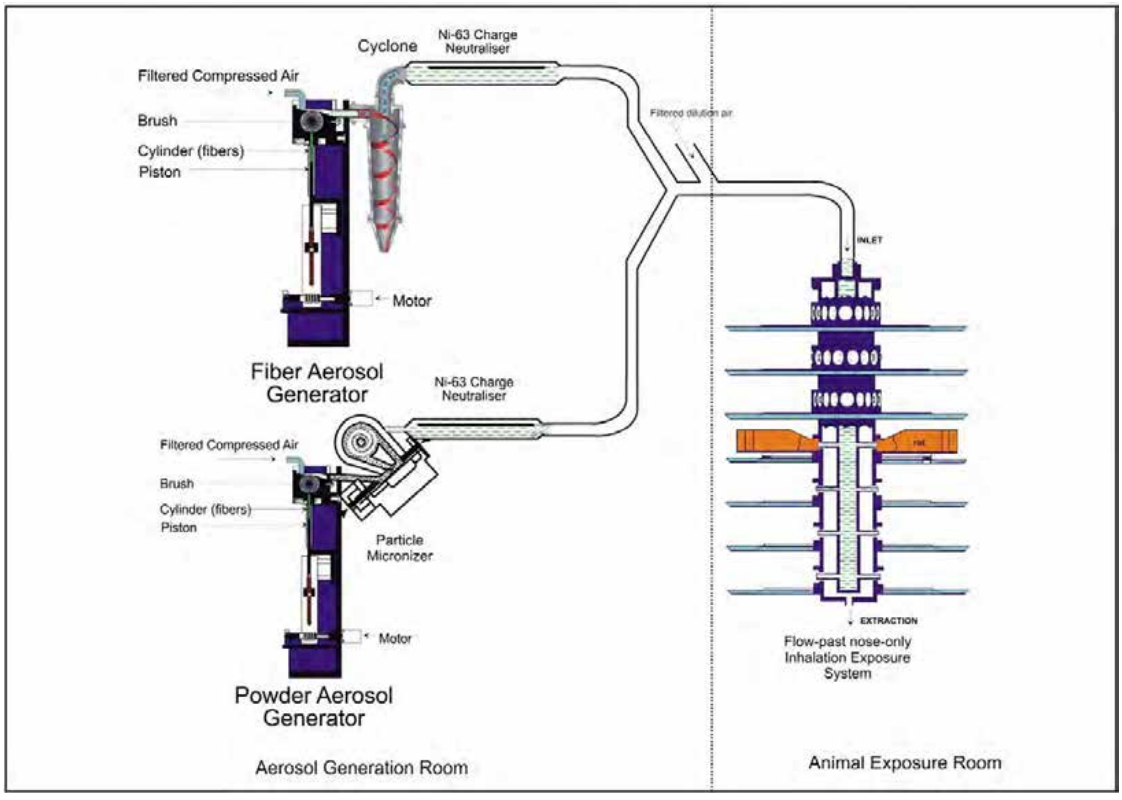



**Fig. 2.** Schematic diagram of the aerosol generation and exposure system used for the combined chrysotile and brake dust exposure.

for measuring airborne man made mineral fibers (MMMF) WHO (1985) and the VDI Guideline 3492 (2004).

All objects seen at the magnification of 10,000× (acceleration voltage 25 kV) were sized with no lower or upper limit imposed on either length or diameter. The bivariate length and diameter was recorded individually for each object measured. Fibers were defined as any object that had an aspect ratio of at least 3:1. The diameter was determined at the greatest width of the object. All other objects were considered as non-fibrous particles. Fibers with both ends in the field of view had the "weight" of one fiber (=2 fiber ends), fibers with only one end within the field had the "weight" of a half fiber (=1 fiber end). Fibers without any fiber end in the field of view were not counted nor measured.

The stopping rules for counting each sample were defined as follows.

Fibrous objects: The minimum numbers of fibers examined were:

a) fibers with a length <5 μm = 100 fibers (200 fiber ends)
b) fibers with a length between 5 and 20 μm = 200 fibers (400 fiber ends)
c) fibers with a length >20 μm = 100 fibers (200 fiber ends)

Fields of view were examined for each length category until the defined minimum number of fibers for each length category was recorded or a maximum of 1 $mm^2$ of the filter surface was examined in case the fiber minimum number for the length category was not reached. For samples of the control group an area of 0.5 $mm^2$ of the filter was evaluated. These counting rules were based on the number of fibers per sample necessary in order to have statistical reproducibility of the means (EUR, 18748 EN, 1999). For non-fibrous objects, fields of view were examined until a total of 100 particles were recorded or the aforementioned stopping criteria for fibers were reached.

Particle Size of Dust Aerosol: The particle size of the brake dust aerosol was measured using a Marple Series 290 Cascade Impactor (TSE Systems, Germany). The impactor determines the aerodynamic particle size distributions from 0.52 to 21.3 μm.

*Clinical examination and body weights*

Animals were observed for mortality/morbidity and for other clinical symptoms at least once daily during the acclimatization period and the 12-month post exposure observation period and twice daily during the treatment period. Once a week, animals were examined for clinical symptoms, i.e. abnormalities concerning their general condition. This included inspection of skin, fur, eyes, visible mucous membranes, examination for patho-morphological changes (e.g. unusual breathing pattern, masses, nodules), abnormal behavior and central nervous symptoms (e.g. changes in gait, posture or grooming activity, unusual response to handling, secretion/excretion abnormalities, clonic/tonic movements, stereotypies) and/or other clinical abnormalities. Body weight was recorded weekly starting on day 1 of the acclimatization period, on days 1, 3 and 5 of the treatment period, once weekly during the first month post exposure and every second week thereafter.

*Gross pathology and organ weights*

Animals were anesthetized with an overdose of pentobarbital sodium (Narcoren™) and humanely killed by cutting the vena cava caudalis. The abdominal cavity was opened and the diaphragm cut carefully allowing the lungs to collapse. Heart, esophagus, upper half of trachea, thymus and lung associated lymph-nodes were sampled. The physical condition of the animals prior to euthanasia and the examination of the internal organs were recorded. Moribund animals or those found dead were necropsied as soon as possible and the findings recorded.

*Tissue preparation for lung ashing, histopathology and confocal microscopy*

In animals assigned to biopersistence/lung burden, the lungs and the lower half of the trachea were collected with the attached mediastinal tissue. The mediastinal tissue containing the mediastinal lymph nodes was resected and immediately inserted into appropriate labeled plastic bags and deep-frozen. All lung lobes were removed by transection of the bronchi and were weighed, inserted into appropriately labeled plastic tubes and deep frozen (−70 °C) without fixation. Particular attention was given to avoid contamination of the dissected organs by fibers from the fur or deposited on dissecting instruments.

In animals assigned to lung histopathology and confocal microscopy, the lungs and the lower half of the trachea were collected with the attached mediastinal tissue. In addition, the diaphragm was collected for confocal microscopy. Macroscopic abnormalities of the lungs were recorded and the lung weight measured and recorded.

The lung lobes were dried by freeze drying (Christ, Osterode, Germany) and subjected to low temperature ashing (200-G, Plasma Technics, Kirchheim, Germany) with the ash weight calculated for each lung. An aliquot was suspended in filtered water, sonicated (Sonorex RK 510H at 35 kHz and 160 W, Bandelin, Germany) until the suspension was homogenous (~60 s) and filtered onto a Nuclepore filter (pore size 0.2 μm). The filter was mounted on an aluminum stub and sputtered (Balzers SCD 030, Balzers Hochvakuumtechnik, Germany) with ~10 nm of gold.

*Histopathology*

The left lung lobe of each animal assigned to lung histopathology was filled with neutral buffered 4% formaldehyde solution by gentle instillation under a hydrostatic pressure of 20 cm. In addition, the mediastinal tissue containing the lymph nodes was fixed in neutral buffered 4% formaldehyde solution except at the 91, 181 and 365 day terminations as mentioned below.

The lung tissues and lymph nodes were processed, embedded in paraffin, cut at a nominal thickness of 2–4 μm and stained with hematoxylin and eosin. Special stains were used at the discretion of the pathologist. The slides were examined by light microscopy and the observations recorded.

The mediastinal lymph nodes from animals terminated at days 91, 181 and 365 were analyzed for fiber number and size. The lymph nodes were frozen, dried and plasma ashed as described for the lung lobes above and analyzed for bivariate fiber size distribution and counting by SEM.

*Lung and diaphragm tissue preparation for confocal microscopy*

The bronchus to the left lung was ligated, left lung removed for sampling as previously described. Right lung lobes were fixed via intratracheal instillation of modified Karnovski's fixative (with PBS) at a hydrostatic pressure of 20 cm for at least 10 min. The trachea was then ligated and the inflated lung stored in the same fixative. Diaphragms were excised from the chest wall, pinned flat to stiff filter paper with parietal surface facing up and immersion fixed in modified Karnovski's fixative. Specimens were then dispatched to Rogers Imaging Corporation (Needham Heights, MA, USA) for processing and analysis. Upon arrival, the fixed right lung samples were weighed, piece dissected, dehydrated, stained and embedded in Spurr epoxy (Rogers et al., 1999).

*Chestwall preparation for confocal microscopy*

In all animals assigned to low temperature microscopy, following termination, a gross dissection of the animal proceeded as follows: A short PE 190 tube was inserted into the trachea and fixed with silk thread. Skin surrounding the thoracic cage, the forelimbs at the brachial

plexus and the lower part of the body below the diaphragm were surgically removed. Intact chestwalls were immediately lowered into liquid nitrogen diaphragm-first over a one minute period. Frozen chestwalls were sealed in ziplock bags and stored at −80 °C, then shipped to Rogers Imaging Corporation (RIC) on dry ice for freeze substitution processing.

*Chest wall processing*

For each chestwall a series of cross sectional slabs 4–5 mm thick were cut using a band saw. Slabs were placed on a dry ice cooled copper plate, then put into wire mesh processing baskets, labeled, and immersed by group in freeze-dry transition fluid (anhydrous methanol (75%), acetone (25%)) in 1 l cryo containers and stored at −80 °C. Cyro fluids were replaced weekly for up to two months. Then specimens were stored in cryofluid at −20 °C with weekly fluid replacement until solution cleared, usually after one month.

*Staining and preparation of specimens for microscopic evaluation*

Following freeze substitution, cross sections of chestwall were transferred to anhydrous methanol at −20 °C and brought to room temperature. Chestwall slabs and lung pieces were then stained with Lucifer yellow-CH (0.0001%) (Rogers et al., 1999), infiltrated in Spurr epoxy resin then heat cured. Undisturbed surfaces of chestwall slabs were exposed within the Epoxy-embedment using a belt sander, or in the case of lung, 2 milimeter-thick sections were cut using a thin kerf rock saw blade. Exposed surfaces were polished using a diamond lapidary wheel until glass smooth.

*Confocal microscopy*

Confocal microscopy imaging was performed on four lung sample pieces or chestwall slabs from each animal for each time point using Sarastro 2000 or 2010 (Molecular Dynamics, Inc.) laser scanning microscopes fitted with 25 mW argon-ion lasers and an upright Zeiss Axiophot or upright Nikon or inverted Nikon Diphot2 microscopes, modified for reflected light imaging in dual channel reflected and fluorescent imaging mode. The cellular constituents and fibers (and particles) were imaged simultaneously with this arrangement with each "exposure" producing two digital images in perfect register with one another (Bernstein et al., 2010). Images were recorded through 60× objectives for Nikon-fitted confocal microscopes with voxel dimensions of 0.16 μm, 0.16 μm, and 0.60 μm (x, y, and z dimensions, respectively). Voxel dimensions were 0.16 μm, 0.16 μm, and 0.50 μm (x, y, and z dimensions, respectively) obtained from 63× objective for the Zeiss-fitted confocal microscope.

*Morphometric methods for confocal analysis*

*Fiber load and fiber distribution.* All fiber profiles in length classes of 3 μm or longer were recorded. The true length of individual fibrils was recorded if the fiber profile was oriented such that two free ends were present. Particulate material is viewed as a single spot or profile that does not deviate along X or Y axis when serial section data is scrolled up and down along the z-axis. Sampling strategies were designed to permit the determination of the number of fibers retained within the lung parenchyma and conducting airways.

*Sampling strategy for parenchyma.* The sampling strategy for the parenchyma has been described previously (Bernstein et al., 2010). Each volume was recorded by obtaining 25 optical sections separated by 0.5 μm along the z-axis. On average, the real-world dimensions of a volume, therefore, were 86.6 μm × 86.6 μm × 13.75 μm in x, y, and z, respectively. For this phase of the study, well over 240,000 micrographs were recorded to obtain the necessary quantitative information from this compartment. The number of fibers in each volume was counted by scrolling up and down through the depth series of images while looking for the characteristic bright points or lines which indicated a reflective or refractile particle or fiber. The number of fibers/volume of parenchyma, μm$^3$ was recorded and then extrapolated to the whole lung based upon the volume of parenchyma (including airspaces).

Fibers in parenchyma were classified as occurring:

- in alveoli, alveolar ducts, or terminal bronchioles, in contact with the surface of tissue,
- in ducts or alveoli, but not in contact with tissue in the recorded volume,
- wholly or partly inside alveolar macrophages.

No fibers were observed in other parenchymal contexts.

*Sampling strategy for airways.* The sampling strategy for the airways has been described previously (Bernstein et al., 2010). Ten depth series (dimensions identical to parenchymal depth series) were recorded from each of 4 samples per animal, with these stacks holding, on average, 95 μm of airway wall profile each. Well over 24,000 micrographs were recorded for this phase of the study. The average airway diameter in these lungs was estimated at 300 μm, with airway volume, ca. 10% of lung volume. These numbers allow a further estimate of the length of an equivalent cylinder and its wall area, which is an estimate of the total airway wall area in the lungs. The total fiber burden in the airway compartment was estimated.

*Sampling strategy for connective tissue in parenchyma*

The same images used to collect quantitative measurements of pulmonary fiber load and distribution were used to measure connective tissue (Ct) present per field of view (FOV) to obtain a percentage of connective tissue occupied in a given area of lung tissue (%CT/FOV). For each FOV, the midpoint of the volume was examined and the area occupied by lung tissue was measured by adjusting the threshold detection. Fluorescent specificity imparted to the lung tissue in general, with the highest affinity to connective tissue produced images with distinct pixel intensity maps as follows; airspace was represented by pixel intensity units from 0 to 18, lung tissue occupied pixel intensities 19–180, and connective tissue (elastin and collagen) were shown in the pixel intensity range of 181–255. Overall variations of pixel intensity resulting from different depth from the block surface were adjusted by the examiner of the dataset on an individual basis by "scroll up and down" through the depth series of images. The area occupied by connective tissue was divided by the total lung tissue area from each field of view then the average %Ct/FOV was determined for each group by time point and compared. These measurements produced the fraction of connective tissue per area lung tissue for all FOVs examined.

*Statistical analyses*

Comparison of lung digestion and confocal methodologies was performed using concordance correlation, Lin (1989) using MedCalc Software (Version 12.7, Belgium).

The fiber clearance half-times were determined using the statistical procedures specified in the EC protocol (EUR, 18748 EN, 1999). The clearance curve was fitted to the data using nonlinear regression techniques with a single or double exponential (StatSoft, Inc. (2011). STATISTICA (data analysis software system), version 12. www.statsoft.com).

The confocal fibrotic response data was analyzed using analysis of variance (MedCalc, ver 12.7, Ostend, Belgium).

## Results

### *Validation of the lung digestion and counting procedures*

Validation of lung digestion and counting procedures is essential to the legitimacy of this type of study, although it was often absent from early studies. Such validation provides confidence that there is no significant alteration of the fiber counting and size distribution during fiber recovery. Comparative CM was used to assure that the lung digestion and SEM procedures used in this study did not affect the fiber dimensions of the chrysotile and crocidolite present in the lung (Bernstein et al., 2004).

The results of this analysis confirmed that there is a very good correlation between the length distribution as measured by the lung digestion procedure/SEM and the confocal methodology with a concordance correlation coefficient $\rho_c$ (Lin, 1989, 2000) of 0.8384 (0.9972 with outlier removed) for group 2, 0.9956 for group 3 and 0.9823 for group 4.

### *Inhalation exposure*

The aerosol concentrations and the size distributions of the fibers of all groups are shown in Table 1. The aerosol concentrations of groups 2 (chrysotile and brake dust) and 4 (crocidolite asbestos) were set based upon the number of fibers/cm$^3$ longer than 20 μm. Group 3 (brake dust alone) was included as a comparative exposure of the brake dust particulate material (with the relatively low concentration of chrysotile fibers present) with groups 2 and 3 having similar gravimetric concentrations of brake dust. The difference between the total gravimetric concentration in group 2 (3.48 mg/m$^3$) compared to group 3 (1.52 mg/m$^3$) was largely due to the gravimetric concentration of the chrysotile aerosol added to group 2. The mean gravimetric concentration for group 4 (crocidolite asbestos) was 6.34 mg/m$^3$ which was a result of the crocidolite fibers having a larger diameter than the chrysotile.

### *Bivariate length and diameter distributions of the exposure aerosols*

The bivariate length and diameter size distributions of the chrysotile and brake dust, brake dust and crocidolite aerosol are shown in Fig. 3. As mentioned above, all measurements were made at the position of the animal's nose in the exposure system. The fiber distribution in the chrysotile and brake dust aerosol included a larger number of shorter fibers with 84% less than 5 μm. For those fibers longer than 20 μm there was a mean of 189 fibers/cm$^3$ ranging from 20 to 160 μm in length. As summarized in Fig. 4, 99% of the long fibers were less than 1 μm in diameter (rat-respirable) with 95% less than 0.4 μm in diameter.

The crocidolite-exposure atmosphere had considerably fewer short fibers with 66% less than 5 μm. For those fibers longer than 20 μm there was a mean of 93 fibers/cm$^3$ ranging in length from 20 to 190 μm. 88% of the long fibers were less than 1 μm in diameter (rat-respirable) with 21% of the fibers less than 0.4 μm in diameter.

In the brake dust exposure group there were fewer chrysotile fibers present without the added chrysotile with a mean of 3 fibers longer than 20 μm/cm$^3$. These longer fibers ranged in length from 20 to 140 μm with 90% less than 1 μm in diameter (rat respirable) with 63% of the fibers less than 0.4 μm in diameter.

SEM photomicrographs of the aerosol from each exposure group atmosphere are shown in the Supplementary data, Figs. S1–S6.

### *Lung fiber burden (from the lung digestion evaluation)*

The lung fiber burden was evaluated using two independent methods in the study. The first method, the results of which are presented in this section through 91 days post-exposure, was through the digestion of the entire lung without differentiating where in the lung the fibers are located with evaluation of fiber size distribution using scanning electron microscopy. The second method was using confocal microscopy which provided the localization of fibers in the lung compartment, including fiber burden and size.

**Table 1**
Aerosol concentration and size distribution of the exposure atmosphere in the air control group 1, chrysotile and brake dust group 2, brake dust group 3 and crocidolite asbestos group 4.

| Exposure group | Gravimetric concentration mg/m$^3$ (SD) | Total Number of fibers counted on the filter* | Number of total fibers/cm$^3$ | WHO fibers/cm$^3$ | Percent WHO fibers | Number of fibers ≥ 20 μm/cm$^3$ | Percent of all fibers ≥20 μm/cm$^3$ | Mean number particles/cm$^3$ | Diameter Range (μm) | | Length range (μm) | | GMD (μm) (Std. Dev.) | GML (μm) (Std. Dev.) | Mean diameter (μm) Std. Dev. | Mean length (μm) Std. Dev. | Length weighted arthm. dmeter μm) | Length weighted geom. diameter (μm) | Aspect ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (Group 1) Air Control | 0 | 7 | 0.002 | 0.001 | 42.9 | 0 | 0 | 0.006 | 0.5 | 2.3 | 1.11 | 4.49 | 1.20 | 4.70 | 1.34 | 1.23 | 4.07 | | |
| | | | | | | | | | −1.8 | −6.8 | 1.53 | 1.46 | | 1.63 | | | | | |
| (Group 2) Chrysotile & Brake dust | 3.48 | 2454 | 6953 | 1007 | 14.5 | 189 | 2.7 | 3140 | 0.03 | 0.6 | 0.17 | 2.47 | 0.20 | 4.01 | 0.19 | 0.16 | 29.8 | | |
| | 0.36 | | | | | | | | −2.4 | −160 | 1.68 | 2.32 | 0.12 | 6.83 | | | | | |
| (Group 3) Brake dust** | 1.52 | 1623 | 389.3 | 46.0 | 11.8 | 3.6 | 0.9 | 1240 | 0.05 | 0.6 | 0.21 | 2.25 | 0.25 | 3.21 | 0.32 | 0.24 | 16.99 | | |
| | 0.07 | | | | | | | | −2.9 | −140 | 1.79 | 2.00 | 0.20 | 4.25 | | | | | |
| (Group 4) Crocidolite | 6.34 | 1820 | 2013 | 709 | 35.2 | 93 | 4.6 | 602 | 0.05 | 0.7 | 0.34 | 4.07 | 0.39 | 6.50 | 0.50 | 0.39 | 17.62 | | |
| | 0.42 | | | | | | | | −2.6 | −190 | 1.64 | 2.44 | 0.21 | 9.40 | | | | | |

SD: Standard deviation; GMD: Geometric mean diameter; GML: Geometric mean length; MMAD: Mass median aerodynamic diameter.
* The total number of fibers counted on the filter is based upon the rules specified in section "Fiber/Particle Analysis and Lung Digestion" above.
** For the brake dust group 3, the MMAD = 1.89 (Geometric standard deviation = 2.54) as determined by the impactor measurement.

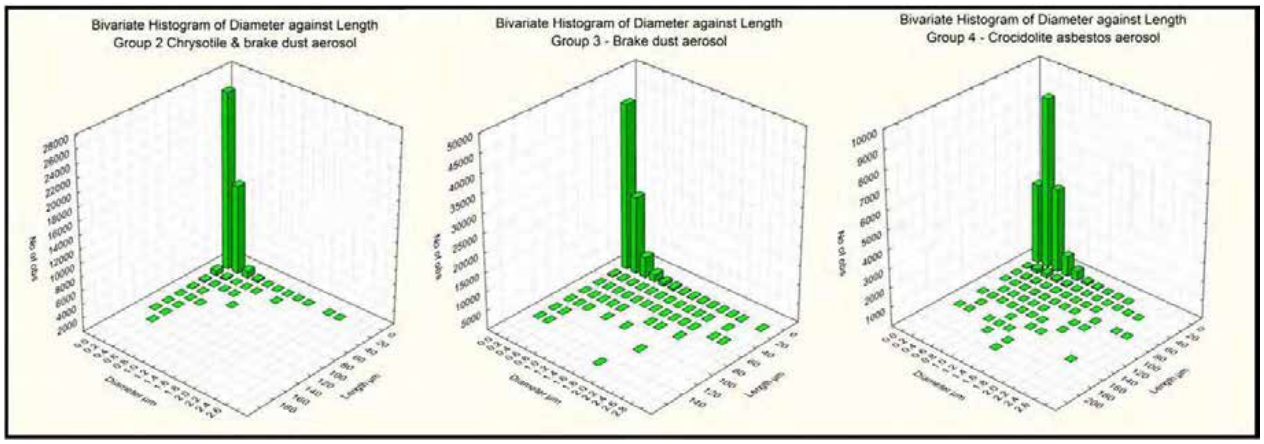



**Fig. 3.** Bivariate length and diameter distributions of the exposure aerosols in the chrysotile and brake dust group 2, brake dust group 3; and crocidolite asbestos group 4.

The mean concentrations and dimensions of the fibers as determined by lung digestion evaluation at each time point through 91 days post exposure for groups 2–4, respectively, are presented in the supplemental data in Tables S3–S5.

The lung burden immediately after cessation of the 5 days of exposure (day 0) is a product of the clearance dynamics of the fibers in the lung since the start of the 5 days of exposure. Immediately following the cessation of the last exposure there was a mean of 0.1 million fibers longer than 20 μm in the lung in the chrysotile and brake dust exposure group (2). In the brake dust group (3) there were 0.016 million fibers longer than 20 μm in the lung which corresponds to the lower exposure concentration of chrysotile in this group. In the crocidolite exposure group (4) there were 3.4 million fibers longer than 20 μm in the lung at day 0.

In the brake dust groups with or without added chrysotile the number of fibers greater than 20 μm in length diminishes rapidly after cessation of exposure. These fibers appear to break apart into shorter fibers with the number of fibers less than 5 μm increasing from a mean of 24 million on day 0 to 36 million on day 1 and 57 million on day 7. The number of fibers 5–20 μm in length also increased from 1.8 million on day 0 to 2.7 million on day 1. The bivariate length and diameter distribution of the fibers in the lung at days 0 and 91 are shown in Fig. 5 for the 3 exposure groups. In the chrysotile and brake dust group the number of longer fibers are reduced as they are broken down into shorter fibers. The number of thin shorter fibers (<10 μm length) are increasing as a result. In the brake dust group the lung concentration immediately following cessation of exposure was correspondingly less with the shorter chrysotile fibers present also clearing.

By 91 days after cessation of exposure approximately 90% of the longer fibers have been cleared from the lung in group 2 with an average of only nine fibers detected on the filter in the SEM microscopic analysis. In group 3, between 1 and 5 fibers were detected at 91 days on the filter in the SEM microscopic analysis. The regional localization of fibers using confocal analysis as discussed below provides a basis for assessing that these few fibers observed are likely in the bronchial tree and not in the alveolar region of the lung. By 91 days, 95% of all fibers observed in the lung in the chrysotile and brake dust group were less than 5 μm in length. The geometric mean length of the fibers in the lung at 91 days was 2.3 μm. As the lung digestion procedure provides only a total estimate of the number of fibers in all the lung compartments without the ability to differentiate where in the lung each fiber is located, this procedure cannot address the question of where these fibers are in the lung.

In the crocidolite asbestos exposure group the fibers longer than 20 μm which are less than 1 μm in diameter (rat-respirable to the lung parenchyma), have approximately the same distribution pattern at 91 days as compared to 0 days. It is only the shorter thinner crocidolite fibers which show clearance. Due to the insolubility of the crocidolite fibers, the longer fibers do not disintegrate into shorter fibers as occurs in the chrysotile exposure groups.

As described above, the air control group was exposed to filtered air without any fibers using similar aerosol generation and exposure systems. The lungs from the control animals were processed and analyzed using the same procedures. A low level background of shorter chrysotile fibers was detected in some of the air control lungs samples on days 0 and 14 that were low-temperature ashed which was less than 0.001 of the exposed values. No fibers longer than 20 μm were found in any of the control sample. No crocidolite fibers were found in any of the control samples. The confocal microscopy measurements found no fibers in any of the air control lungs indicating that the background was due to the ashing procedure and not from inhalation exposure.

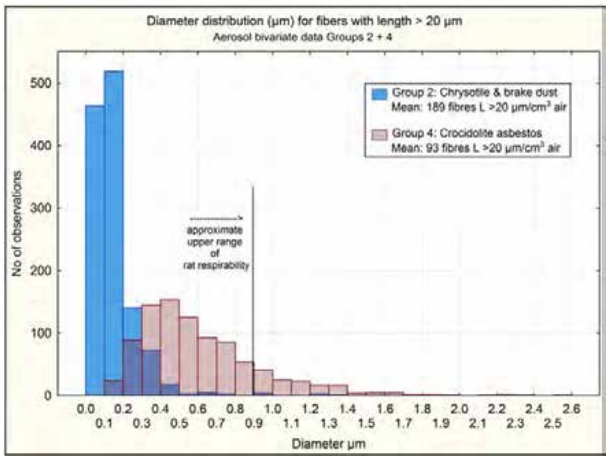



**Fig. 4.** Diameter distribution of the fibers longer than 20 μm in groups 2 and 4.

*Fiber clearance (from the lung digestion evaluation)*

The clearance half-times for each fiber range are shown in Table 2. The clearance curves for each fiber range are shown in Figs. S-7 through S-10. These were determined using the statistical procedures specified in the EC protocol (EUR, 18748 EN, 1999). The clearance curves were

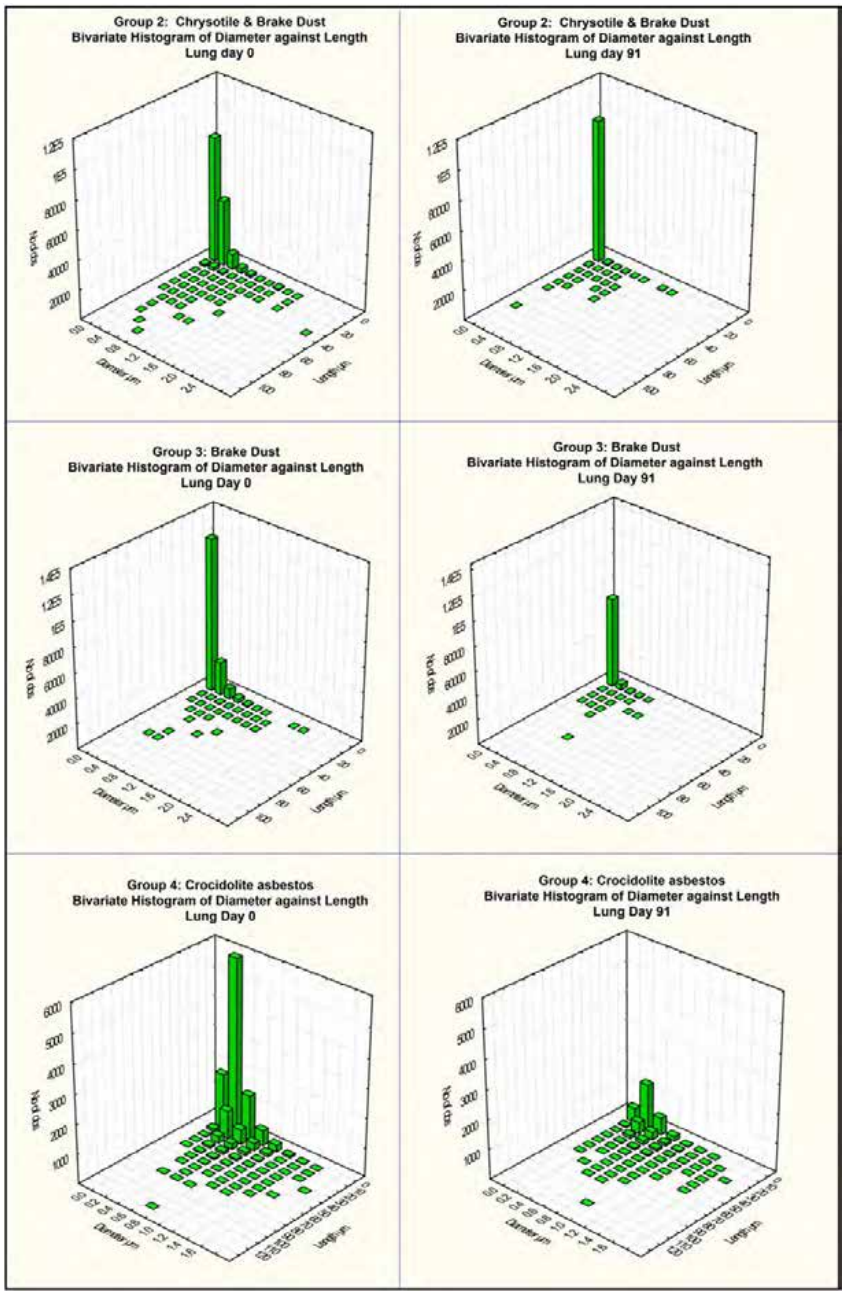



**Fig. 5.** Bivariate length and diameter distributions of fibers in the lung in the chrysotile and brake dust group 2; brake dust group 3; and crocidolite asbestos group 4 at 0 days and 91 days after cessation of exposure. (The No. obs. is number of fibers.)

fitted to the data using nonlinear regression techniques with a single or double exponential (StatSoft, Inc., 2011) and were based upon the initial results available through 91 days post exposure.

The clearance rate from the lung of the chrysotile fibers longer than 20 μm is similar in the chrysotile-brake dust group and the brake dust alone group with estimated half-times of 33 and 30 days.

The impact of the larger number of short fibers and relatively insoluble particles in the chrysotile and brake dust group is reflected in the estimated clearance half-times with of 52 days for the shorter fibers and 510 days for the particles. In contrast, the brake dust group which had nearly 20 times fewer shorter fibers and 4 times fewer particles than the chrysotile-brake dust group, also had faster clearance half-times for the fibers less 5 μm in length of 45 days and of particles of 47 days.

In contrast, in the crocidolite asbestos group, following the clearance of the fibers longer than 20 μm which deposit in the tracheal–bronchial tree, those longer fibers remaining in the parenchyma persist in the lung with a clearance half-time estimated to be greater than 1000 days. Following cessation of exposure, the short crocidolite fibers begin to clear, however, by 32 days the clearance rate has diminished and plateaus

**Table 2**
Estimated Fiber Clearance Half Times in days (through 91 days post exposure) (SE: Standard error).

| Group | Exposure | Fibers L > 20 μm | Fibers 5 – 20 μm | Fibers<5μm | Particles |
|---|---|---|---|---|---|
| | | (days) | | | |
| 2 | Chrysotile & brake dust | 33 (SE:11) | 23 (SE: 12) | 52 (SE: 42) | 510 (SE: 1922) |
| 3 | Brake dust | 30 (SE: 11) | 41 (SE: 15) | 45 (SE: 23) | 47 (SE: 42) |
| 4 | Crocidolite asbestos* | ≻ 1000 | ≻ 1000 | ≻ 1000 | ≻ 1000 |

* The standard errors for the crocidolite estimates could not be calculated due to the lack of clearance.

through 91 days. The clearance half time of the remaining shorter fibers and particles is estimated also to be greater than 1000 days.

*Pathological findings in the lung*

*Histopathological examination*

The fiber clearance results clearly differentiate chrysotile fiber retention in both the chrysotile-brake dust group and the brake dust alone group from that of crocidolite asbestos; the histopathology findings provide a basis for determining the biological relevance of this difference.

The summary of histopathological findings in the lung through 91 days after cessation of exposure is presented in Table S-6 (supplemental data) which shows the specific histological findings seen in the lung and the mean grade observed for each finding. Severity was scored on the following scale: no lesions, minimal, slight, moderate, marked, or massive (grades 0–5 respectively). A summary of the key lung histopathology scores through 91 days post exposure is presented in Fig. 6.

There were no exposure-related histopathological findings in animals exposed to filtered air. In the chrysotile-brake dust group and brake dust alone group, slight accumulation of particle laden macrophages were observed from 7 through 91 days post exposure. At 32 days post exposure, very slight (multi)focal particle laden microgranulomas at the bronchiole–alveolar junctions were observed in groups 2 and 3, however, there were no associated giant cells. These findings are illustrated at 0 and 91 days in micrographs in Fig. 7.

In the crocidolite asbestos exposure group, accumulation of fiber laden macrophages was observed already at day 0, immediately after cessation of exposure. This increased at day 7 and was associated with the formation of (multi)focal fiber laden microgranulomas at the bronchiolo–alveolar junctions with multinucleate (syncytial) giant cells within these microgranulomas. By day 32, interstitial fibrosis was observed. These findings persisted through 91 days post exposure (Fig. 7). The concurrent development of the fibrosis is illustrated in the micrographs stained with Masson's tri-chrome at 91 days post exposure (Fig. 8, panel D). Masson's tri-chrome is specific to collagen which is shown as blue in the images. Panels A, B, and C of Fig. 8 show the air control brake dust with chrysotile and brake dust alone groups, all of which are similar in appearance. The development of the fibrotic lesions in the crocidolite asbestos exposure group is also reflected in the interstitial fibrosis score (Fig. 6). No fibrosis was observed in the other groups.

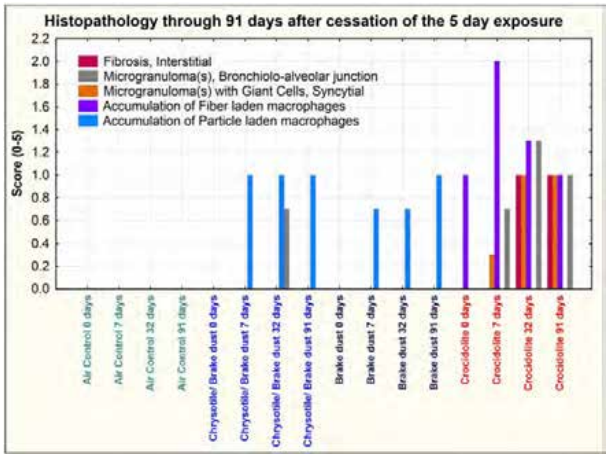



**Fig. 6.** Summary of lung histopathology scores through 91 days post exposure.

The lungs were also evaluated by the pathologist for the Wagner score (McConnell et al., 1984; McConnell and Davis, 2002). The Wagner score specified that "the grading system made a clear differentiation (break) between Grade 3 and Grades 4–8, with the former representing "cellular change" (inflammatory and reversible) and the latter progressive degrees of fibrosis (not totally reversible)".

The Wagner score for both the chrysotile and brake dust group and the brake dust alone group ranged between 1 at day 0 and up to 2 through 91 days post exposure, where 1 is no lesion observed and 2 is a few macrophages in the lumen of the terminal bronchioles and alveoli. With the crocidolite asbestos exposed animals, the Wagner score started at 2 on day 0 and increased up to 4 through 91 days post exposure (Grade 4: Minimal collagen deposition at the level of the terminal bronchiole and alveolus. Increased bronchiolization with associated mucoid debris suggesting glandular pattern.)

*Pulmonary fibrosis analysis — confocal microscopy*

The connective tissue (elastin and collagen) present in the lung was measured by confocal microscopy to obtain the percentage of the elastin and collagen per area of lung tissue (%CT/FOV). For each group and time point 300 cubic lung tissue volumes of 112,550 $\mu m^3$ each were imaged, the amount of connective tissue measured and the number and length of the fibers observed were recorded. Fields of view that contained a blood-vessel of diameter greater than 50 μm were not included due to the high amount of collagen in the blood-vessel walls.

The percent fibrosis is shown in Fig. 9 for each group at 0, 32 and 91 days after cessation of the 5 day exposure. In the air control group the percent connective tissue ranged from a mean of 3.8 ± 2.9 at day 0; 6.4 ± 4.4 at day 32 and 2.9 ± 2.2 at day 91 with a range in individual values over this period of 0.2 to 27%. Compared to the air control group, there were no statistically significant trends in the chrysotile & brake dust group or in the brake dust group alone with or without fibers present. The chrysotile fibers present in the tissues had no impact on the development of connective tissue and did not cause a fibrotic response through 91 days post exposure.

In the crocidolite asbestos exposure group, there is a consistent statistically significant increase in the mean amount of connective tissue present compared to day 0 (mean 4.4 ± 3.4) with means of 8.0 ± 4.4 at 32 days and 14.7 ± 12.8 at 91 days post exposure compared to day zero as determined by analysis of variance (Table S-5, supplemental data). At 91 days, when compared to the air control group the mean connective tissue in the crocidolite asbestos exposure group increased by 5 times. The range of individual measurements shows that at day 0 the percent connective tissue in the crocidolite asbestos exposure group was similar to that found in the air control, however, by 91 days

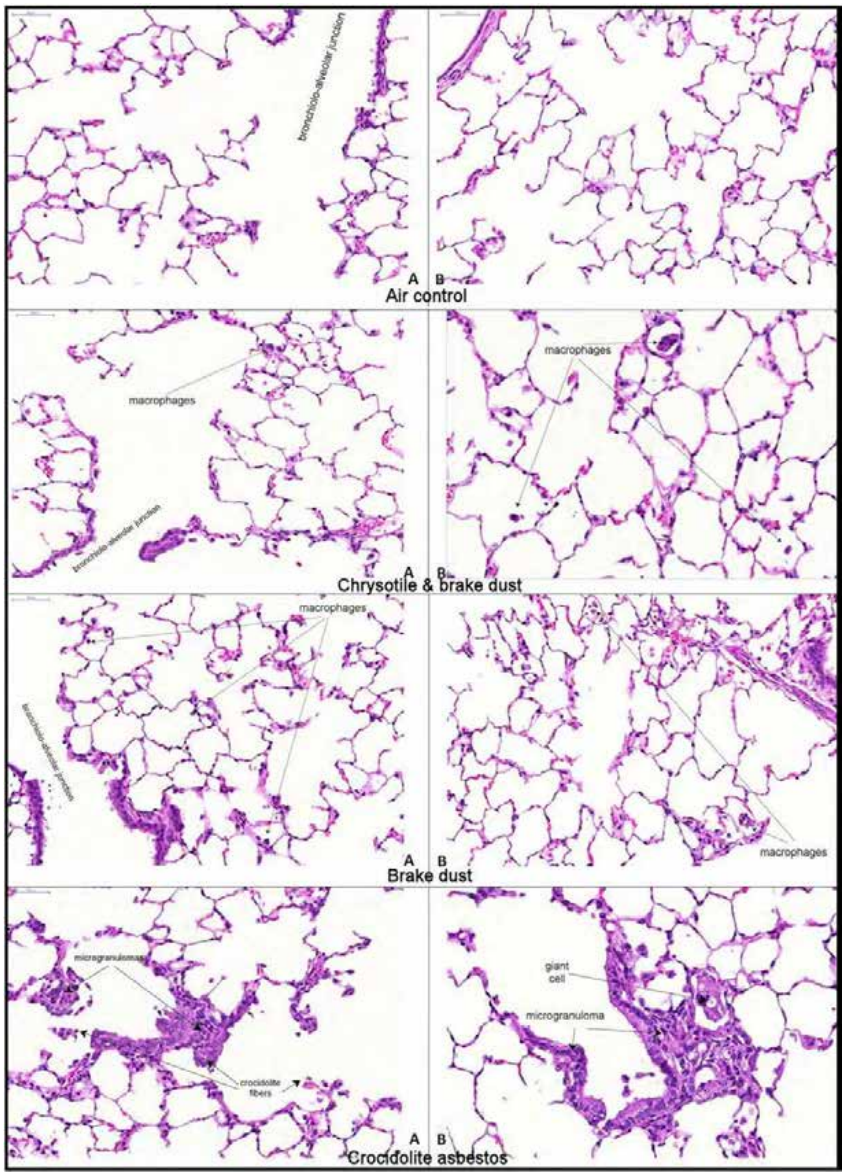



**Fig. 7.** Histopathological photomicrographs of groups 1–4 at 0 and 91 days post exposure: Group 1 Air control: A: day 0 showing bronchiolo-alveolar junction and adjacent alveoli (400×). B: 91 days showing alveoli (400×). Group 2 Brake dust & chrysotile: A: 0 days showing bronchiolo-alveolar junction and adjacent alveoli with a few macrophages (400×). B: 91 days showing alveoli with a few macrophages (530×). Group 3 Brake dust: A: 0 days showing bronchiolo-alveolar junction and adjacent alveoli with a few macrophages (400×). B: 91 days showing alveoli with a few macrophages (530×). Group 4 Crocidolite asbestos: A: 0 days showing inflammatory response with microgranulomas. Crocidolite fibers are also observed (400×). B: 91 days showing alveoli filled with inflammatory cells forming microgranulomas with giant cell (530×).

post exposure, the connective tissue range in group 4 increased in fields of view to up to 87%.

The confocal microscopy images (Figs. 10 and 11) show the comparative connective tissue response. The connective tissue is imaged as the bright white structures. The parenchyma immediately after cessation of exposure (day 0) is shown in Fig. 10. A normal interstitial wall of the lung lined with a thin layer of collagen is seen in the air control group 1 with occasional alveolar macrophages in the alveolus. In the chrysotile and brake dust group 2 and the brake dust alone group 3 similar patterns of collagen are also observed with a few additional macrophages that responded to clear the inhaled dust (shown in red). In the crocidolite asbestos group 4, an alveolus is seen filled with inflammatory cells and interlaced with a collagen matrix. A long crocidolite fiber is seen in the adjacent alveolus surrounded by a few macrophages.

By 91 days post exposure, the air control, chrysotile/brake dust and brake dust groups (1, 2 and 3) are very similar in appearance with a few macrophages observed on the distal ciliated airway (Fig. 11). In the crocidolite asbestos group 4, a collagen-interlaced hyperplasia marked by interstitial fibrosis is observed in the region of the alveolar duct (panel a). The intensive collagen matrix obscures the normal structure and is associated with crocidolite fibers intertwined within the matrix. Only the ends of the fibers are seen as this is a 2 dimensional image. In panel (b) long crocidolite fibers are observed in a terminal bronchial hyperplasia which is interlaced with a dense collagen matrix.

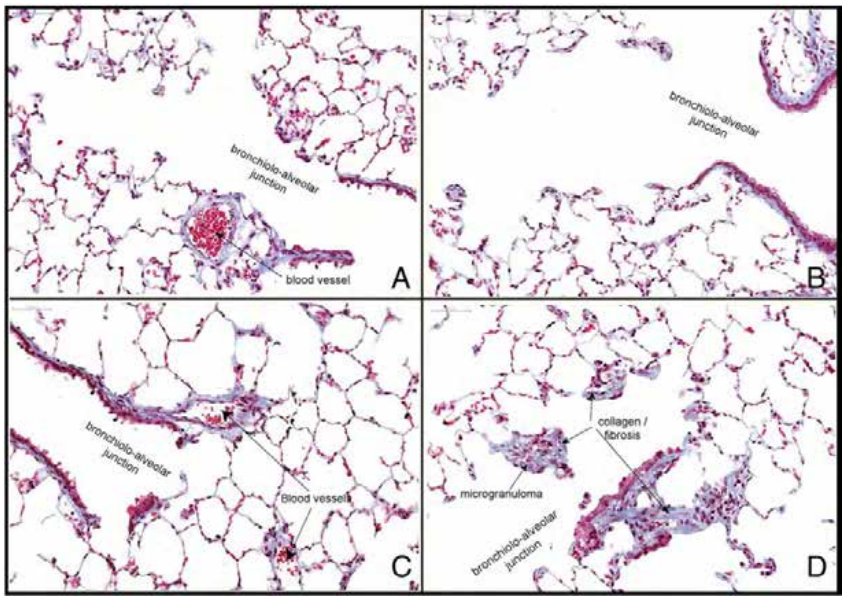



**Fig. 8.** Histopathological photomicrographs with Masson's trichrome stain at 91 days (400×). Panels A, B, & C: Group 1 Air control, group 2 Chrysotile and brake dust and group 3 Brake dust are all similar in appearance. In groups 1 and 3, blood vessel(s) can also be seen. Panel D: Group 4 Crocidolite asbestos. The Masson's trichrome stain shows inflammatory response with micrograniuloma with extensive collagen and fibrosis (blue color).

*Regional quantification of fibers in the lung*

*Airway versus parenchyma.* An issue that is often of concern with inhalation toxicology studies is to assure that the test material being evaluated has reached the site in the lung were disease can develop. The confocal microscopy procedures used in the study provide the ability to determine not only the fiber size distribution in the lung but where within the lung compartments these fibers are located.

The number of fibers deposited in the airways and parenchyma was estimated through the confocal microscopy fiber measurements. The

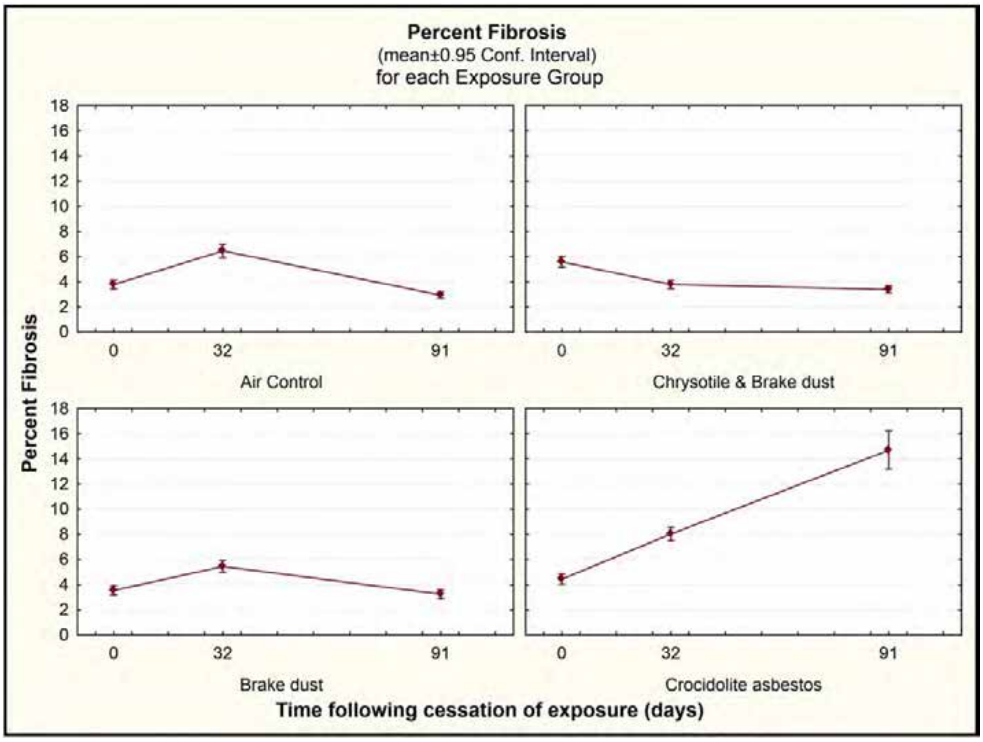



**Fig. 9.** Confocal microscopy: Percent connective tissue in the lung per field of view (Animal means ± 0.95 Conf. Interval) for groups 1 Air control, 2 Chrysotile & brake dust, 3 Brake dust and 4 Crocidolite asbestos. For crocidolite asbestos, the correlation coefficient is $r = 0.83$, $p = 0.0055$.

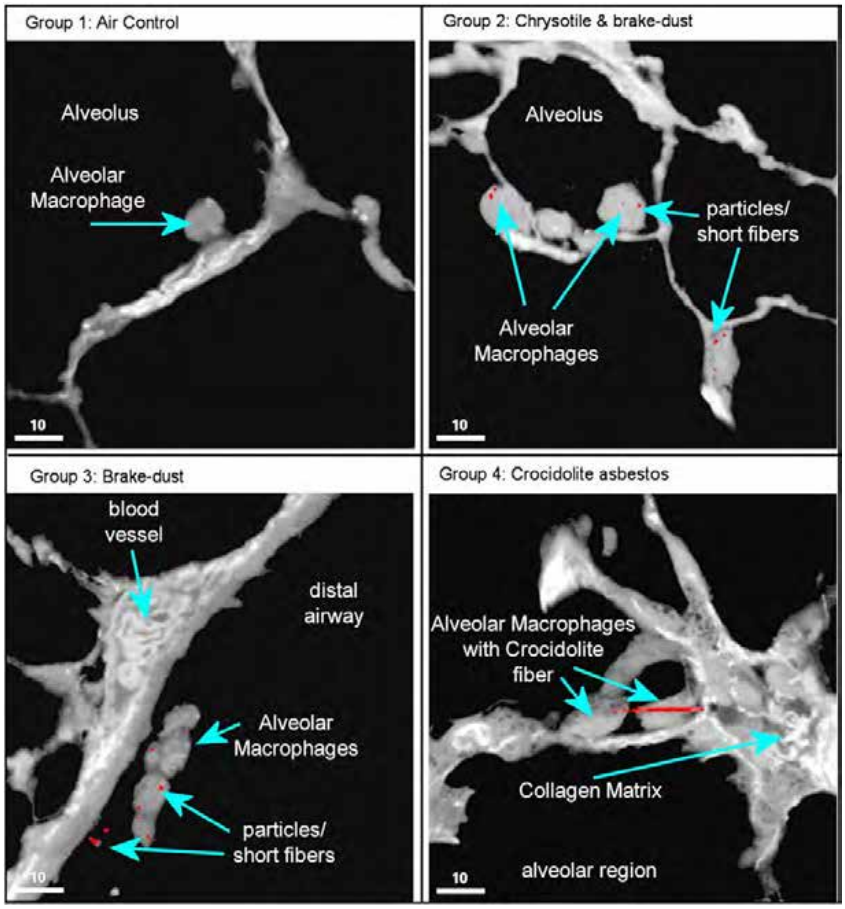



**Fig. 10.** Confocal microscopy Interstitial Fibrosis Assay — day 0: Group 1 Air control: A normal interstitial wall of the lung lined with a thin layer of collagen is seen in the air control group 1 with occasional alveolar macrophages in the alveolus. Group 2 Chrysotile & brake dust: A few macrophages are observed in the alveoli. Chrysotile or brake dust particles can be observed within the macrophages. Group 3 Brake dust: A few macrophages are observed in the distal airway. Chrysotile or brake dust particles can be observed within the macrophages. A blood vessel is also seen. Group 4 Crocidolite asbestos. An alveolus is seen filled with inflammatory cells and interlaced with collagen (fibrosis) shown in white. A long crocidolite fiber (~20 μm) is observed in an adjacent alveolus with macrophages at each end.

number of fibers in the total lung volume was estimated based upon the regions sampled in the lung by confocal microscopy. These values should be viewed as estimates as they are influenced by the orientation of fibers within the lungs and the degree of inflation of the lungs. As presented above the clearance rates as determined by confocal correlate with those determined by the lung digestion procedure. In each of the three exposure groups, more than 99% of the fibers were observed in the parenchyma immediately after cessation of exposure.

*Fiber clearance by length fraction in the airways versus the parenchyma.* The fiber clearance by length fraction was also estimated from the 3D confocal images in the airways and parenchyma. Table 3 shows for each exposure group the percent of the total number of fibers observed at day 0 in the parenchyma and in the airways for the length fractions <5 μm. 5–20 μm and >20 μm at 0, 7, 32 and 91 days post exposure.

For both the chrysotile and brake dust group and the brake dust alone group, nearly all of the fibers longer than 20 μm were observed in the parenchyma. In both of these groups, by 91 days post exposure the chrysotile fibers were no longer observed. In the airways, chrysotile fibers longer than 20 μm were only observed at 91 days post exposure in the brake dust group.

In the crocidolite asbestos exposure group 15% of the fibers in the airways and 14% of the fibers in the parenchyma were longer than 20 μm immediately after cessation of exposure. As also observed in the lung digestion procedure, these longer crocidolite fibers persisted and accounted for 9% (parenchyma) and 7% (airways) of the fiber present at 91 days post exposure.

*Localization of fibers within the airways and parenchyma*

The location of fibers within the following lung compartments was also determined by confocal microscopy using random search procedures as shown below.

Airway region, if the fiber was:

- Penetrating the airway wall or located completely underneath the airway wall. Partly or fully embedded into the interstitial space, blood vessel, or lymphatics.
- In airway lumen; portion of fiber visualized not touching tissue.
- Wholly or partly inside airway macrophages.
- On surface of or intercalated within ciliated epithelium of conducting airway

Parenchyma region, if a fiber was:

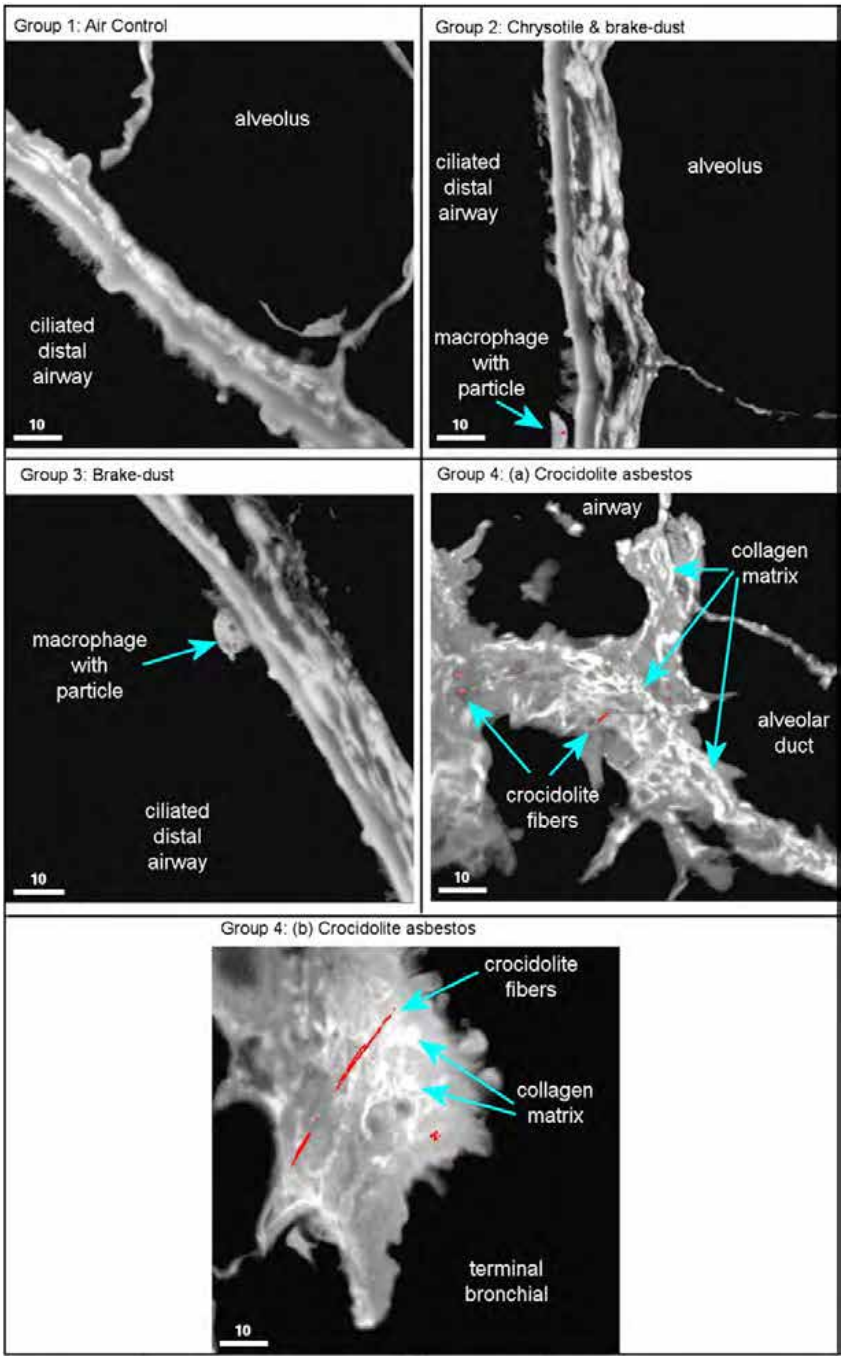



**Fig. 11.** Confocal microscopy Interstitial Fibrosis Assay — 91 days: Group 1 Air control shows a typical profile of ciliated airway epithelium. Group 2 Chrysotile & brake dust: A few macrophages are observed on the ciliated distal airway. A few chrysotile or brake dust particles can be observed within the macrophages. Group 3 Brake dust: A macrophage is observed on the ciliated distal airway. Chrysotile or brake dust particles can be observed within the macrophage. Group 4 Crocidolite asbestos: Intense inflammatory response interlaced with collagen (white) to the crocidolite fibers in the region adjacent to the airway and alveolar duct.

- Partly or intercalated within the interstitial space, blood vessel, or lymphatic vessel.
- Observed in alveolar ducts but not in contact with tissue.
- Wholly or partly inside alveolar macrophages.
- In contact with epithelium, alveoli, alveolar ducts, or terminal bronchioles.

The distribution of fibers in each group within the airway and the parenchyma are shown in Figs. 12 and 13, respectively.

In the chrysotile and brake dust exposure group, immediately following cessation of exposure a mean of 260,000 (SD[2]: 97,000) fibers

[2] SD: Standard deviation.

**Table 3**
Confocal microscopy analysis: Percentage of total fibers at day 0 observed in the parenchyma and the airways by the random analysis with confocal microscopy for groups 1 Air control, 2 Chrysotile & brake dust, 3 Brake dust and 4 Crocidolite asbestos as a function of time through 91 days.

| Group | Day | Parenchyma % | | | | Airways % | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | All parenchyma fibers | Fibers Length <5 μm | Fibers Length 5–20 μm | Fibers Length >20 μm | All Airway Fibers | Fibers Length <5 μm | Fibers Length 5–20 μm | Fibers Length >20 μm |
| Chrysotile & Brake dust | 0 | **100%** | 76.5 | 22.6 | 0.9 | **100%** | 64.86 | 35.14 | 0.00 |
| Chrysotile & Brake dust | 7 | 25 | 19.6 | 5.2 | 0.3 | 25 | 16.22 | 18.92 | 0.00 |
| Chrysotile & Brake dust | 32 | 37 | 33.6 | 3.4 | 0.3 | 37 | 5.41 | 0.00 | 0.00 |
| Chrysotile & Brake dust | 91 | 1.5 | 1.5 | 0.0 | 0.0 | 1.5 | 0.00 | 5.41 | 0.00 |
| Brake dust | 0 | **100%** | 24.3 | 64.9 | 10.8 | **100%** | 36 | 64 | 0 |
| Brake dust | 7 | 105 | 59.5 | 45.9 | 0.0 | 4 | 0 | 4 | 0 |
| Brake dust | 32 | 27 | 13.5 | 10.8 | 2.7 | 12 | 4 | 8 | 0 |
| Brake dust | 91 | 11 | 8.1 | 2.7 | 0.0 | 8 | 4 | 0 | 4 |
| Crocidolite | 0 | **100%** | 25 | 61 | 14 | **100%** | 28 | 58 | 15 |
| Crocidolite | 7 | 91 | 22 | 58 | 10 | 77 | 30 | 41 | 6 |
| Crocidolite | 32 | 73 | 26 | 36 | 11 | 24 | 3 | 17 | 4 |
| Crocidolite | 91 | 31 | 3 | 20 | 9 | 26 | 2 | 17 | 7 |

longer than 5 μm were observed in the airways with the majority located either within airway macrophages or on the airway lumen (Fig. 12). By 91 days, the majority of fibers in the airways had cleared with the remaining fibers observed on the ciliated epithelium which suggests that these fibers were in the process of being cleared as well.

In the parenchyma, in the chrysotile-brake dust group more than 300 million (SD: 99 million) fibers were estimated to be in the parenchyma immediately after cessation of exposure (Fig. 13). The majority of these fibers were either in airway macrophages or in contact with epithelium, alveoli, alveolar ducts, or terminal bronchioles suggesting that they were actively involved in being cleared from the lung. By 91 days, 97% of the fibers had been cleared with those remaining found either in airway macrophages or in contact with epithelium, alveoli, alveolar ducts, or terminal bronchioles.

In the brake dust group, immediately following cessation of exposure a mean of approximately 170,000 (SD: 115,000) fibers longer than 5 μm were observed in the airways corresponding to the lower fiber exposure in this group. The majority of fibers were located either within airway macrophages or on the airway lumen (Fig. 12). By 91 days, most of the fibers in the airways had cleared with the remaining fibers observed on the airway lumen.

In the parenchyma, in the brake dust group approximately 40 million (SD: 38 million) fibers were observed in the parenchyma immediately after cessation of exposure (Fig. 13). The majority of these fibers were either in airway macrophages or in contact with epithelium, alveoli, alveolar ducts, or terminal bronchioles. By 91 days, approximately 90% of the fibers had been cleared with those remaining found either in airway macrophages, alveolar ducts or in contact with epithelium, alveoli, alveolar ducts, or terminal bronchioles.

In the crocidolite asbestos exposure group a different pattern was observed. After cessation of exposure, more than 2 million (SD: 0.5 million) crocidolite fibers were estimated to be in the airways located wholly or partly inside airway macrophages, on the surface or intercalated within ciliated epithelium of the conducting airway, in airway lumen and also penetrating the airway wall or located completely underneath the airway wall, partly embedded in the interstitial space, blood vessel or lymphatics (Fig. 13). By 91 days, while more than 80% of the fiber had cleared, 350,000 (SD: 150,000) crocidolite fibers were estimated to remain in the airways largely either in airway macrophages or on the surface or intercalated within the ciliated epithelium of the conducting airways.

In the parenchyma at day 0, more than 200 million (SD: 19 million) crocidolite fibers were estimated to be either in alveolar macrophages or in contact with epithelium, alveoli, alveolar ducts or terminal bronchioles. At 32 days, while the majority of fibers were still found in alveolar macrophages or in contact with epithelium, alveoli, alveolar ducts or terminal bronchioles, a significant portion of fibers were now observed partly or intercalated within the interstitial space, blood vessels or lymphatic vessels. By 91 days, there were approximately 86 million (SD: 20 million) crocidolite fibers remaining in the parenchyma with most in similar compartments as at 32 days post-exposure. As presented in Table 3, approximately 1/3 of the observed fibers in the parenchyma at 91 days post exposure were longer than 20 μm.

## Discussion

This study was designed to determine the persistence, translocation and pathological response of the lung and pleural cavity to dust emitted from grinding of drum brakes that incorporated chrysotile into the matrix. This is the first study to evaluate brake dust by inhalation in an animal model. The interim results presented here on the lung provide a basis for evaluating the biopersistence and pulmonary response of both brake dust alone and brake dust combined with added chrysotile in comparison to the amphibole crocidolite asbestos following short term exposure. The results through 365 days including pleural translocation and response will be presented in a subsequent paper.

This study has evaluated the exposure to the full brake dust matrix and the combined exposure of the brake dust with added chrysotile. This study has not examined the possible effect of any co-exposures which may occur in parallel with brake dust exposure. The choice of exposure groups and concentrations was based upon the European Commission (EC) and the US Environmental Protection Agency (USEPA) (EUR, 18748 EN, 1999; ILSI, 2005) recommendations of having at least 100 fibers with length >20 μm/cm$^3$ in the exposure atmosphere. Given this requirement, we included group 2 as a mixed brake dust and added chrysotile exposure to address the 'worst case' scenario of the relatively insoluble particles from brake dust matrix interacting in the lung to produce an added or synergistic effect with the clearance of the chrysotile and the potential for pathological response. Group 3, brake dust, was included to provide a comparative group of exposure to the brake dust matrix alone, in order to evaluate particle effect from this matrix.

Crocidolite asbestos was included as a positive control to provide comparison to an amphibole asbestos. The crocidolite asbestos sample used in the study was unique as it was obtained as a commercial crocidolite asbestos sample (from Voorspoed mine, South Africa) which facilitated aerosol generation of the recommended exposure concentration

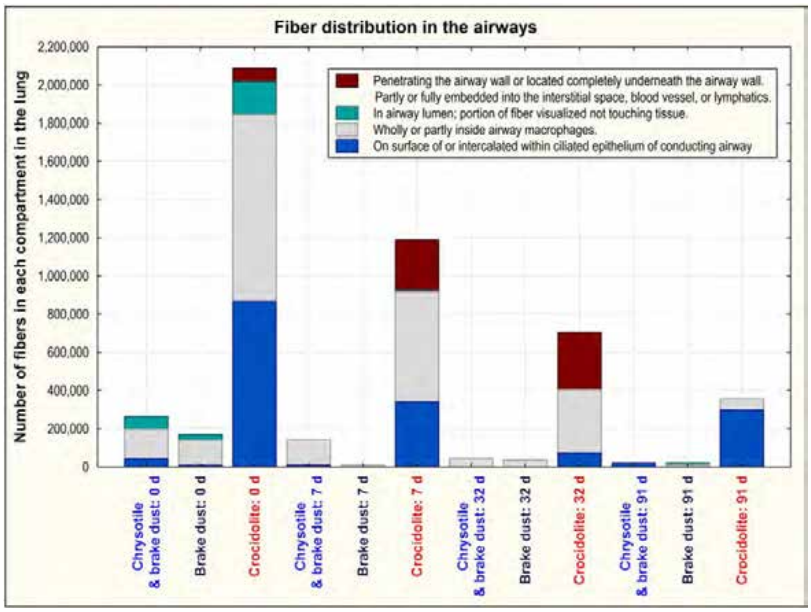



**Fig. 12.** Confocal microscopy: Compartmental fiber distribution in the airways for groups 1 Air control, 2 Chrysotile & brake dust, 3 Brake dust and 4 Crocidolite asbestos as a function of time through 91 days.

of fibers longer than 20 μm. The exposure concentration was chosen to meet the USEPA and EC recommendations.

The findings of the study are consistent with the current understanding of the differences in mineralogy and biopersistence of chrysotile fibers in brake dust compared to amphibole asbestos. Chrysotile is an acid soluble (Kobell, 1834) sheet silicate and is formed with rolled or concentric thin sheets (7.3 Å thick) composed of silicate and brucite layers with the magnesium hydroxide part of each layer closest to the fiber surface (Whittaker, 1963, 1957; Tanji et al., 1984; Titulaer et al., 1993). The magnesium is readily attacked by acid milieu such as occurs inside the macrophage (pH 4–4.5), and dissociates from the crystalline structure, leaving an unstable silicate sheet. This process causes the thin rolled sheet of the chrysotile fiber to break apart and decompose into smaller pieces. These pieces can then be readily cleared from the lung by macrophages through mucociliary and lymphatic clearance.

In contrast, crocidolite asbestos is formed as solid rods/fibrils with the silica on the outside of the fibrils which makes it very strong and durable (Skinner et al., 1988; Whittaker, 1960). Due to the structural matrix of amphibole fibers, they are formed as solid fibrils and lack acid-soluble surface groups, resulting in negligible solubility at any pH

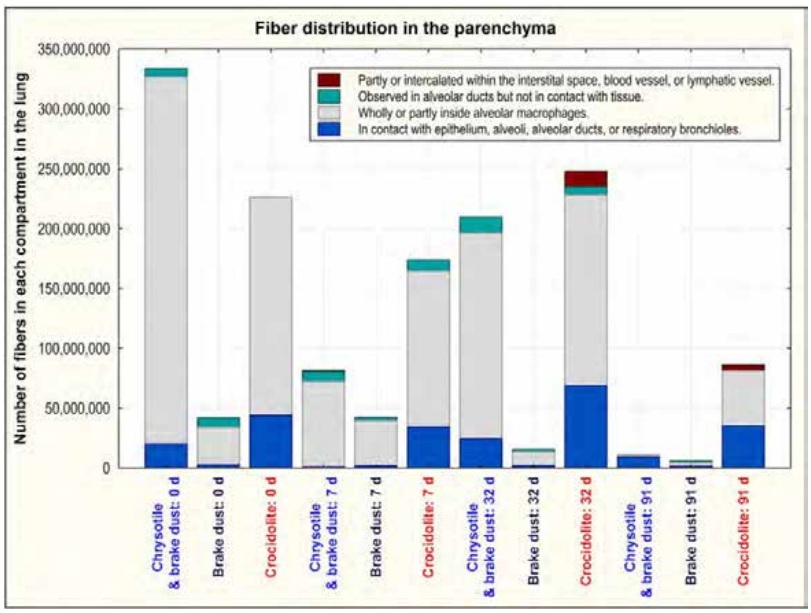



**Fig. 13.** Confocal microscopy: Compartmental fiber distribution in the parenchyma for groups 1 Air control, 2 Chrysotile & brake dust, 3 Brake dust and 4 Crocidolite asbestos as a function of time through 91 days.

**Table 4**
Fiber concentration and diameter and length range of the aerosol in the current study and Bernstein et al. (2010).

| Study | Fiber grade | Number of total fibers/cm$^3$ | Number WHO fibers/cm$^3$ | Number of fibers ≥20 μm/cm$^3$ | Diameter range (μm) | Length range (μm) |
|---|---|---|---|---|---|---|
| Chrysotile & brake dust (current study) | Added chrysotile 5R04 | 6953 | 1007 | 189 | 0.03–2.4 | 0.6–160 |
| Brake dust (current study) | Section 3.3 | 389.3 | 46.0 | 3.6 | 0.05–2.9 | 0.6–140 |
| Chrysotile & sanded material (Bernstein et al., 2010) | Added chrysotile 7RF3 | 6543 | 1496 | 295 | 0.01–0.6 | 1.0–180 |

that might be encountered in an organism (Speil and Leineweber, 1969).

One of the key factors in evaluating inhalation biopersistence/toxicology studies is accessing the respirability of the fibers (rat respirable: <~1 μm, Morgan, 1995; Weir and Meraz, 2001) and the resulting comparative exposure. In the chrysotile and brake dust aerosol 99% of the fibers L > 20 μm were <1 μm in diameter (rat-respirable) with 95% <0.4 μm in diameter. In the brake dust group, 90% were <1 μm in diameter (rat respirable) with 63% of the fibers <0.4 μm in diameter. In the crocidolite asbestos group, 88% of the long fibers were <1 μm in diameter (rat-respirable) with 21% <0.4 μm in diameter (Fig. 4). Thus fibers in all groups were largely respirable in the rat with chrysotile fibers in group 2 having the highest percentage of thinner fibers.

While the samples in this study were prepared in a similar fashion as those in Bernstein et al. (2010), Table 4 shows that the range of fibers diameters was greater in the 5R04 grade chrysotile in the current study compared to the grade 7RF3 chrysotile used in the earlier study. While in Bernstein et al. (2010) the largest diameter fiber was 0.6 μm, in the current study fibers up to 2.9 μm were in the aerosol. These larger diameter fibers as observed by SEM were either bundles of fibrils or fibers with attached brake dust particles and were of diameters that would not be rat respirable to the lung parenchyma (<~1 μm, Morgan, 1995; Weir and Meraz, 2001).

The estimated clearance times of the fibers from the lung >20 μm were 32 days for the chrysotile and brake dust group and 30 days for the brake dust alone group. In comparison to the crocidolite asbestos exposure group which had an estimated clearance half-time >1000 days, the longer chrysotile fibers cleared rapidly. As a result of their geological formation, chrysotile can vary in characteristic depending upon the mine and processing of the ore. In earlier studies of chrysotile alone or of chrysotile mixed with a joint compound (Bernstein et al., 2003, 2004, 2005a, 2005b, 2008, 2011) the clearance half-time of the longer fibers ranged from 0.7 to 11.4 days. The lack of a fiber related response in the histopathological findings strongly suggests that the few (group 2: 6.5–10 fibers counted on the filter; group 3: 1–5 fibers counted on the filter) remaining fibers longer than 20 μm in the current study were in the airways and not in the parenchyma.

Of the fibers that were > 1 μm in diameter on day 0 in the group 2 lungs, 46% were longer than 20 μm and ranged in length up to 71.5 μm. In group 3 lungs on day 0, 11% of the fibers > 1 μm in diameter were longer than 20 μm and ranged in length up to 51 μm. Such fibers could become trapped in the lower airways and with the whole lung digestion procedure could not be differentiated in terms of location in the lung. The few longer fibers observed by confocal microscopy were observed in the airways. The clearance half-time of fibers longer than 20 μm that were less than 1 μm in diameter was 1.6 days for the chrysotile and brake dust group which is within the range of the earlier studies.

Another factor which differentiates this study from an earlier comparative product study of a chrysotile with and without joint compound (Bernstein et al., 2008) is the nature of the particulate matter in the product. With the joint compound, the particles were composed primarily of calcium carbonate and easily dissolved in the lung. The effect reported by Bernstein et al. (2008) for these particles was to stimulate the sequestering of macrophage. These particles did not accumulate and were not observed by either histopathology or confocal microscopy. The matrix required for the brakes is composed of heat resistant materials designed for endurance (resistance to wear and severe use) such as epoxies and other strong binders which because of the product application could not be readily soluble (Bosch, 2011). In addition, to assure respirability in the rat, the brake dust was micronized in an in-line jet mill reducing the average diameter of the particles from that which occurs through the grinding process. With a MMAD of 1.9 μm in the brake dust group, density of approximately 2 g/cm$^3$ (Blau, 2001) and brake dust exposure concentration of 1.5 mg/m$^3$, there would have been approximately $10^{14}$ brake dust/cm$^3$ in the brake dust exposure aerosol (assuming spherical particles). In the combined chrysotile and brake dust exposure group, the total would increase to ~ $10^{19}$ particles-fibers/cm$^3$. Following exposure these particles were observed in macrophages in the lung. The particle clearance half-time for group 2 of 510 days suggests that this combined exposure concentration together with the high rate of decomposition into shorter fibers of the longer chrysotile fibers which adds to this fiber pool exceeded the ability of the lung to effectively clear the particles. High concentrations of insoluble nuisance dusts can result in lung overload which compromises the clearance mechanisms of the lung though reduced macrophage function and clearance, and even result in inflammation and a tumorigenic response in the rat (Bolton et al., 1983; Muhle et al., 1988; Morrow, 1988, 1992; Oberdörster, 1995).

The durability of longer chrysotile fibers compared to amphibole fibers has been investigated in-vitro by Osmond-McLeod et al. (2011). The durability of a number of fibers including long fiber amosite and long fiber chrysotile in a Gambles solution was assessed. The pH of the Gambles solution was adjusted to 4.5 to mimic that inside the macrophage phagolysosomes which the authors described as "potentially the most degradative environment that a particle should encounter following lung deposition and macrophage uptake". The authors reported that the data indicate that long fiber chrysotile showed 70% mass loss and a marked decrease in length with long-term incubation in the Gambles solution, with a concomitant mitigation of the pathogenicity seen in mice injected with samples. In contrast, the amphibole asbestos amosite showed no fiber shortening and did not lose its pathogenicity. This and other studies assessing the difference between the serpentine mineral chrysotile and amphibole mineral crocidolite asbestos have been reviewed recently by Bernstein et al. (2013).

In contrast, to the chrysotile fibers the long crocidolite asbestos had an estimated clearance half time of >1000 days. In previous biopersistence studies with amphibole asbestos, the clearance half-time was reported to range from 418 to >1000 days (Musselman et al., 1994; Hesterberg et al., 1996, 1998; Bernstein et al., 2005b, 2011).

No significant pathological response was observed at any time point in response to the added chrysotile or the brake dust. A low level macrophage response was observed in response to the large number of particles in the exposure atmosphere, with no further evolution observed. The absence of pathological response in chrysotile/brake dust and brake dust groups was confirmed through classical histopathological examination which determined that the Wagner score ranged between 1 (unexposed normal-air control) and 2 (minimal cellular change). Quantitative fibrotic response was also evaluated through confocal microscopy which determined that the amount of connective tissue/fibrosis present following exposure in the chrysotile/brake dust

and brake dust groups was similar to that measured in the air control group.

Following 5 days of exposure to crocidolite asbestos, a notable inflammatory response occurred immediately following cessation of exposure with numerous long fibers observed in both the airways, distal airways and the parenchyma and associated with a cellular/macrophage response with collagen formation. The crocidolite fibers in the parenchyma and in the distal airways persisted with the inflammatory response progressing to Wagner grade 4 interstitial fibrosis within 32 days. The confocal assessment of the collagen in the connective tissue revealed a progression in connective tissue proliferation which increased linearly through 91 days ($r = 0.83$, $p = 0.0055$) with a 5-fold increase in mean collagen levels in the crocidolite group at 91 days, compared to the air control group.

As the exposure concentration for fibers longer than 20 μm in groups 2 and 4 were in the same range, if both fibers were equally biopersistent, the mean concentration of fibers longer than 20 μm in the lung would have been similar. As presented above, the fibers longer than 20 μm in the group 2 exposure aerosol were thinner than those in the group 4 aerosol with 98% less than 1 μm in diameter. This would suggest that the large majority of the longer chrysotile fibers would have deposited in the lung parenchyma and had cleared quickly with only the fibers in bundles which were most likely in the airways clearing more slowly. The clearance half-time of the long chrysotile fibers that were less than 1 μm in diameter was 1.6 days.

The results from this study provide a scientific basis that following short term exposure the no observable effect level for chrysotile in brake dust is less than 1000 WHO fibers/cm$^3$ (including 189 fibers L > 20 μm/cm$^3$). In addition, these results support the lung burden and epidemiological studies reviewed by Marsh et al. (2011), Paustenbach et al. (2004), Laden et al. (2004), and Butnor et al. (2003), which differentiate that the chrysotile in brake dust is not associated with disease. These studies have taken into consideration possible confounders such as smoking and occupational exposures.

The pathological response following short term crocidolite asbestos exposure at a concentration of 709 WHO fibers/cm$^3$ (including 93 fibers L > 20 μm/cm$^3$) emphasizes the importance of even small exposures to amphibole asbestos such as crocidolite in the etiology of asbestos related disease.

## Conclusions

The interim results of this study show that there is an important difference in biopersistence and pathological response in the lung between brake dust derived from brake pads manufactured with chrysotile in comparison to the amphibole, crocidolite asbestos. The pathological response was determined using two independent methods. Classical histopathological examination was performed on thin lung sections with scoring of the collagen level at the bronchoalveolar junctions as well as the Wagner score. In addition, the collagen deposition in the connective tissue of the lung was evaluated using confocal microscopy in order to assess the fibrotic response.

No significant pathological response was observed at any time point in the brake dust or chrysotile/brake dust exposure groups. Slight macrophage accumulation was noted in response to the high particle exposure levels in the test atmospheres and the decomposition of the longer chrysotile fibers into shorter fibers or particles. This was reflected as well in the Wagner score which ranged from 1 to 2 (with one being the level in the air control group). The long chrysotile fibers cleared quickly with clearance halftimes estimated as 30 and 33 days respectively in the brake dust and the chrysotile/brake dust exposure group. Using the quantitative evaluation of fibrotic response with confocal microscopy, there was no statistically significant difference trend between the air control group and either the brake dust alone or the brake dust with chrysotile exposure group at any time point through 91 days after cessation of exposure.

The crocidolite asbestos sample used in the study was unique as it was obtained as a commercial crocidolite asbestos sample from the mine such as would have been destined to be shipped to the manufacturing end-user. The crocidolite asbestos produced inflammatory response from day 0 which progressed to Wagner grade 4 interstitial fibrosis within 32 days following cessation of exposure. In addition, the confocal microscopy evaluation of the fibrotic response in the connective tissue showed a linear increase in fibrotic response through 91 days after cessation of exposure. When compared to the air control group at 91 days, the mean level of fibrotic response was 5 times greater. The long crocidolite fibers had a clearance half-time of greater than 1000 days.

There are many brake linings still in use worldwide that contain chrysotile. This study provides in-vivo toxicological support that brake dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung following short term inhalation.

## Conflict of interest statement

This study was funded by Honeywell International Inc. The affiliations of the authors are as shown on the cover page and include research laboratories, government institute, corporate affiliations, as well as independent toxicology consultant. This publication is the professional work product of the authors and may not necessarily represent the views of the corporate sponsor. One of the authors, David Bernstein, has appeared as an expert witness in litigation concerned with alleged health effects of exposure to chrysotile. Honeywell is a defendant in asbestos-product litigation and its predecessor manufactured the automotive brakes used in this study. There have been periodic communications between Honeywell and the authors concerning the status of this study. The contribution of Prof JI Phillips is based on research supported by the National Research Foundation.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.taap.2014.01.016.

## References

Antonini, J.M., Charron, T.G., Roberts, J.R., et al., 1999. Application of laser scanning confocal microscopy in the analysis of particle-induced pulmonary fibrosis. Toxicol. Sci. 51 (1), 126–134 (Sep).

Bernstein, D.M., Riego-Sintes, J.M.R., 1999. Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF). In: Bernstein, D.M., Riego-Sintes, J.M.R. (Eds.), Vol. EUR 18748 EN, April 93. European Commission Joint Research Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau (Available from: http://ecb.ei.jrc.it/DOCUMENTS/Testing-Methods/mmmfweb.pdf).

Bernstein, D.M., Mast, R., Anderson, R., et al., 1994. An experimental approach to the evaluation of the biopersistence of respirable synthetic fibers and minerals. Environ. Health Perspect. 102 (Supplement 5), 15–18.

Bernstein, D.M., Rogers, R., Smith, P., 2003. The biopersistence of Canadian chrysotile asbestos following inhalation. Inhal. Toxicol. 15, 101–128.

Bernstein, D.M., Rogers, R., Smith, P., 2004. The biopersistence of Brazilian chrysotile asbestos following inhalation. Inhal. Toxicol. 16, 745–761.

Bernstein, D.M., Rogers, R., Smith, P., 2005a. The biopersistence of Canadian chrysotile asbestos following inhalation: final results through 1 year after cessation of exposure. Inhal. Toxicol. 17, 1–14.

Bernstein, D.M., Chevalier, J., Smith, P., 2005b. Comparison of Calidria chrysotile asbestos to pure tremolite: final results of the inhalation biopersistence and histopathology following short term exposure. Inhal. Toxicol. 17, 427–449.

Bernstein, D.M., Donaldson, K., Decker, et al., 2008. A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. Inhal. Toxicol. 20, 1009–1028.

Bernstein, D.M., Rogers, R.A., Sepulveda, R., et al., 2010. The pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite asbestos following short term inhalation exposure — interim results. Inhal. Toxicol. 22, 937–962.

Bernstein, D.M., Rogers, R.A., Sepulveda, R., et al., 2011. Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine

particles compared to amosite-asbestos following short-term inhalation exposure. Inhal. Toxicol. 23, 372–391.
Bernstein, D., Dunnigan, J., Hesterberg, T., et al., 2013. Health risk of chrysotile revisited. Crit. Rev. Toxicol. 43 (2), 154–183 (Feb).
Blau, P.J., 2001. Compositions, functions, and testing of friction brake materials and their additives. Oak Ridge National Laboratory report ORNL/TM-2001/64, Oak Ridge, Tennessee.
Bolton, R.E., Vincent, J.H., Jones, A.D., et al., 1983. An overload hypothesis for pulmonary clearance of UICC amosite fibers inhaled by rats. Br. J. Ind. Med. 40, 264–272.
Bosch, R., 2011. Bosch Automotive Handbook, Updated 8th Edition. Robert Bosch GmbH Bentley Publishers (ISBN-13: 978-0-8376-1686-5).
Butnor, K.J., Sporn, T.A., Roggli, V.L., 2003. Exposure to brake dust and malignant mesothelioma: a study of 10 cases with mineral fiber analyses. Ann. Occup. Hyg. 47 (4), 325–330.
Cannon, W.C., Blanton, E.F., McDonald, K.E., 1983. The flow-past chamber: an improved nose-only exposure system for rodents. Am. Ind. Hyg. Assoc. J. 44 (12), 923–928.
Cossette, M., Delvaux, P., 1979. Technical evaluation of chrysotile asbestos ore bodies. In: Ledoux, R.C. (Ed.), Short Course in Mineralogical Techniques of Asbestos Determination. Mineralogical Association of Canada, Toronto, Canada, pp. 79–109 (May).
Dodson, R.F., Hammer, S.P., 2012. Asbestos: Risk Assessment, Epidemiology and Health Effects, 2nd edition. CRC Press, Taylor & Francis Group, Boca Raton, Florida.
EUR 18748 EN, 1999. Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF). In: Bernstein, D.M., Riego-Sintes, J.M.R. (Eds.), Vol. EUR 18748 EN, April. 93. European Commission Joint Research Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau (http://ecb.ei.jrc.it/DOCUMENTS/Testing-Methods/mmmfweb.pdf).
Finley, B.L., Pierce, J.S., Paustenbach, D.J., et al., 2012. Malignant pleural mesothelioma in US automotive mechanics: reported vs expected number of cases from 1975 to 2007. Regul. Toxicol. Pharmacol. 64 (1), 104–116.
Freeman, M.D., Kohles, S.S., 2012. Assessing specific causation of mesothelioma following exposure to chrysotile asbestos-containing brake dust. Int. J. Occup. Environ. Health 18 (4), 329–336 (Oct–Dec).
Harper, G.A., 1998. Brakes and Friction Materials: The History and Development of the Technologies. Mechanical Engineering Publications Limited, London, England.
Hesterberg, T.W., Miiller, W.C., Musselman, R.P., et al., 1996. Biopersistence of man-made vitreous fibers and crocidolite asbestos in the rat lung following inhalation. Fundam. Appl. Toxicol. 29 (2), 269–279.
Hesterberg, T.W., Chase, G., Axten, C., et al., 1998. Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. Toxicol. Appl. Pharmacol. 151 (2), 262–275.
ILSI, Bernstein, D., Castranova, V., Donaldson, K., et al., 2005. Testing of fibrous particles: short-term assays and strategies. Inhal. Toxicol. 17, 497–537.
Kobell, F., 1834. Ueber den schillernden Asbest von Reichenstein in Schlesien. J. Prakt. Chem. 2, 297–298.
Laden, F., Stampfer, M.J., Walker, A.M., 2004. Lung cancer and mesothelioma among male automobile mechanics: a review. Rev. Environ. Health 19 (1), 39–61.
Lemen, R.A., 2004. Asbestos in brakes: exposure and risk of disease. Am. J. Ind. Med. 45 (3), 229–237.
Lin, L.I-K., 2000. A note on the concordance correlation coefficient. Biometrics 56, 324–325.
Lin, Lawrence I-Kuei, 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics (Int. Biom. Soc.) 45 (1), 255–268.
Marsh, G.M., Youk, A.O., Roggli, V.L., 2011. Asbestos fiber concentrations in the lungs of brake repair workers: commercial amphiboles levels are predictive of chrysotile levels. Inhal. Toxicol. 23 (12), 681–688.
McConnell, E.E., Davis, J.M., 2002. Quantification of fibrosis in the lungs of rats using a morphometric method. Inhal Toxicol. 14 (3), 263–272.
McConnell, E.E., Wagner, J.C., Skidmore, et al., 1984. A comparative study of the fibrogenic and carcinogenic effects of UICC Canadian chrysotile B asbestos and glass microfibre (JM 100). In: Biological Effects of Man-made Mineral Fibres. World Health Organization, pp. 234–252.
Morgan, A., 1995. Deposition of inhaled asbestos and man-made mineral fibers in the respiratory tract. Ann. Occup. Hyg. 39 (5), 747–758.
Morrow, P.E., 1988. Possible mechanisms to explain dust overloading of the lung. Fundam. Appl. Toxicol. 10, 369–384.
Morrow, P.E., 1992. Dust overloading of the lungs: update and appraisal. Toxicol. Appl. Pharmacol. 113, 1–12.
Muhle, H., Bellman, B., Heinrich, U., 1988. Overloading of lung clearance during chronic exposure of experimental animals to particles. Ann. Occup. Hyg. 32, 141–147.
Musselman, R.P., Miiller, W.C., Eastes, W., et al., 1994. Biopersistences of man-made vitreous fibers and crocidolite fibers in rat lungs following short-term exposures. Environ. Health Perspect. 102 (Suppl. 5), 139–143.
Oberdörster, G., 1995. Lung particle overload: implications for occupational exposures to particles. Regul. Toxicol. Pharmacol. 21, 123–135.
Osmond-McLeod, M.J., Poland, C.A., Murphy, F., et al., 2011. Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibers. Part Fiber Toxicol. 8, 15.
Paustenbach, D.J., Finley, B.L., Lu, E.T., et al., 2004. Environmental and occupational health hazards associated with the presence of asbestos in brake linings and pads (1900 to present): a "state-of-the-art" review. J. Toxicol. Environ. Health B Crit. Rev. 7 (1), 25–80 (Jan-Feb).
Richter, R.O., Finley, B.L., Paustenbach, D.J., et al., 2009. An evaluation of short-term exposures of brake mechanics to asbestos during automotive and truck brake cleaning and machining activities. J. Expo. Sci. Environ. Epidemiol. 19 (5), 458–474 (Jul).
Rogers, R.A., Antonini, J.M., Brismar, H., et al., 1999. In situ microscopic analysis of asbestos and synthetic vitreous fibers retained in hamster lungs following inhalation. Environ. Health Perspect. 107 (5), 367–375 (May).
Shedd, K.B., 1985. Fiber dimensions of crocidolites from Western Australia, Bolivia, and the Cape and Transvaal Provinces of South Africa. U.S. Bureau of Mines Report of Investigations 8998United States Department of the Interior.
Skinner, H.C.W., Ross, M., Frondel, C., 1988. Asbestos and Other Fibrous Materials — Mineralogy, Crystal Chemistry, and Health Effects. Oxford University Press, New York (NY).
Speil, S., Leineweber, J.P., 1969. Asbestos minerals in modern technology. Environ. Res. 2, 166–208.
StatSoft, Inc., 2011. STATISTICA (data analysis software system), version 12. www.statsoft.com.
Tanji, T., Yada, K., Akatsuka, Y., 1984. Alternation of clino- and orthochrysotile in a single fiber as revealed by high-resolution electron microscopy. Clay. Clay Miner. 32 (5), 429–432 (October 1984).
Titulaer, M.K., van Miltenburg, J.C., Jansen, J.B.H., et al., 1993. Characterization of tubular chrysotile by thermoporometry, nitrogen sorption, drifts, and TEM. Clay. Clay Miner. 41, 496–513.
VDI Guideline 3492, 2004. Indoor Air Measurement, Ambient Air Measurement, Measurement of Inorganic Fibrous Particles, Scanning Electron Microscopy Method. Verein Deutscher Ingenieure e.V, Düsseldorf.
Virta, R.L., 2002. Asbestos: Geology, Mineralogy, Mining, and Uses. Prepared in Cooperation with Kirk-Othmer Encyclopedia of Chemical Technology. USGS Open file 02-149, Online Edition. Wiley-Interscience, a division of John Wiley & Sons, Inc., New York (NY).
Weir, F.W., Meraz, L.B., 2001. Morphological characteristics of asbestos fibers released during grinding and drilling of friction products. Appl. Occup. Environ. Hyg. 16 (12), 1147–1149.
Whittaker, E.J.W., 1957. The structure of chrysotile. V. Diffuse reflexions and fiber texture. Acta Crystallogr. 10, 149–156.
Whittaker, E.J.W., 1960. The crystal chemistry of the amphiboles. Acta Crystallogr. 13, 291–298.
Whittaker, E.J.W., 1963. Research report: Chrysotile fibers — filled or hollow tubes? Mathematical interpretation may resolve conflicting evidence. Chem. Eng. News 41, 34–35 (September 30, 1963).
WHO, 1985. Reference Methods for Measuring Airborne Man-Made Mineral Fibers (MMMF), WHO/EURO MMMF Reference Scheme. World Health Organisation, Copenhagen.

STUDY No. 7

# EVALUATION OF THE FATE AND PATHOLOGICAL RESPONSE IN THE LUNG AND PLEURA OF BRAKE DUST ALONE AND IN COMBINATION WITH ADDED CHRYSOTILE COMPARED TO CROCIDOLITE ASBESTOS FOLLOWING SHORT-TERM INHALATION EXPOSURE

**STUDY No. 7**

# EVALUATION OF THE FATE AND PATHOLOGICAL RESPONSE IN THE LUNG AND PLEURA OF BRAKE DUST ALONE AND IN COMBINATION WITH ADDED CHRYSOTILE COMPARED TO CROCIDOLITE ASBESTOS FOLLOWING SHORT-TERM INHALATION EXPOSURE

**Toxicology and Applied Pharmacology 283 (2015) 20-34**

**D. M. Bernstein, R. A. Rogers, R. Sepulveda, P. Kunzendorf, B. Bellmann, H. Ernst, O. Creutzenberg, J. I. Phillips**

This study was designed to provide an understanding of the biokinetics and potential toxicology in the lung and pleura following inhalation of brake dust following short term exposure in rats. The deposition, translocation and pathological response of brake dust derived from brake pads manufactured with chrysotile was evaluated in comparison to the amphibole, crocidolite, asbestos. Rats were exposed by inhalation 6 h/day for 5 days to either brake dust obtained by sanding of brake drums manufactured with chrysotile, a mixture of chrysotile and the brake dust or crocidolite asbestos. The chrysotile fibers were relatively biosoluble whereas the crocidolite asbestos fibers persisted through the life-time of the animal. This was reflected in the lung and the pleura where no significant pathological response was observed at any time point in the brake dust or chrysotile/ brake dust exposure groups through 365 days post exposure. In contrast, crocidolite asbestos produced a rapid inflammatory response in the lung parenchyma and the pleura, inducing a significant increase in fibrotic response in both of these compartments. Crocidolite fibers were observed embedded in the diaphragm with activated mesothelial cells immediately after cessation of exposure. While no chrysotile fibers were found in the mediastinal lymph nodes, crocidolite fibers of up to 35 $\mu$m were observed. These results provide support that brake dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung or the pleural cavity following short term inhalation.

Toxicology and Applied Pharmacology 283 (2015) 20–34

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

# Evaluation of the fate and pathological response in the lung and pleura of brake dust alone and in combination with added chrysotile compared to crocidolite asbestos following short-term inhalation exposure

D.M. Bernstein [a,*], R.A. Rogers [b], R. Sepulveda [b], P. Kunzendorf [c], B. Bellmann [d,1], H. Ernst [d], O. Creutzenberg [d], J.I. Phillips [e]

[a] Consultant in Toxicology, Geneva, Switzerland
[b] Rogers Imaging, Needham, MA, USA
[c] GSA Gesellschaft für Schadstoffanalytik mbH, Ratingen, Germany
[d] Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany
[e] National Institute for Occupational Health, National Health Laboratory Service, Johannesburg South Africa and Department of Biomedical Technology, Faculty of Health Sciences, University of Johannesburg, Johannesburg, South Africa

ARTICLE INFO

*Article history:*
Received 17 September 2014
Revised 26 November 2014
Accepted 23 December 2014
Available online 2 January 2015

*Keywords:*
Brake dust
Chrysotile
Crocidolite asbestos
Fiber inhalation toxicology
Lung
Pleura

ABSTRACT

This study was designed to provide an understanding of the biokinetics and potential toxicology in the lung and pleura following inhalation of brake dust following short term exposure in rats. The deposition, translocation and pathological response of brake-dust derived from brake pads manufactured with chrysotile were evaluated in comparison to the amphibole, crocidolite asbestos. Rats were exposed by inhalation 6 h/day for 5 days to either brake-dust obtained by sanding of brake-drums manufactured with chrysotile, a mixture of chrysotile and the brake-dust or crocidolite asbestos. The chrysotile fibers were relatively biosoluble whereas the crocidolite asbestos fibers persisted through the life-time of the animal. This was reflected in the lung and the pleura where no significant pathological response was observed at any time point in the brake dust or chrysotile/brake dust exposure groups through 365 days post exposure. In contrast, crocidolite asbestos produced a rapid inflammatory response in the lung parenchyma and the pleura, inducing a significant increase in fibrotic response in both of these compartments. Crocidolite fibers were observed embedded in the diaphragm with activated mesothelial cells immediately after cessation of exposure. While no chrysotile fibers were found in the mediastinal lymph nodes, crocidolite fibers of up to 35 μm were observed. These results provide support that brake-dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung or the pleural cavity following short term inhalation.



## Introduction

The study is unique in that it has examined the pathological response and fiber distribution in the lung and in the pleural cavity of brake dust from chrysotile containing brake drums. In the interim results on the lung which were presented in Bernstein et al. (2014), the brake dust from chrysotile containing brake drums was shown to produce no pathological response in the lung through 91 days following short-term exposure in rats. The study also demonstrated the importance of amphibole asbestos exposure in comparison to chrysotile in the etiology of asbestos related lung disease. This study was continued through 365 days post exposure in order to assess the evolution of these findings and includes further results from the lung analyses and from the analysis of the pleural cavity from the study including assessment of the visceral and parietal pleural surfaces.

Chrysotile fibers were found to be effective since the 1900s in manufacturing brake materials with the ability to withstand heat and control speed. The surface of the brake drums often needed to be sanded to assure a proper fit. This study was designed to evaluate the hypothesis of whether brake dust from sanded chrysotile containing brake drums

*Abbreviations:* Crl: Wi(Han), Wistar rats, Specific Pathogen Free from Charles River Deutschland; SEM, scanning electron microscope; WHO, World Health Organization; MMMF, man made mineral fibers; VDI, Verein Deutscher Ingenieure (English, Association of German Engineers); GMD, Geometric mean diameter; GML, Geometric mean length; GSD, Geometric standard deviation; MMAD, Mass median aerodynamic diameter; %CT/FOV, percentage of the elastin and collagen per area of lung tissue; CM, Confocal microscopy; TGF-ß, Transforming growth factor (TGF)-β; bFGF, Basic fibroblast growth factor; PDGF, Platelet-derived growth factor; CTGF, connective tissue growth factor

* Corresponding author. Fax: +41 22 735 1463.
*E-mail addresses:* davidb@itox.ch (D.M. Bernstein), rarogers5@yahoo.com (R.A. Rogers), Peter.Kunzendorf@GSA-Ratingen.de (P. Kunzendorf), Heinrich.ernst@item.fraunhofer.de (H. Ernst), jim.phillips@nioh.nhls.ac.za (J.I. Phillips).

[1] Deceased.

http://dx.doi.org/10.1016/j.taap.2014.12.012

will produce a pathological response following short term exposure in rats. Brake dust has not been previously evaluated in animal studies.

The techniques used in this study have been designed to sample the thin pleural surfaces with minimal alteration of the homeostatic balance and fiber location. Two independent methods were developed for examining the translocation of fibers to the pleural cavity and any associated inflammatory response following exposure to either brake dust with added chrysotile, brake dust alone or crocidolite asbestos. These methods included the in situ examination of the lungs and pleural space including the visceral and parietal pleural obtained from freeze substituted tissue in deep frozen rats and the examination of the diaphragm as a parietal pleural tissue.

In examining the visceral pleural environment, including the subpleural lung, the visceral pleural itself, and the pleural space, a non-invasive method for determining fiber location, size, inflammatory and fibrotic response was used on rats which were deep frozen immediately after killing. In addition, the visceral pleural wall thickness and the amount of collagen per field of view (fibrotic response) in the visceral pleura was quantified using confocal microscopy procedures.

The diaphragm was chosen as the parietal pleural tissue for examination because it can be quickly removed at necropsy with minimal alteration of the visceral lung surface and it has a high density of lymphatic stomata (Negrini et al., 1991; Negrini and Moriondo, 2013). A fixed area which included lymphatic drainage sites (stomata) on the diaphragmatic surface was selected for examination of possible inflammatory response using scanning electron microscopy (SEM) and for the presence of fibers.

## Methods

The aerosol generation/exposure, in-life and pathology phases of this study were performed by the Fraunhofer Institute for Toxicology and Experimental Medicine (Hannover, Germany) in compliance with the Principles of Good Laboratory Practice (German Chemicals Act §19a, Appendix 1, July 02, 2008, Federal Law Gazette I, No. 28, p. 1146) and the German animal protection law (Tierschutzgesetz of May 18, 2006, German Federal Law Gazette I, page 1206, 1313). The fiber counting and sizing was performed by Gesellschaft für Schadstoffanalytik mbH (Ratingen, Germany). The confocal microscopy was performed by Rogers Imaging (Needham, Massachusetts, USA).

### *Brake dust*

The brake dust was produced directly from chrysotile-containing friction products (automotive drum brake shoes) by the RJ LeeGroup Ltd. (Monroeville, PA, USA). The shoes were designed to fit the drum brakes of mid-1960's Chevrolet Impala model cars. The friction material was evaluated and found to contain approximately 30% (by area) chrysotile asbestos (analyzed in accordance with EPA 600/R-93/116). No amphibole asbestos minerals have been observed in any of the aerosol or lung samples from these brake shoes or in the added chrysotile used in this study.

The brake drums were ground using a commercial AMMCO arc grinder (Model 8000, S/N 24788) with a modified dust collection system. The arc grinder is a motorized sander that is swept across the surface of the brake shoe with the dust collected on an attached $8 \times 10$ inch quartz micro-fiber filter that was used in place of a collection bag. A Tisch high volume air sampler sampling pump (Tisch Environmental Inc., Ohio, USA) was used following the filter to provide uniform sampling suction over the course of the grinding operation. All brake dust preparation took place at the RJ LeeGroup facility in a room equipped with an Aramsco Comanche® HEPA ventilation unit (Model 55011) with a nominal flowrate of 1800 cfm (50 $m^3$/min). The brake dust was produced directly from asbestos-containing friction products (automotive drum brake shoes) by the RJ LeeGroup Ltd. (Monroeville, PA, USA) as described previously (Bernstein et al., 2014).

### *Chrysotile*

The chrysotile fiber used in this study had the mineralogical grade of 5R04 according to the Canadian chrysotile asbestos classification (Cossette and Delvaux, 1979). The chrysotile grade 5R04 sample was chosen based upon an evaluation of which chrysotile grade was ordered or supplied for use in brake manufacturing in a random search of 67 formulations dating from 1964 to 1986. All of the grade 5R04 chrysotile in these brakes was supplied by Johns-Manville. The chrysotile sample used in this study was obtained directly from Mine Jeffery Canada (formerly the Johns-Manville Mine).

### *Crocidolite asbestos*

The crocidolite asbestos sample used in this study was from the Voorspoed mine in South Africa was obtained from the National Institute of Occupational Health – NIOH, South Africa. This mine is located in Limpopo Province which at the time when mining took place was called Transvaal Province. The chemical compositions of chrysotile, a serpentine asbestos, and crocidolite, an amphibole asbestos, have been described previously (Shedd, 1985; Virta, 2002). A key difference with this crocidolite asbestos sample is that it was received as produced without subsequent grinding. The crocidolite asbestos used previously in animal studies has been largely either the Union for International Cancer Control (UICC) or US National Institute of Environmental Health Sciences (NIEHS) prepared crocidolite. Both of these samples were ground extensively more than 30 years ago using large scale industrial mills resulting in size distribution not typical of the commercial product (Bernstein et al., 2013).

## Experimental design

The experimental design of the study has been illustrated in the flowchart in Fig. 1 of Bernstein et al., 2014. All end points were analyzed for each group with the exception that lung digestion was not performed in the control group on Days 1, 2 and 7 in order to limit animal use.

### *Animal exposure*

Groups of laboratory rats (Groups 1, 2, 3 and 4) were exposed for 6 h per day for 5 days to:

Group 1: Filtered air (negative control group) (Total 65 animals).
Group 2: Brake dust powder mixed with chrysotile 5R04 (Total 100 animals).
Group 3: Brake dust powder (Total 100 animals).
Group 4: Crocidolite asbestos (Total 100 animals).

For groups 2 and 4, the exposure concentrations were set based upon the number of fibers longer than 20 μm/$cm^3$. In group 2, the chrysotile concentration was increased over that recommended by the EC Biopersistence Protocol (Bernstein and Riego-Sintes, 1999) of 100 fibers L > 20 μm/$cm^3$ due to the tendency of chrysotile to clump (this was minimized through the use of the cyclone, see below). Group 3 was included as a comparative exposure of the brake dust particulate material (with a relatively low aerosol concentration of chrysotile fibers) using a similar gravimetric exposure concentration as the brake dust component of group 2. A negative control group 1 was exposed in a similar fashion to filtered air.

Weanling (8–10 weeks old at exposure) male Wistar rats [Crl: WI(Han)], Specific Pathogen Free from Charles River Deutschland, Sulzfeld, Germany) were used. The rats were exposed by flow-past nose-only exposure for 6 h/day for a period of 5 consecutive days. In groups 2, 3, and 4; 7 animals per sub-group were allocated for lung burden evaluation at each time point. In the control group 1; 5 animals per sub-group were allocated for lung burden evaluation (no animals at days 1, 2 & 7). For the Confocal lung and histopathology, 3 animals per

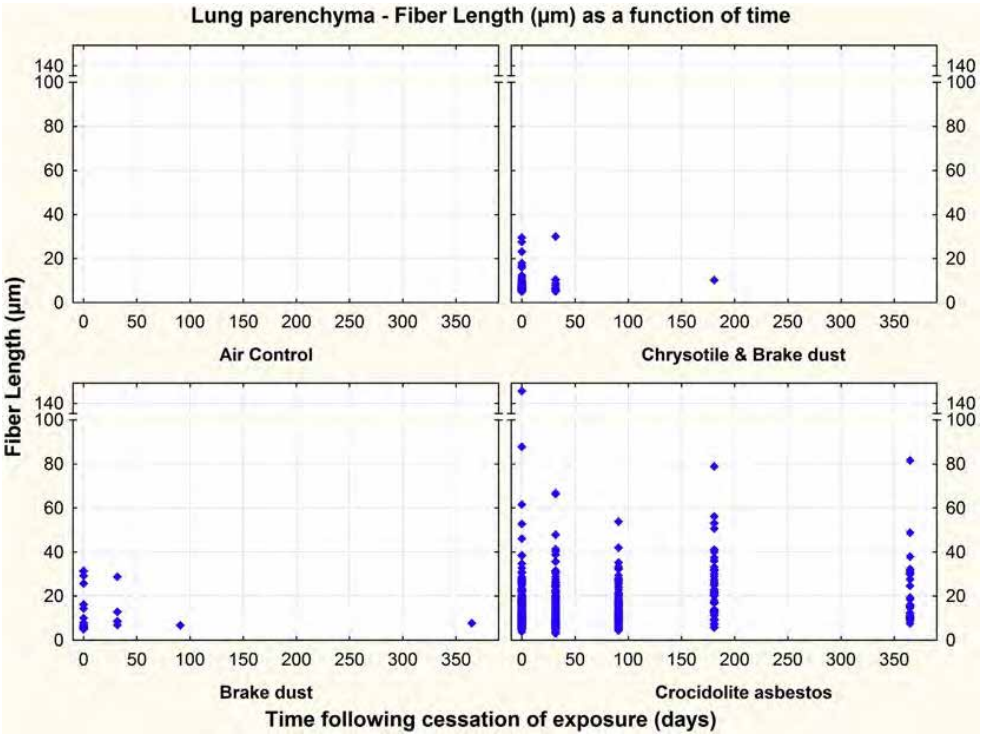



**Fig. 1.** Number and length of fibers observed in the lung parenchyma by confocal microscopy.

sub-group were allocated at each time point. For the low temperature Confocal microscopy, 3 animals per sub-group were allocated at each time point.

*Exposure system*

The fiber aerosol generation system (Model CR 3020, CR Equipments, Switzerland) was designed to loft the bulk fibers without breaking, grinding or contaminating the fibers (Bernstein et al., 1994). The animals were exposed using stainless steel flow-past nose/snout-only inhalation exposure systems with 16 animals per level. This system was derived from Cannon et al. (1983) and is different from conventional nose-only exposure systems in that fresh fiber aerosol is supplied to each animal individually and exhaled air is immediately exhausted. The exposure units were placed in separate ventilated chambers connected to the animal room to avoid cross contamination between the groups.

For group 2 (chrysotile fiber 5R04 mixed with brake dust powder), a fiber aerosol was generated from chrysotile fiber 5R04 and separately a dust aerosol from the brake dust using individual rotating brush aerosol generators (Bernstein et al., 2014). The fiber aerosol generator used was followed by a 500 mL pyrex glass cyclone to assist in the elimination of any remaining fiber bundles from the aerosol. The brake dust aerosol generator was followed by a micronising jet mill to reduce the particle size to be rat respirable. Following each generator, in-line $^{63}$Ni charge neutralisers reduced the electrostatic charge from fibers and particulate material in the generated aerosols. Following the charge neutralizers, the fiber and powder aerosols were mixed through a Y-piece connection and then delivered directly into the nose-only flow-past exposure chamber. The group 3 brake dust aerosol was generated using only the 'powder aerosol generator'.

The group 4 crocidolite asbestos aerosol was generated using only the 'fiber aerosol generator'. A pre-study technical trial revealed stronger electrostatic properties of the crocidolite fiber aerosol which resulted in losses in the transfer tubing. To achieve a similar degree of neutralization with similar fiber transfer efficiency as with the chrysotile an electronic charge neutraliser at the brush head of the aerosol generator (WEKO Model AP230, Weitmann & Konrad GmbH, Germany) was used in addition to the $^{63}$Ni charge neutraliser.

*Exposure system monitoring*

The aerosol concentration was monitored continuously using an aerosol photometer developed by Fraunhofer ITEM. Actual concentrations were measured in the breathing zone of the animals as described below. The temperature and the relative humidity of the exposure atmosphere were monitored continuously with data on temperature, relative humidity, and air flow rate collected by the Fraunhofer ITEM animal exposure facility computer system.

Gravimetric Determination of Aerosol Concentrations: Gravimetric determinations of aerosol concentration were performed at least once daily for each group with samples collected on a Millipore® glass fiber filter (Type 13400-25-J) for approximately 4–6 hours per day.

Fiber number and size distribution of Aerosol Concentrations: Aerosol samples for bivariate analysis of fiber size distribution and counting were collected onto NUCLEPORE® filters (PC membrane, 25 mm, pore size 0.8 µm – SN 110.609, Whatman Ltd.) for approximately 2 hours successively during each exposure period in parallel with the gravimetric sampling. For group 1 (air control) one sample per treatment day was collected over approximately 5 hours per day. These samples were analysed for bivariate fiber size distribution and counting (# fibers/cm$^3$ aerosol) using analytical Scanning Electron Microscopy (SEM) with Energy Dispersive x-ray analysis (EDAX).

Counting rules for the evaluation of air and lung samples by scanning electron microscopy (Fiber/Particle Analysis and Lung Digestion): Unless otherwise specified, the basis for the evaluation using the scanning electron microscope (SEM) was WHO-Reference Methods for measuring airborne man made mineral fibers (MMMF) WHO (1985) and the VDI Guideline 3492 (2004).

All objects seen at the magnification of 10,000× (acceleration voltage 25 kV) were sized with no lower or upper limit imposed on either length or diameter. The bivariate length and diameter was recorded individually for each object measured. Fibers were defined as any object that had an aspect ratio of at least 3:1. The diameter was determined at the greatest width of the object. All other objects were considered as non-fibrous particles. Fibers with both ends in the field of view had the "weight" of one fiber (=2 fiber ends), fibers with only one end within the field had the "weight" of a half fiber (=1 fiber end). Fibers without any fiber end in the field of view were not counted nor measured.

The stopping rules for counting each sample were defined as follows.

Fibrous objects: The minimum numbers of fibers examined were:

a) fibers with a length <5 μm = 100 fibers (200 fiber ends)
b) fibers with a length between 5 and 20 μm = 200 fibers (400 fiber ends)
c) fibers with a length >20 μm = 100 fibers (200 fiber ends)

Fields of view were examined for each length category until the defined minimum number of fibers for each length category was recorded or a maximum of 1 $mm^2$ of the filter surface was examined in case the fiber minimum number for the length category was not reached. For samples of the control group an area of 0.5 $mm^2$ of the filter was evaluated. These counting rules were based on the number of fibers per sample necessary in order to have statistical reproducibility of the means (EUR, 18748 EN, 1999). For non-fibrous objects, fields of view were examined until a total of 100 particles were recorded or the aforementioned stopping criteria for fibers were reached.

The details of the procedures for clinical examination and body weights; gross pathology and organ weights; tissue preparation for lung ashing; histopathology and for the confocal microscopy of the lung are presented in Bernstein et al., 2014.

*Diaphragm tissue preparation*

Diaphragms were excised from the chest wall, pinned flat to stiff filter paper with parietal surface facing up and immersion fixed in modified Karnovski's fixative. Specimens were then dispatched to Rogers Imaging Corporation (Needham Heights, MA, USA) for processing and analysis. Upon arrival, the fixed diaphragms were piece dissected by 10 mm biopsy punch, dehydrated and prepared for scanning electron microscopy.

*Chest wall processing*

For each chestwall a series of cross sectional slabs 4–5 mm thick were cut using a band saw. Slabs were placed on a dry ice cooled copper plate, then put into wire mesh processing baskets, labeled, and immersed by group in freeze-dry transition fluid (anhydrous methanol (75%), acetone (25%)) in 1 l cryo containers and stored at −80 °C. Cryo-fluids were replaced weekly for up to two months. Then specimens were stored in cryo-fluid at − 20 °C with weekly fluid replacement until solution cleared, usually after one month.

*Staining and preparation of specimens for microscopic evaluation*

Following freeze substitution, cross sections of chestwall were transferred to anhydrous methanol at −20 °C and brought to room temperature. Chestwall slabs and lung pieces were then stained with Lucifer yellow-CH (0.0001%) (Rogers et al., 1999), infiltrated in Spurr epoxy resin then heat cured. Undisturbed surfaces of chestwall slabs were exposed within the Epoxy embedment using a belt sander, or in the case of lung, 2 milimeter-thick sections were cut using a thin kerf rock saw blade. Exposed surfaces were polished using a diamond lapidary wheel until glass smooth.

*Confocal microscopy*

Confocal microscopy imaging was performed on chestwall slabs from each animal for each time point using Sarastro 2000 or 2010 (Molecular Dynamics, Inc.) laser scanning microscopes fitted with 25 mW argon-ion lasers and an upright Zeiss Axiophot or upright Nikon or inverted Nikon Diphot 2 microscopes, modified for reflected light imaging in dual channel reflected and fluorescent imaging mode. The cellular constituents and fibers (and particles) were imaged simultaneously with this arrangement with each "exposure" producing two digital images in perfect register with one another (Bernstein et al., 2010). Images were recorded through 60× objectives for Nikon-fitted confocal microscopes with voxel dimensions of 0.16 μm, 0.16 μm, and 0.60 μm (x, y, and z dimensions, respectively). Voxel dimensions were 0.16 μm, 0.16 μm, and 0.50 μm (x, y, and z dimensions, respectively) obtained from 63× objective for the Zeiss-fitted confocal microscope.

*Mediastinal lymph nodes*

One of the principle routes of clearance of the lymphatic fluid which is drained by the stomata on the parietal pleura is through the mediastinal lymph nodes. The mediastinal lymph nodes were collected at necropsy and pooled for each exposure group at each time point. They were then processed by lung digestion and then analyzed for fiber number and size distribution by SEM using similar procedures as was performed for the lung.

*Statistical analyses*

The confocal data was analyzed using analysis of variance (StatSoft, Inc. (2013) STATISTICA (data analysis software system), version 12 www.statsoft.com; MedCalc, ver 12.7, Ostend, Belgium). The fiber clearance half-times were calculated using StatSoft, Inc. (2013) and GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com).

## Results

The validation of the lung digestion and counting procedures has been presented in Bernstein et al., 2014.

The exposure conditions are summarized in Table 1. The exposure conditions and the bivariate length and diameter distributions have been presented in Bernstein et al. (2014).

The fiber distribution in the chrysotile and brake dust aerosol included a larger number of shorter fibers with 84% less than 5 μm. For those fibers longer than 20 μm there was a mean of 189 fibers/$cm^3$ ranging from 20 to 160 μm in length. Of the long fibers, 99% were less than 1 μm in diameter (rat-respirable) with 95% less than 0.4 μm in diameter.

The crocidolite-exposure atmosphere had considerably fewer short fibers with 66% less than 5 μm. For those fibers longer than 20 μm there was a mean of 93 fibers/$cm^3$ ranging in length from 20 to 190 μm. 88% of the long fibers were less than 1 μm in diameter (rat respirable) with 21% of the fibers less than 0.4 μm in diameter.

In the brake dust exposure group there were fewer chrysotile fibers present without the added chrysotile with a mean of 3 fibers longer than 20 μm/$cm^3$. These longer fibers ranged in length from 20 to 140 μm with 90% less than 1 μm in diameter (rat respirable) with 63% of the fibers less than 0.4 μm in diameter.

*Fiber clearance (from the lung digestion evaluation)*

The fiber clearance was determined through 365 days post exposure using the whole lung digestion procedures described earlier (Bernstein et al., 2014). The clearance half-times based on the results through 91 days post exposure which were presented in that publication, have

**Table 1**
Aerosol concentration and size distribution of the exposure atmosphere in the air control group 1, chrysotile and brake dust group 2, brake dust group 3 and crocidolite asbestos group 4.

| Exposure Group | Gravimetric Concentration mg/m$^3$ / SD | Total Number of Fibers counted on the filter* | Number of total fibers/cm$^3$ | WHO Fibers/cm$^3$ | Percent WHO fibers | Number of fibers ≥20 μm/cm$^3$ | Percent of all fibers ≥20 μm/cm$^3$ | Mean Number Particles/cm$^3$ | Diameter Range (μm) | Length Range (μm) | GMD (μm) / GSD | GML (μm) / GSD | Mean Diameter (μm)/SD | Mean Length (μm)/SD | Length weighted arthm. diameter μm) | Length weighted geom. diameter (μm) | Aspect ratio |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (Group 1) Air Control | 0 | 7 | 0.002 | 0.001 | 42.9 | 0 | 0 | 0.006 | 0.5–1.8 | 2.3–6.8 | 1.11<br>1.53 | 4.49<br>1.46 | 1.20<br>0.44 | 4.70<br>1.63 | 1.34 | 1.23 | 4.07 |
| (Group 2) Chrysotile & Brake dust | 3.48<br>0.36 | 2,454 | 6,953 | 1,007 | 14.5 | 189 | 2.7 | 3,140 | 0.03–2.4 | 0.6–160 | 0.17<br>1.68 | 2.47<br>2.32 | 0.20<br>0.12 | 4.01<br>6.83 | 0.19 | 0.16 | 29.8 |
| (Group 3) Brake dust** | 1.52<br>0.07 | 1,623 | 389.3 | 46.0 | 11.8 | 3.6 | 0.9 | 1240 | 0.05–2.9 | 0.6–140 | 0.21<br>1.79 | 2.25<br>2.00 | 0.25<br>0.20 | 3.21<br>4.25 | 0.32 | 0.24 | 16.99 |
| (Group 4) Crocidolite | 6.34<br>0.42 | 1,820 | 2,013 | 709 | 35.2 | 93 | 4.6 | 602 | 0.05–2.6 | 0.7–190 | 0.34<br>1.64 | 4.07<br>2.44 | 0.39<br>0.21 | 6.50<br>9.40 | 0.50 | 0.39 | 17.62 |

SD: Arithmetic standard deviation; GMD: Geometric mean diameter; GML: Geometric mean length; GSD: Geometric standard deviation; MMAD: Mass median aerodynamic diameter.
* The total number of fibers counted on the filter is based upon the rules specified in section "Fiber/Particle Analysis and Lung Digestion" above.
** For the brake dust group 3, the MMAD = 1.89 (Geometric standard deviation = 2.54) as determined by the impactor measurement.

been updated based upon the data through 365 days after cessation of exposure as shown below (Table 2).

The 5R04 grade of chrysotile used in the current study, according to the Quebec Standard Testing system (Cossette and Delvaux, 1979) this grade fiber had more than 60% of the fibers by weight larger than 0.13 cm (screen opening in the selection apparatus). Even though these bundles were largely separated prior to use in this study, 11% of the fibers > 1 μm in diameter were longer than 20 μm and ranged in length up to 51 μm. As presented in Bernstein et al. (2014) such fibers could become trapped in the lower airways and with the whole lung digestion procedure could not be differentiated in terms of location in the lung. The few longer fibers observed by confocal microscopy were observed in the airways. In addition, the large number of shorter fibers remaining in the lung accumulate preferentially in the lymphatic system which again cannot be differentiated through the lung digestion procedure. This resulted in group 2 in a two phase clearance and as such the weighted clearance half-time (as described in Bernstein and Riego-Sintes, 1999; Bernstein et al., 2001) was determined as shown in Table 2. As presented in Fig. 1, below, the confocal microscopy analysis confirmed that the long chrysotile fibers do not persist in the lung parenchyma.

*Fiber clearance (from the confocal microscopy evaluation)*

With the confocal microscopy examination of the lung parenchyma in which the amount of connective tissue (presented below) was determined, the number and length of any fiber in these regions was also determined. These results are shown in Fig. 1 with the summary statistics presented in Table S1 (supplemental data). In the brake dust with added chrysotile group as well as the brake dust alone group the fibers longer than 20 μm were rapidly cleared with only one fiber observed at 32 days post exposure and none thereafter. In the crocidolite asbestos exposed animals, numerous long fibers were observed which persisted through 365 days post exposure. The maximum crocidolite fiber length observed in the parenchyma was 146 μm.

*Pathological findings in the lung*

*Histopathology*

Histopathological findings reported earlier through 91 days after cessation of exposure clearly differentiated the response to brake dust with added chrysotile and brake dust alone as compared to crocidolite asbestos. The histopathological findings in the lung through 365 days are presented in Table S-2 with the key histopathological scores illustrated in Fig. 2. These results through 365 days post exposure continue to reinforce the differentiation between brake dust with added chrysotile and brake dust alone as compared to crocidolite asbestos.

There were no exposure-related histopathological findings in animals exposed to filtered air. In the chrysotile-brake dust group and brake dust alone group, slight accumulation of particle laden macrophages were observed from 7 through 91 days post exposure which decreased at 181 and 365 days post exposure. At 32 days post exposure, very slight (multi)focal particle laden micro-granulomas at the bronchiolo-alveolar junctions were observed in groups 2 and 3, however, there were no associated giant cells. There were no fiber related findings observed in groups 2 or 3 throughout the 365 day observation period.

In the crocidolite asbestos exposure group, accumulations of fiber laden macrophages were observed already at day 0, immediately following cessation of exposure. This incidence increased at day 7 and was associated with the formation of (multi)focal fiber laden micro-granulomas at the bronchiolo-alveolar junctions with multinucleate (syncytial) giant cells within these micro-granulomas. Interstitial fibrosis was observed by day 32. These findings persisted through 365 days post exposure. In addition, pleural fibrosis was also observed at 365 days post exposure in response to the crocidolite fibers.

**Table 2**
Estimated fiber clearance half times in days (through 365 days post exposure) (SE: standard error).

| Group | Exposure | Fibers L > 20 μm | Fibers 5–20 μm | Fibers < 5 μm | Particles |
|---|---|---|---|---|---|
| | | (days) | | | |
| 2 | Chrysotile & brake dust | 42* (SE: 12) | 52* (SE: 29) | 109* (SE: 52) | 399 (SE: 426) |
| 3 | Brake dust | 29 (SE: 9) | 46 (SE: 17) | 54 (SE: 20) | 110 (SE: 49) |
| 4 | Crocidolite asbestos** | >1000 | >1000 | >1000 | >1000 |

SE: standard error.
* Weighted $T_{1/2}$ based on double exponential fit to the data (Bernstein and Riego-Sintes, 1999; Bernstein et al., 2001).
** The standard errors for the crocidolite estimates could not be calculated due to the lack of clearance.

*Pulmonary fibrosis analysis – confocal microscopy*

The connective tissue (elastin and collagen) present in the lung was measured by confocal microscopy to obtain the percentage of the elastin and collagen per area of lung tissue (%CT/FOV). These measurements were performed on the same lung parenchyma volumes which were examined for fiber length in Fig. 1. For each group and time point 300 cubic lung tissue volumes of 112,550 $\mu m^3$ each were imaged, the amount of connective tissue measured and the number and length of the fibers observed were recorded. Fields of view that contained a blood-vessel of diameter greater than 50 μm were not included due to the high amount of collagen in the blood-vessel walls.

The percent fibrosis is shown in Fig. 3 for each group at 0, 32, 91, 181 and 365 days after cessation of the 5 day exposure. The summary statistics are presented in Table S-3. In the air control group the percent connective tissue ranged from a mean of 3.8 ± 2.9 at day 0 to 7.1 ± 4.2 at day 365 with a range in individual values over this period of 0.1–24%. Compared to the air control group, there were no statistically significant (analysis of variance) trends in the chrysotile & brake dust group or in the brake dust group alone with or without fibers present. The chrysotile fibers present in the tissues had no impact on the development of connective tissue compared to the air control group and did not cause a fibrotic response through 365 days post exposure.

In the crocidolite asbestos exposure group, there is a consistent statistically significant increase in the mean amount of connective tissue present compared to day 0 (mean 4.5 ± 3.5) through 91 days post exposure (mean 14.7 ± 12.7) which then persisted through 365 days (mean 13.5 ± 8.9 ) as determined by analysis of variance. At 91 days, when compared to the air control group the mean connective tissue in the crocidolite asbestos exposure group increased by ~ 5 times.

The range of individual measurements shows that at day 0 the percent connective tissue in the crocidolite asbestos exposure group was similar to that found in the air control (0.2 - 23%), however, by 91 days post exposure, the connective tissue range in group 4 increased up to 87%.

*Translocation of fibers to the pleural cavity and pathological response*

One of the main objectives of this study was to examine the translocation of fibers to the pleural cavity using non-invasive techniques and to evaluate whether these fibers produce a pathological response.

Two methods were used to perform this analysis. The examination of the visceral pleural and the sub-visceral pleural regions of the lung was performed on the deep frozen tissues on subgroups of animals at 14, 91, 181 and 365 days post-exposure.

The examination of the diaphragm as a representative parietal pleural tissue was performed on the same animals that were examined for lung histopathology and CM. These animals were examined at time points starting at 0 days, immediately following cessation of exposure, through 365 days post-exposure. The 0 day results are presented here.

*Visceral pleural examination and analysis*

The visceral pleura barrier is a key boundary in the transport of fibers from the lung to the pleural cavity. Examination of the visceral pleural

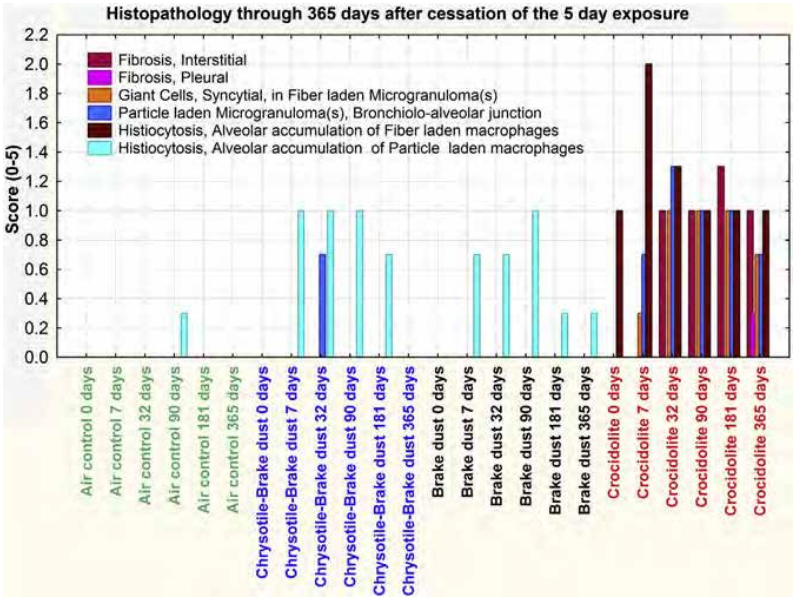



**Fig. 2.** Histopathological scores through 365 days after cessation of exposure.

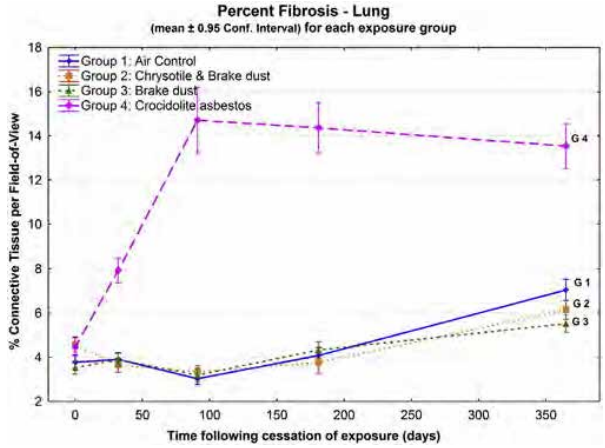



**Fig. 3.** Percent fibrosis (connective tissue per field of view) measured in the lung parenchyma by confocal microscopy.

region provides an initial and important indication of whether fibers reach this area and can produce pathological response.

The visceral pleural was systematically examined from cross-sections of rats that were frozen in liquid nitrogen immediately following sacrifice. This procedure was used in order to avoid possible artefacts that could stem from cross-contamination of fibers from the lung to the pleural cavity when tissues are manipulated at necropsy. The examination included a systematic survey using CM of the visceral pleural wall, the adjacent subpleural alveoli and the pleural space. The features of the tissues were evaluated and the location and length of any fibers present were determined.

In addition, the thickness of the pleural wall was measured at between 5 and 10 points in each section examined and the amount of collagen was quantified using the confocal microscopy procedures similar to which was reported above for the lung.

The visceral wall thickness is influenced by inflammation and fibrotic development. Disordered fibrin turnover plays a central role in the pathogenesis of pleural fibrosis (Bignon and Gee, 1985). A progression of increased vascular permeability, formation of a transitional fibrin gel, and remodelling and organization of the fibrinous neomatrix are common to the pathogenesis of lung inflammation and neoplasia, including tumors of the lung and pleural space (Idell et al., 2001).

As shown in Fig. 4 (summary statistics are presented in Table S-5), in the air control group, the mean visceral wall thickness ranges from 2.5 ± 0.6 μm on day 14–3.4 ± 0.7 μm on day 365 post exposure. For the chrysotile with brake dust and the brake dust alone groups there were no statistically significant differences (analysis of variance) between the mean visceral wall thicknesses as compared to the air control.

With the crocidolite asbestos exposure group a rapid increase in the mean visceral wall thickness was observed from 14 days through 272 days post exposure at which time it plateaued through 365 days post exposure. The mean visceral wall thickness in the crocidolite asbestos exposed rats increased from a mean on day 14 of 3.1 ± 0.7 μm to 5.8 ± 0.8 μm on day 272 which then persisted through day 365 (5.8 ± 2.0) and ranged from 2.9 μm to 19 μm.

The amount of connective tissue (elastin and collagen) present in the visceral pleura was also examined as to whether the increase in visceral pleura thickness was accompanied by an increase in collagen.

As shown in Fig. 5 (summary statistics are presented in Table S-6), the mean percent connective tissue per field of view was not statistically different in the chrysotile with added brake dust and the brake dust alone groups in comparison to the air control group (analysis of variance). In the crocidolite asbestos exposed group, the mean percent connective tissue per field of view increased from a mean of 6.5 ± 2.4% on day 14 to 16.1 ± 9.4% on day 365 which was statistically different from the air control and the brake dust groups (analysis of variance, $p < 0.01$). It is interesting to note that while the visceral pleural wall thickness levels off from 272 to 365 days post exposure, the percent connective tissue continued to increase indicative of a continuing inflammatory response to the crocidolite asbestos fibers.

With the confocal analysis of the chestwall, the length of any fibers observed within the fields of view analysed was also recorded. Fig. 6 shows length of each fiber observed in the visceral pleura space examined for thickness and percent connective tissue above (summary statistics are presented in Table S-7). No fibers were observed at any time point in the visceral pleura region of air control group and one short fiber of 3.3 μm was observed in the brake dust group at 365 days. In the chrysotile and brake dust group fibers up to 17.9 μm were observed at day 14, however, only 2 short fibers of 3 and 4.7 μm were observed at day 91 and at day 365 one fiber 4.3 μm was observed which is coherent with the disintegration of the longer chrysotile fibers and their subsequent clearance.

In the crocidolite asbestos exposed rats, fibers up to 26 μm were observed at 14 days post exposure with no systematic clearance of the fibers through 365 days post exposure. At 365 days post exposure, 15 fibers were observed in the visceral pleura region surveyed ranging in length up to 22.2 μm.

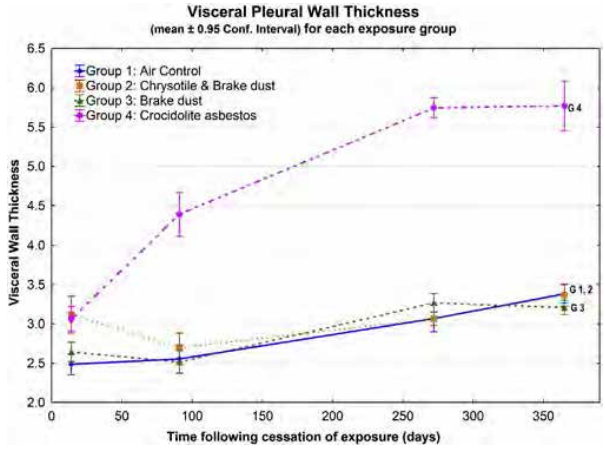



**Fig. 4.** Visceral pleural wall thickness measured by confocal microscopy.

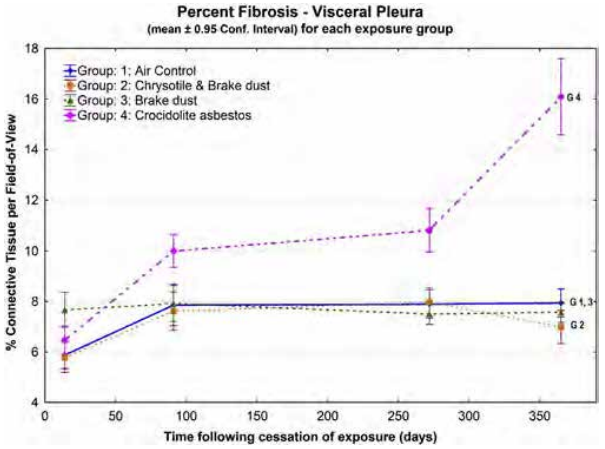



**Fig. 5.** Percent fibrosis (% conenctive tissue) in the visceral pleural wall measured by confocal microscopy.

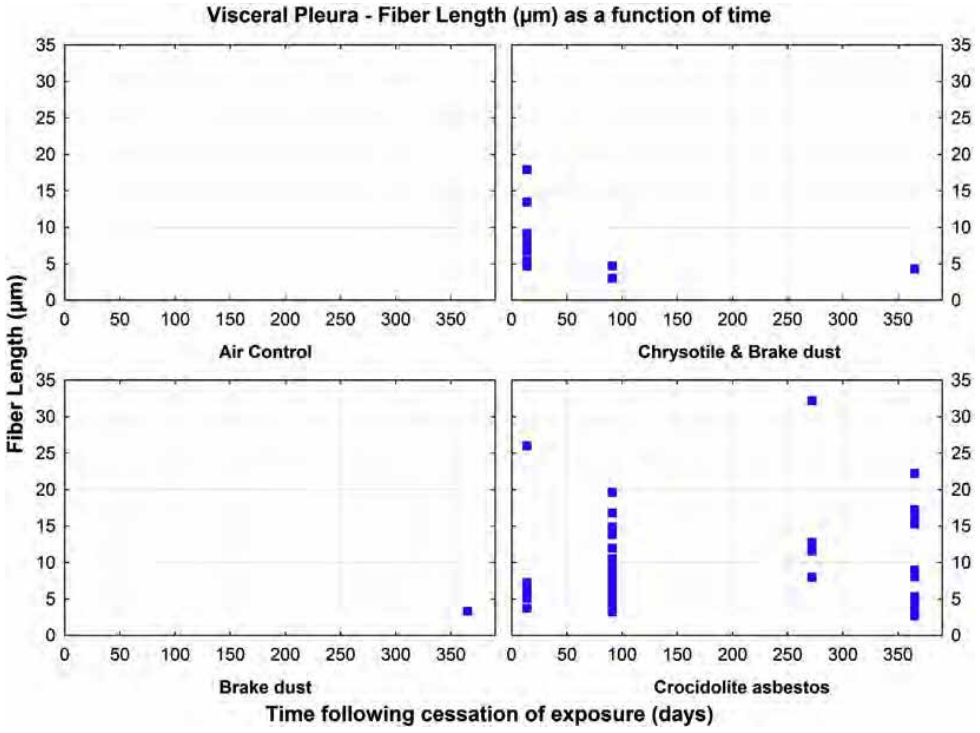



**Fig. 6.** Visceral pleural wall – number and length of fibers observed by confocal microscopy.

*Mediastinal lymph nodes*

One of the principle routes of clearance of the lymphatic fluid which is drained by the stomata on the parietal pleura is through the mediastinal lymph nodes (Negrini and Moriondo, 2013). The number and length and diameter distribution of the fibers present in the mediastinal lymph nodes is shown in Fig. 7 with the results summarized in Table S8. At 91 day after cessation of exposure, no chrysotile fibers were observed in the mediastinal lymph nodes of the brake dust or chrysotile/brake dust exposed groups. In the crocidolite exposed animals, at 91 days post exposure, a total of 58 crocidolite fibers were recovered from the mediastinal lymph nodes with lengths up to 35 µm (diameter 0.65 µm) and diameters up to 1.1 µm (length 8.7 µm).

*Confocal microscopy images of the pleura*

The snap frozen chest walls that were collected from animals in each of the exposure groups at 14, 91, 272 and 365 days following cessation of exposure were processed as described above and imaged using confocal microscopy. This process preserved the tissue, cellular and spatial orientation of any particles or fibers present in the visceral and parietal pleura as well as in the pleural space. There was some minor contraction of the lung during the snap freezing process which was observed in the wavy orientation of the visceral pleural surface.

The confocal microscopy images (Figs. 8–10) show the comparative response in the visceral and parietal pleura for each group. Sub pleural alveolar septa appear as grayscale in color. Visceral pleural surface and

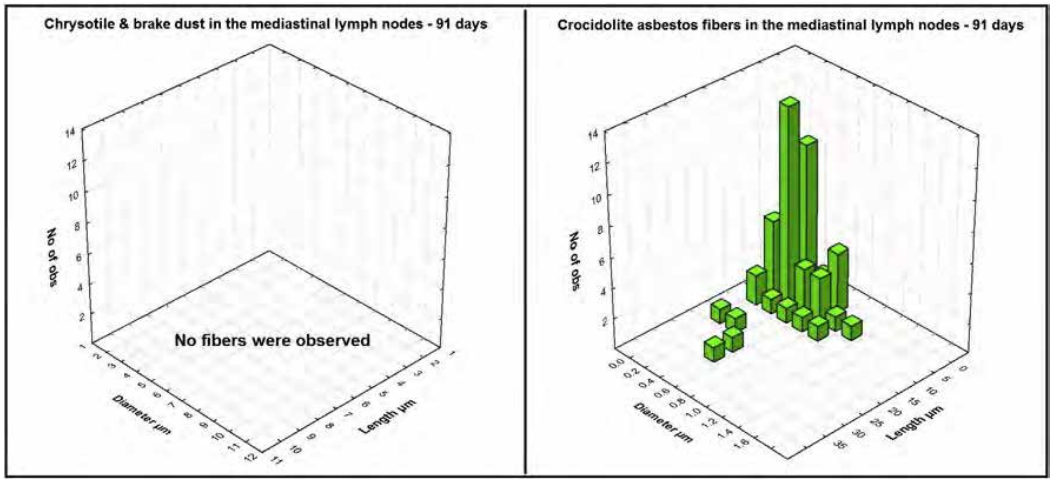



**Fig. 7.** Mediastinal lymph nodes — fiber length & diameter distribution at 91 days post exposure.

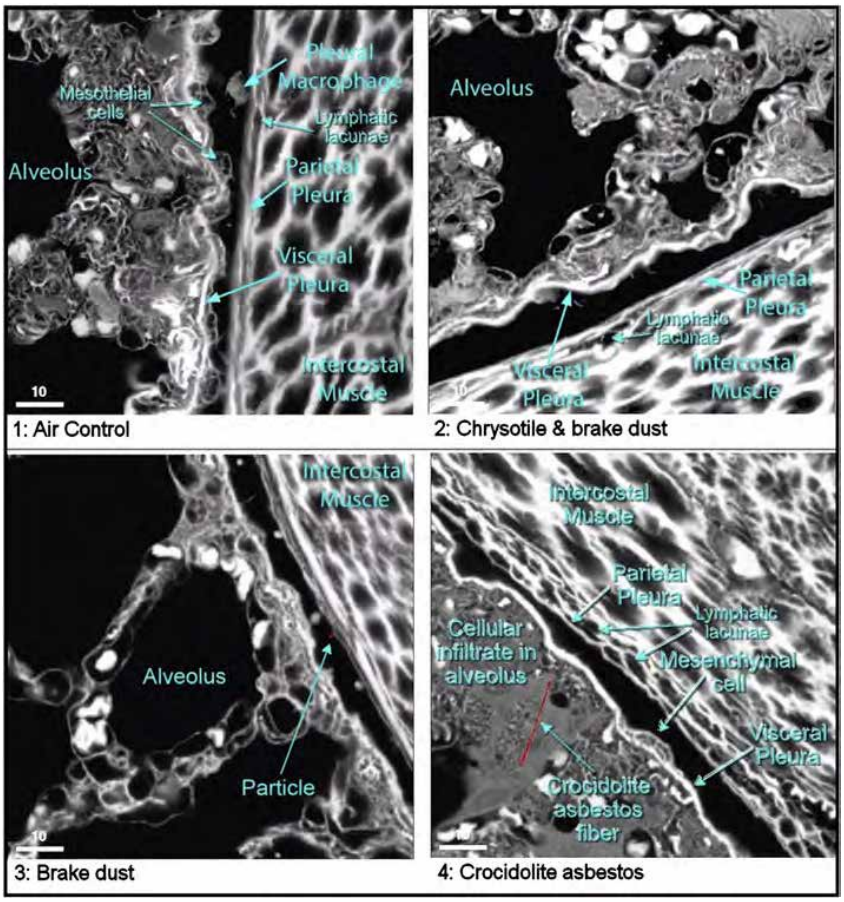



**Fig. 8.** Confocal images of the pleural cavity: 14 days after cessation of exposure. The images were obtained from snap frozen chestwall sections which preserved the tissue, cellular and spatial orientation of any particles or fibers present. The intercostal muscle which runs between the ribs and is mainly involved in the mechanical aspect of breathing is seen on the right, adjacent to the parietal pleura (when present). Opposite is the visceral pleura wall and the alveolar region of the lung. Details of each image are provided in the text.

(when present) parietal pleura surfaces high in collagen content appear as bright white linear profiles. Pleural space cells and particles and or fibers (red, if present) in context with lung tissue and pleura are also shown.

A typical image of the air control is shown in Fig. 8, panel 1 (14 days post exposure). The intercostal muscle which runs between the ribs and is mainly involved in the mechanical aspect of breathing is seen on the right, below the parietal pleura. Also indicated are submesothelial lymphatic lacunae into which the pleural fluid flows through the stomata. The pressure oscillations in the lacunae, related either to tissue motion or contractile properties of myogenic cells, represent the main mechanism for lymph propulsion toward larger lymphatic collecting ducts (Negrini et al., 1992). A pleural macrophage is observed within the pleural space. The normal collagen of the visceral pleura is observed as a solid white line on which can be seen mesothelial cells. Below the visceral pleura are the sub-pleural alveoli. Red blood cells can also be seen in the blood vessels surrounding the alveoli.

At 14 days following exposure to brake dust with added chrysotile (Fig. 8, panel 2), the visceral and parietal pleura, sub-pleural alveoli and pleural space have nearly the same appearance as the air control group. Similarly, the brake dust exposed group has a similar appearance with the exception of a single particle in the pleural space (Fig. 8, panel 3). No fibers were observed.

At 14 days following the end of exposure to crocidolite asbestos, a very different image is observed (Fig. 8, panel 4). A long crocidolite asbestos fibers (~25 μm) can be seen within a sub-pleural alveolus adjacent to the visceral pleura. Partial profiles of a number of other fibers can also be seen. A dense cellular infiltrate fills the alveolus and the surrounding alveoli. On the visceral pleura, a mesenchymal cell can be observed. Below the parietal pleura, the lymphatic lacunae which drain the pleural fluid through the stomata appear enlarged suggestive of increased pleural fluid flow.

At 91 days after cessation of exposure, the visceral and parietal pleura architecture of the brake dust with added chrysotile and the brake dust group alone appears similar to that of the air control group (Fig. 9, plates 1–3). In the brake dust group, a particle is seen adjacent to a pleural macrophage. No fibers were observed.

In the crocidolite asbestos exposure group at 91 days post exposure (Fig. 9, plate 4), a crocidolite fiber is seen within the pleural space. In addition, numerous pleural macrophages and neutrophils are also present surrounded by greyish wisps, which is likely coalesced pleural protein that was a result of the freezing process. Activated mesothelium is seen on the visceral pleura, which now has a dense collagen matrix (bright white area). The parietal pleura is not seen in this image.

At 272 and 365 days after cessation of exposure images of only the crocidolite asbestos exposure group are shown (Fig. 10) as the brake dust with added chrysotile and the brake dust group alone continue to appear similar to that of the air control group. In Fig. 10, plate 1, a crocidolite fiber is observed in the sub-pleural alveolus around which a granuloma has formed with a collagen capsule around the fiber. In Fig. 10, plate 2, an extended collagen matrix is seen along the visceral pleura. The parietal pleura is activated with enlarged mesothelial

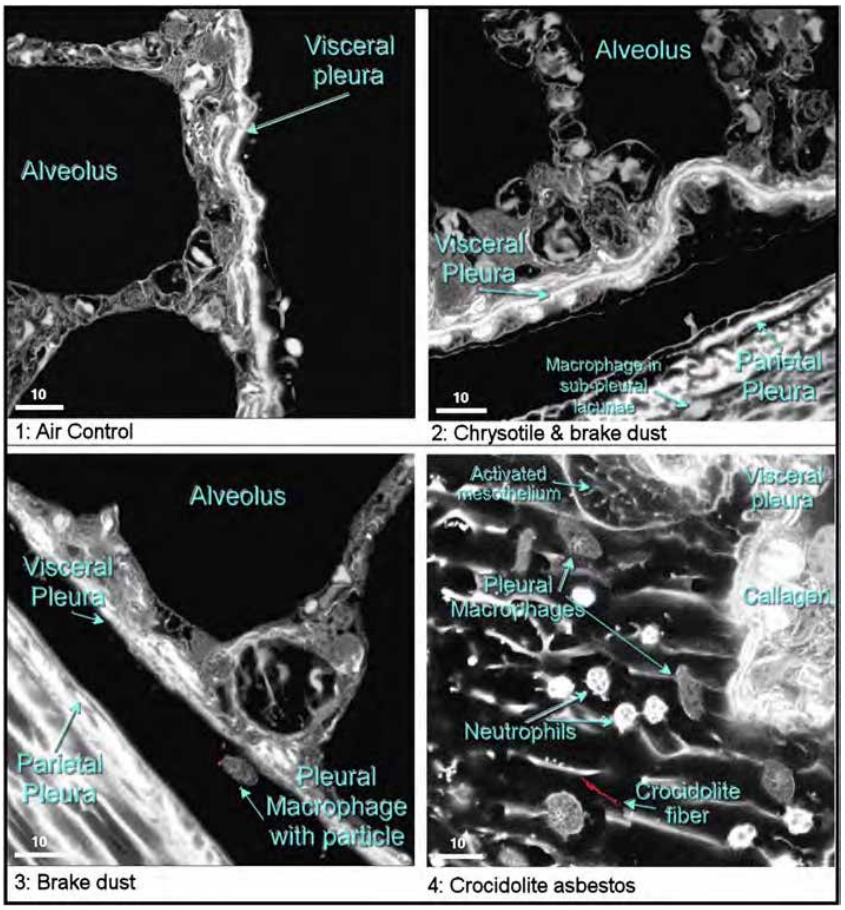



**Fig. 9.** Confocal images of the pleural cavity: 91 days after cessation of exposure: The images were obtained from snap frozen chestwall sections which preserved the tissue, cellular and spatial orientation of any particles or fibers present. The intercostal muscle which runs between the ribs and is mainly involved in the mechanical aspect of breathing is seen on the right, adjacent to the parietal pleura (when present). Opposite is the visceral pleura wall and the alveolar region of the lung. Details of each image are provided in the text.

cells with philapodia extensions. The sub-pleural lymphatic lacunae are again enlarged suggestive of a continuing increase in pleural fluid flow.

At 365 days post exposure (Fig. 10, plate 3) a crocidolite fiber is observed in the dense collagen matrix of the visceral pleura. In addition, there is a strong localized protein response adjacent to the visceral pleura. The parietal pleura is not shown in this image. In Fig. 10, plate 4, both the visceral and parietal pleura have extensive collagen networks with fibrin adhesions bridging the visceral and parietal pleura.

*Parietal pleura (diaphragm) examination*

The lymphatic stomata on the diaphragm represent a major site for pleural and peritoneal liquid drainage (Negrini et al., 1991). The diaphragm was chosen as a representative parietal pleura sample as it also could be readily removed from the animal quickly after sacrifice thus reducing the possibility of contamination through post-mortem body fluids.

Scanning electron microscopy was included to image the surface of the diaphragm and confocal microscopy to image the sub-pleural structure. Thus far, the SEM images at 0 days after cessation of exposure are available as shown in Fig. 11. The brake dust with added chrysotile and the brake dust alone were found to be similar in appearance to the air control group. Shown in each of these images are lymphatic stomata which are the parietal pleura lymphatic drainage portals.

In the crocidolite asbestos exposed group, immediately following cessation of the 5 day exposure, two crocidolite fibers are observed sticking out of the diaphragmatic surface (plate 4). These fibers are likely embedded in the stomata one of which is partially seen below the lower fiber. Activated mesothelial cells are also observed in the vicinity of these fibers. Two additional SEM images of the diaphragm from the crocidolite asbestos group at day 0 are shown in Fig. 12. Plate 1 shows a cluster of neutrophils on the mesothelial parietal surface. Plate 2 shows a 5.3 μm crocidolite fiber being transported by the mesothelial microvilli towards a stomata. What is notable is that there is no mesothelial activation or inflammatory cells present in response to this short fiber.

## Discussion

While our understanding of fiber pathogenesis continues to evolve, this study confirms the importance of fiber characteristics on the potential for producing a pathological response following inhalation. The biosolubility of the chrysolite fibers appears to affect their clearance and toxicity, especially the initial inflammatory response and tissue injury, and the resulting fibrogenic response. Chrysotile is a thin rolled sheet of magnesium on the outside and silica on the inside which is acid soluble (Kobell, 1834; Whittaker, 1957, 1963; Tanji et al., 1984; Titulaer et al., 1993). In contrast, amphibole asbestos fibers such as

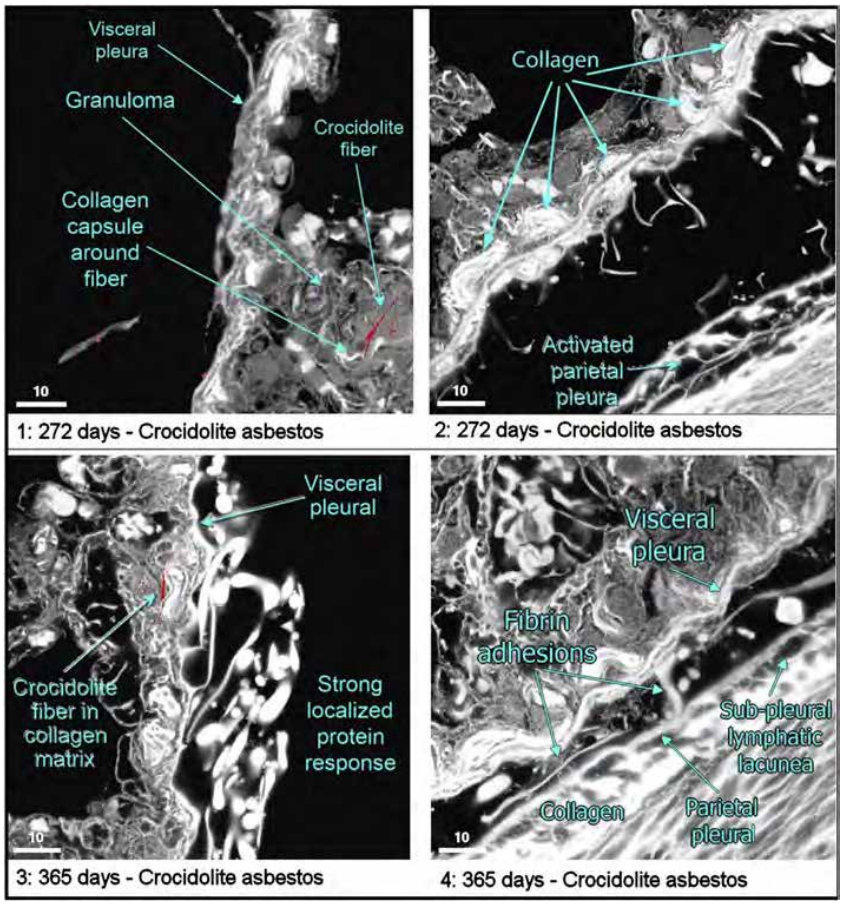



**Fig. 10.** Confocal images of the pleural cavity: Crocidolite asbestos 272 and 365 days after cessation of exposure: The images were obtained from snap frozen chestwall sections which preserved the tissue, cellular and spatial orientation of any particles or fibers present. The intercostal muscle which runs between the ribs and is mainly involved in the mechanical aspect of breathing is seen on the right, adjacent to the parietal pleura (when present). Opposite is the visceral pleura wall and the alveolar region of the lung. Details of each image are provided in the text.

crocidolite, are encased in silica and are insoluble at any pH that can occur in physiological conditions (Skinner et al., 1988; Whittaker, 1960).

This is the first study to assess the effects of chrysotile containing brake-dust. This study is also unique in that it included as a positive control a crocidolite sample that was not previously ground in preparation and thus contained a fiber size distribution more typical of what would have been encountered in commercial use.

The aerosol exposures to the chrysotile fibers used in this study were well above those that have been reported historically for mechanics working with brakes. Paustenbach et al. (2003) reviewed the historical exposures of mechanics to asbestos in brake dust and reported that the estimated and measured 8-hour time weighted averages (TWAs) for mechanics servicing automobiles and light trucks ranged from <0.002 to 0.68 f/cm$^3$, with a mean of 0.04 f/cm$^3$. The personal sampling data on which these TWAs were based showed that the concentrations ranged from <0.004 to 2.33 f/cm$^3$. For mechanics servicing heavy truck and bus brakes, the 8-hour TWAs ranged from 0.002 to 1.75 f/cm$^3$, with a mean of 0.2 f/cm$^3$. The corresponding personal sample concentrations ranged from <0.004 to 7.09 f/cm$^3$.

Blake et al. (2003) evaluated the dust emissions from four nearly identical automobiles from 1960s that were fitted with new replacement asbestos-containing brake shoes and then driven over a predetermined public road course for about 2253 km. Each car was brought separately into a repair facility; the brakes removed and replaced with new asbestos-containing shoes that were filed, sanded and ground as required. The authors reported that the airborne chrysotile fiber exposures for each test remained below currently applicable limit of 0.1 fiber/ cm$^3$ (eight-hour time-weighted average). The authors also measured the total dust for brake changing tests expressed as 8-h TWA that ranged from 0.193 to 0.708 mg/m$^3$ with a mean of 0.333 mg/m$^3$. The cleaning test resulted in less than 0.102 mg/m$^3$ total dust exposure. The respirable dust fraction expressed as 8-h TWA indicated concentrations below the 0.095 mg/m$^3$ detection limit for all but filing and the second arc-grinding tests, where 0.243 and 0.103 mg/m$^3$ were found, respectively. The mean respirable dust exposure concentration was <0.121 mg/m$^3$ or about one third of that for the total dust.

In this study the chrysotile aerosol exposure concentration was 1,007 f(WHO)/cm$^3$ in the combined chrysotile and brake dust group 2 and 46 f(WHO)/cm$^3$ in the brake dust group 3. Compared to the historical mean TWA for servicing automobiles and light trucks of 0.04 f/cm$^3$, the chrysotile fiber exposure concentration in group 2 of this study was 25,000 times the mean historical TWA exposure. The mean respirable gravimetric dust exposure concentration in this study was 3.48 mg/m$^3$ for group 2 and 1.52 mg/m$^3$ for group 3. Compared to the historical mean gravimetric TWA concentration for changing brakes of <0.121 mg/m$^3$, the gravimetric exposure concentration in group 2 of this study was more than 29 times the mean historical gravimetric exposure.

The estimated clearance times of the fibers >20 μm from the lung through 365 days were 42 days for the chrysotile and brake dust group and 29 days for the brake dust alone group. In comparison to

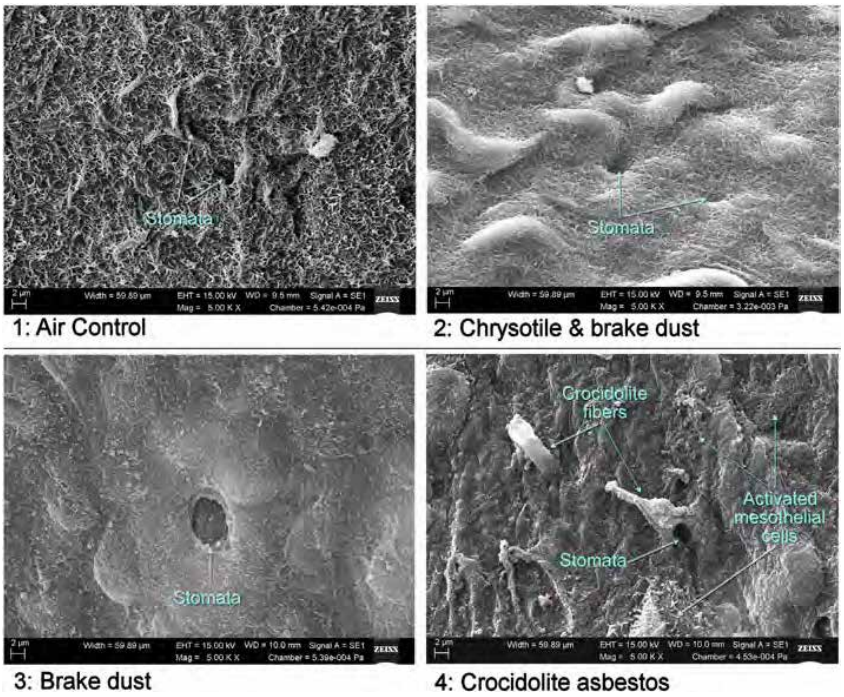



**Fig. 11.** Scanning electron micrographs of the diaphragm at 0 days following cessation of exposure. Exposure groups 1–4 are shown in each of the corresponding plates. Plates 1, 2, and 3 show a normal diaphragm structure with a stomata. Plate 4 shows crocidolite fibers penetrating the stomata of the diaphragm with adjacent activated mesothelial cells.

the crocidolite asbestos exposure group which had an estimated clearance half-time >1000 days, the longer chrysotile fibers cleared rapidly. Chrysotile can vary in characteristic depending upon the mine and processing of the ore. In earlier studies of chrysotile alone or of chrysotile mixed with a joint compound (Bernstein et al., 2003, 2004, 2005a, 2005b, 2008, 2011) the clearance half-time of the longer fibers ranged from 0.7 to 11.4 days. The lack of a fiber related response in the histopathological findings in either the lung or the pleural cavity strongly suggests that the few (group 2: 6.5–10 fibers counted on the filter; group 3: 1–5 fibers counted on the filter) remaining fibers longer than 20 μm in the current study were in the airways and not in the parenchyma. The clearance half-time of fibers longer than 20 μm that were less than 1 μm in diameter was 1.6 days for the chrysotile and brake dust group which is within the range of the earlier studies.

With the crocidolite asbestos exposure group 4, following the early clearance of the shorter fibers most likely from the tracheobronchial region, the intense inflammatory response induced by the longer fibers effectively locked-up further clearance. For all fibers lengths there was no subsequent clearance and the clearance half-time was estimated as greater than 1000 days. In previous biopersistence studies with amphibole asbestos, the clearance half-time was reported to range from 418 to >1000 days (Musselman et al., 1994; Hesterberg et al., 1996, 1998; Bernstein et al., 2005b, 2011).

The biopersistence study on amosite asbestos reported by Musselman et al. (1994) and Hesterberg et al. (1996, 1998) did not evaluate histopathological response or fiber translocation to the pleura. The Bernstein et al. (2005b) study which included tremolite asbestos did examine the histopathological response in the lung. The authors reported that following 5 days of exposure tremolite asbestos produced a pronounced inflammatory response with the rapid development of granulomas followed by the development of fibrosis characterized by collagen deposition within these granulomas and by 90 days even mild interstitial fibrosis. In the Bernstein et al. (2011) study amosite asbestos was evaluated as a positive control. The study was designed to evaluate the pathological response

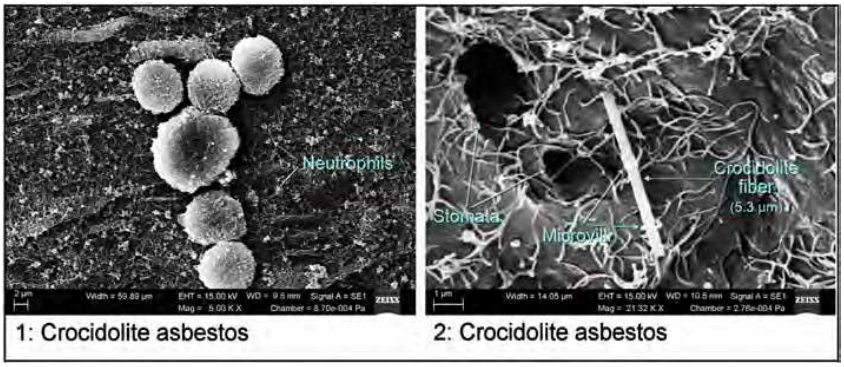



**Fig. 12.** Scanning electron micrographs of the diaphragm at 0 days following cessation of exposure. Crocidolite asbestos exposure group. Plate 1 shows neutrophils of the surface of the diaphragm. Plate 2 shows a 5.3 μm crocidolite fiber supported by the mesothelial microvilli adjacent to a stomata.

and fiber distribution within the lung and pleural cavity. The authors reported that amosite fibers were found to remain partly or fully imbedded in the interstitial space through 1 year and quickly produced granulomas (0 days) and interstitial fibrosis (28 days). Amosite fibers were observed penetrating the visceral pleural wall and were found on the parietal pleural within 7 days post-exposure with a concomitant inflammatory response seen by 14 days. In this study, the visceral wall thickness was measured for the first time using confocal microscopy. There was no difference in visceral wall thickness between the air control group and a group exposed to chrysotile mixed with sanded joint compound. In contrast the amosite asbestos exposed group had more than twice the visceral wall thickness.

In the current study, the design has been further enhanced with the inclusion of the measurement of not only the visceral wall thickness but also the quantification of collagen in the lung and in the visceral pleural wall. In the brake dust and the brake dust with added chrysotile groups, there was no inflammatory response to the chrysotile and no increase in connective tissue in the lung in comparison to the air control group. The only finding reported was a mild macrophage response to the particles present.

In contrast, in the crocidolite asbestos exposure group, the accumulation of fiber laden macrophages observed immediately following cessation of exposure quickly progressed to interstitial fibrosis by seven days and which persisted through 365 days post exposure at which time pleural fibrosis was also observed. There was a linear increase in fibrotic response in the lung parenchyma through 91 days post exposure after which the level of fibrotic response persisted through 365 days post exposure. The mean visceral pleural wall thickness increased by 180% through 272 days post exposure with a doubling in the amount of collagen present at 365 days.

The confocal images of the pleural cavity and the visceral and parietal pleural walls as well as the SEM images of the parietal pleural (diaphragm), provide a comparative basis for the observations reported in this study in comparison to those described in the scientific literature.

One of the goals of this study was to provide insight into the translocation of fibers from the lung to the pleural cavity through the use of non-invasive techniques. While between the lung and the visceral pleura wall there is a network of lymphatic capillaries and collecting vessels, the larger sub-visceral lymphatic vessels contain one-way valves, directing flow of lymph away from the pleura towards the hilar regions of the lung (Bernaudin and Fleury, 1985) and suggesting that this would not be a primary route of entry of fibers into the pleural cavity. Crocidolite fibers are observed in the sub pleural alveoli as well in the visceral pleural region. Due to the rapid onset of inflammatory response from these fibers it is likely that they have deposited following inhalation directly in these areas. What is remarkable in this study is the rapidity in which the crocidolite fibers can penetrate into the pleura and lodge in the stomata of the parietal pleural surface initiating an inflammatory response as observed immediately after the termination of the five day exposure. This was not observed for the brake dust and chrysotile fibers.

It appears that once fibers reach the pleural cavity, the pleural fluid which is produced by the parietal pleura, originating from the systemic circulation, is resorbed mainly through lymphatic drainage via the stomata exclusively on the parietal pleural side (Rahman and Wang, 2008). This flow would result in crocidolite fibers present in the pleural cavity entering the stomata and if they are either too large or are initiating an inflammatory response getting blocked at this point.

From the stomata the lymphatic drainage is facilitated by the lymphatic lacunae which are located in the sub-mesothelial region of the parietal pleura and with the movement of breathing serve to pump the lymph (Negrini et al., 1992; Negrini and Moriondo, 2011). Negrini and Moriondo (2011) have described how the initial lymphatics run through sub-mesothelial connective tissue composed of loose collagen fibers organized in bundles adjacent to the skeletal muscular fibers and that these contain two types of unidirectional valves which regulates fluid entrance into the lumen (Galie and Spilker, 2009) and prevents fluid back-flow (Negrini et al., 1992; Negrini and Moriondo, 2011, 2013).

In the rat, Parungo et al. (2005) have shown that the mediastinal lymph nodes are the sentinel lymph nodes of the pleural space. This study is also unique in assessing fiber number and dimensions in the mediastinal lymph nodes. At 91 days post exposure to crocidolite, fibers up to 35 μm were found in the mediastinal lymph nodes while no chrysotile fibers were observed.

The mesothelial cells which line the pleura are covered with microvilli and have been shown to phagocytize foreign substances such as bacteria, mineral particles such as asbestos fibers and quartz or latex beads (Jaurand and Fleury-Feith, 2008). The microvilli are associated with pinocytotic vessels, implying an important role in transcellular transport (Madison et al., 1979) and have a role in enmeshing glycoproteins rich in hyaluronic acid to lubricate the pleural surface and lessen friction between the lung and thorax (Andrews and Porter, 1973). Images in the current study show that the microvilli can encircle the shorter crocidolite fibers and appear to function to transport fibers towards the stomata as part of the clearance mechanism similar to the cilia in the tracheal bronchial tree. This transport of shorter fibers appears to occur without inducing an inflammatory response.

The mesothelial cell plays a critical role in the initiation of inflammatory responses in the pleural space because it is the first cell to recognize a perturbation in the pleural space. When activated, these cells recruit inflammatory cells (such as neutrophils) and release growth factors for fibroblasts which can lead to subsequent pleural fibrosis (Jantz and Antony, 2006). As observed in this study crocidolite fibers deposit in the stomata, activate the mesothelial cells on the pleural surface and result in the development of pleural fibrosis. This fibrotic response increased over time through the end of the post exposure observation period at 365 days where there was twice the connective tissue present compared to the air control (16.1 vs 7.9%). There was no statistically significant difference between the air control and brake dust and brake dust/chrysotile exposed groups with only a slight increase noted over this same time most likely due to aging.

## Conclusions

This study has demonstrated that there is an important difference in the persistence, translocation and pathological response in the lung and in the pleura between brake dust derived from brakes manufactured with chrysotile compared to the amphibole, crocidolite asbestos. The pathological response was determined using two independent methods. Classical histopathological examination was performed on thin lung sections including visceral pleura with scoring of the collagen level at the bronchoalveolar junctions as well as the Wagner score. In addition, the collagen deposition in the connective tissue of the lung and visceral pleura was evaluated using confocal microscopy in order to assess the fibrotic response.

No significant pathological response was observed at any time point in the brake dust or chrysotile/brake dust exposure groups through 365 days post exposure. Slight macrophage accumulation was noted in response to the high particle exposure levels in the test atmospheres and the decomposition of the longer chrysotile fibers into shorter fibers or particles. This was reflected as well in the Wagner score which ranged from 1 to 2 (with one being the level in the air control group) (Bernstein et al., 2014). The long chrysotile fibers cleared quickly with clearance halftimes estimated as 29 and 42 days respectively in the brake dust and the chrysotile/brake dust exposure group.

This is the first study to quantify the rapid response to fibers and inflammatory development in the pleural cavity following inhalation of crocidolite asbestos and not chrysotile.

Using the quantitative evaluation of fibrotic response in the lung and in the visceral pleura with confocal microscopy, there was no statistically significant difference between the air control group and either the brake dust alone or the brake dust with chrysotile exposure group at

any time point through 365 days after cessation of exposure. In addition, the pleural wall thickness was also not statistically different between these groups.

The crocidolite asbestos produced inflammatory response in the lung parenchyma from day 0 which progressed to Wagner grade 4 interstitial fibrosis within 32 days following cessation of exposure. In addition, the confocal microscopy evaluation of the fibrotic response in the connective tissue showed a marked increase in fibrotic response through 91 days after cessation of exposure (4.8× air control) which persisted through 365 days post exposure. The long crocidolite fibers had a pulmonary clearance half-time of greater than 1000 days.

This study also quantified the evolution of the visceral wall thickness and fibrosis in response to the inhalation of crocidolite asbestos. The pleural wall thickness showed a steady increase through 365 days post exposure. This was accompanied by a corresponding increase in fibrotic response of the visceral pleural wall to 200% that of the air control at 365 days post exposure. The confocal microscopy showed the concomitant inflammatory response in the pleural cavity with the development of the fibrotic response in the pleural walls. In addition, the crocidolite fibers were shown to persist in the vicinity of the visceral pleural wall, and were observed in the pleural space and immediately after the cessation of the five-day exposure on the diaphragm blocking lymphatic stomata. This was accompanied by activation of mesothelial cells, the presence of neutrophils and macrophages and inter-wall adhesions similar to that described in the literature for humans exposed to amphibole asbestos.

There are many brake linings still in use worldwide that contain chrysotile. This study in rats provides in-vivo toxicological support that brake dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung or the pleural cavity following short term inhalation.

## Conflict of interest statement

This study was funded by Honeywell International Inc. The affiliations of the authors are as shown on the cover page and include research laboratories, government institute, corporate affiliations, as well as independent toxicology consultant. This publication is the professional work product of the authors and may not represent the views of the corporate sponsor. The role of the corporate sponsor was limited to provide study funding, identifying the brake dust test article, and supplying the brakes used in the study. There have been periodic communications between Honeywell and the authors concerning the status of this study. One of the authors, David Bernstein, has appeared as an expert witness in litigation concerned with alleged health effects of exposure to chrysotile. Honeywell is a defendant in asbestos-product litigation and its predecessor manufactured the automotive brakes used in this study. The contribution of Prof JI Phillips is based on research supported by the National Research Foundation.

## Acknowledgments

This study was funded by Honeywell International Inc.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.taap.2014.12.012.

## References


Andrews, P.M., Porter, K.R., 1973. The ultrastructural morphology and possible functional significance of mesothelial microvilli. Anat. Rec. 177 (3), 409–426 (Nov).

Bernaudin, J.F., Fleury, J.Y., 1985a. Anatomy of the blood and lymphatic circulation of the pleural serosa. In: Chretien, J., Bignon, J., Hirsch, A. (Eds.), The pleural in health and disease. Marcel Dekker, New York, pp. 101–124.

Bernstein, D.M., Riego-Sintes, J.M.R., 1999. In: Bernstein, D.M., Riego-Sintes, J.M.R. (Eds.), European Commission Joint Research Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau. Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF) vol. EUR 18748 EN (April 93. Available from: http://ecb.ei.jrc.it/DOCUMENTS/Testing-Methods/mmmfweb.pdf).

Bernstein, D.M., Mast, R., Anderson, R., et al., 1994. An experimental approach to the evaluation of the biopersistence of respirable synthetic fibers and minerals. Environ. Health Perspect. 102 (Supplement 5), 15–18.

Bernstein, D.M., Riego Sintes, J.M., Ersboell, B.K., Kunert, J., 2001. Biopersistence of synthetic mineral fibers as a predictor of chronic inhalation toxicity in rats. Inhal. Toxicol. 13 (10), 823–849 (Oct).

Bernstein, D.M., Rogers, R., Smith, P., 2003. The biopersistence of Canadian chrysotile asbestos following inhalation. Inhal. Toxicol. 15, 101–128.

Bernstein, D.M., Rogers, R., Smith, P., 2004. The biopersistence of Brazilian chrysotile asbestos following inhalation. Inhal. Toxicol. 16, 745–761.

Bernstein, D.M., Rogers, R., Smith, P., 2005a. The biopersistence of Canadian chrysotile asbestos following inhalation: final results through 1 year after cessation of exposure. Inhal. Toxicol. 17, 1–14.

Bernstein, D.M., Chevalier, J., Smith, P., 2005b. Comparison of *Calidria chrysotile* asbestos to pure tremolite: final results of the inhalation biopersistence and histopathology following short term exposure. Inhal. Toxicol. 17, 427–449.

Bernstein, D.M., Donaldson, K., Decker, et al., 2008. A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. Inhal. Toxicol. 20, 1009–1028.

Bernstein, D.M., Rogers, R.A., Sepulveda, R., Donaldson, K., Schuler, D., Gaering, S., Kunzendorf, P., Chevalier, J., Holm, S.E., 2010. The pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite asbestos following short-term inhalation exposure: interim results. Inhal. Toxicol. 22 (11), 937–962 (Sep).

Bernstein, D.M., Rogers, R.A., Sepulveda, R., et al., 2011. Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure. Inhal. Toxicol. 23, 372–391.

Bernstein, D., Dunnigan, J., Hesterberg, T., Brown, R., Velasco, J.A., Barrera, R., Hoskins, J., Gibbs, A., 2013. Health risk of chrysotile revisited. Crit. Rev. Toxicol. 43 (2), 154–183 (Feb).

Bernstein, D.M., Rogers, R., Sepulveda, R., Kunzendorf, P., Bellmann, B., Ernst, H., Phillips, J.I., 2014. Evaluation of the deposition, translocation and pathological response of brake dust with and without added chrysotile in comparison to crocidolite asbestos following short-term inhalation: interim results. Toxicol. Appl. Pharmacol. 276 (1), 28–46 (Apr 1).

Bignon, J., Gee, J.B.L., 1985. Pleural fibrogenesis. In: Chretien, J., Bignon, J., Hirsch, A. (Eds.), the Pleura in Health and Disease. Marcel Dekker, New York, pp. 417–443.

Blake, C.L., Van Orden, D.R., Banasik, M., Harbison, R.D., 2003. Airborne asbestos concentration from brake changing does not exceed permissible exposure limit. Regul. Toxicol. Pharmacol. 38 (1), 58–70 (Aug).

Cannon, W.C., Blanton, E.F., McDonald, K.E., 1983. The flow-past chamber: an improved nose-only exposure system for rodents. Am. Ind. Hyg. Assoc. J. 44 (12), 923–928.

Cossette, M., Delvaux, P., 1979. Technical evaluation of chrysotile asbestos ore bodies. In: Ledoux, R.C. (Ed.), Short course in mineralogical techniques of asbestos determination. Mineralogical Association of Canada, Toronto, Canada, pp. 79–109 (May).

EUR 18748 EN, 1999. In: Bernstein, D.M., Riego-Sintes, J.M.R. (Eds.), Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF) vol. EUR 18748 EN. European Commission Joint Research Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau (April. 93, http://ecb.ei.jrc.it/DOCUMENTS/Testing-Methods/mmmfweb.pdf).

Galie, P., Spilker, R.L., 2009. A two-dimensional computational model of lymph transport across primary lymphatic valves. J. Biomech. Eng. 131, 1–9.

Hesterberg, T.W., Miiller, W.C., Musselman, R.P., et al., 1996. Biopersistence of man-made vitreous fibers and crocidolite asbestos in the rat lung following inhalation. Fundam. Appl. Toxicol. 29 (2), 269–279.

Hesterberg, T.W., Chase, G., Axten, C., et al., 1998. Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. Toxicol. Appl. Pharmacol. 151 (2), 262–275.

Idell, S., Mazar, A.P., Bitterman, P., Mohla, S., Harabin, A.L., 2001. Fibrin turnover in lung inflammation and neoplasia. Am. J. Respir. Crit. Care Med. 163 (2), 578–584 (Feb).

Jantz, M.A., Antony, V.B., 2006. Pleural fibrosis. Clin. Chest Med. 27 (2), 181–191 (Jun).

Jaurand, M.C., Fleury-Feith, J., 2008. Mesothelial cells. In: Light, R.W., Lee, Y.C.G. (Eds.), Textbook of pleural diseases, 2nd ed. Arnold Hodder, London, UK, pp. 27–37 (ISBN13: 978-0-340-94017-4).

Kobell, F., 1834. Ueber den schillernden Asbest von Reichenstein in Schlesien. J. Prakt. Chem. 2, 297–298.

Madison, L.D., Bergstrom-Porter, B., Torres, A.R., Shelton, E., 1979. Regulation of surface topography of mouse peritoneal cells. Formation of microvilli and vesiculated pits on omental mesothelial cells by serum and other proteins. J. Cell Biol. 82 (3), 783–797 (Sep).

Musselman, R.P., Miiller, W.C., Eastes, W., et al., 1994. Biopersistences of man-made vitreous fibers and crocidolite fibers in rat lungs following short-term exposures. Environ. Health Perspect. 102 (Suppl. 5), 139–143.

Negrini, D., Moriondo, A., 2011. Lymphatic anatomy and biomechanics. J. Physiol. 589 (Pt 12), 2927–2934 (Jun 15).

Negrini, D., Moriondo, A., 2013. Pleural function and lymphatics. Acta Physiol. (Oxf.) 207 (2), 244–259 (Feb).

Negrini, D., Mukenge, S., Del Fabbro, M., Gonano, C., Miserocchi, G., 1991. Distribution of diaphragmatic lymphatic stomata. J. Appl. Physiol. 70 (4), 1544–1549 (Apr).
Negrini, D., Del Fabbro, M., Gonano, C., Mukenge, S., Miserocchi, G., 1992. Distribution of diaphragmatic lymphatic lacunae. J. Appl. Physiol. (1985) 72 (3), 1166–1172 (Mar).
Parungo, C.P., Colson, Y.L., Kim, S.W., Kim, S., Cohn, L.H., Bawendi, M.G., Frangioni, J.V., 2005. Sentinel lymph node mapping of the pleural space. Chest 127 (5), 1799–1804 (May).
Paustenbach, D.J., Richter, R.O., Finley, B.L., Sheehan, P.J., 2003. An evaluation of the historical exposures of mechanics to asbestos in brake dust. Appl. Occup. Environ. Hyg. 18 (10), 786–804 (Oct).
Rahman, N.M., Wang, N.S., 2008. Anatomy of the pleura. In: Light, R.W., Lee, Y.C.G. (Eds.), Textbook of pleural diseases, 2nd ed. Arnold Hodder, London, UK, pp. 13–25 (ISBN13: 978-0-340-94017-4).
Rogers, R.A., Antonini, J.M., Brismar, H., Lai, J., Hesterberg, T.W., Oldmixon, E.H., Thevenaz, P., Brain, J.D., 1999. In situ microscopic analysis of asbestos and synthetic vitreous fibers retained in hamster lungs following inhalation. Environ. Health Perspect. 107 (5), 367–375 (May).
Shedd, K.B., 1985. Fiber dimensions of crocidolites from Western Australia, Bolivia, and the Cape and Transvaal provinces of South Africa. U.S. Bureau of Mines Report of Investigations 8998. United States Department of the Interior.
Skinner, H.C.W., Ross, M., Frondel, C., 1988. Asbestos and other fibrous materials – mineralogy, crystal chemistry, and health effects. Oxford University Press, New York (NY).
Tanji, T., Yada, K., Akatsuka, Y., 1984. Alternation of clino- and orthochrysotile in a single fiber as revealed by high-resolution electron microscopy. Clay Clay Miner. 32 (5), 429–432.
Titulaer, M.K., van Miltenburg, J.C., Jansen, J.B.H., et al., 1993. Characterization of tubular chrysotile by thermoporometry, nitrogen sorption, drifts, and TEM. Clay Clay Miner. 41, 496–513.
VDI Guideline 3492, 2004. Indoor air measurement, ambient air measurement, measurement of inorganic fibrous particles, scanning electron microscopy method. Verein Deutscher Ingenieure e.V., Düsseldorf.
Virta, R.L., 2002. Asbestos: geology, mineralogy, mining, and uses. Prepared in cooperation with Kirk-Othmer encyclopedia of chemical technology. USGS Open file 02-149 Online edition. Wiley-Interscience, a division of John Wiley & Sons, Inc., New York (NY).
Whittaker, E.J.W., 1957. The structure of chrysotile. V. Diffuse reflexions and fiber texture. Acta Crystallogr. 10, 149–156.
Whittaker, E.J.W., 1960. The crystal chemistry of the amphiboles. Acta Crystallogr. 13, 291–298.
Whittaker, E.J.W., 1963. Research report: Chrysotile fibers – filled or hollow tubes? Mathematical interpretation may resolve conflicting evidence. Chem Eng News 41, pp. 34–35 (September 30, 1963).
WHO, 1985. Reference methods for measuring airborne man-made mineral Fiber (MMMF). WHO/EURO MMMF Reference Scheme. Prepared by the WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF. World Health Organisation, Copenhagen.

STUDY No. 8

# QUANTIFICATION OF THE PATHOLOGICAL RESPONSE AND FATE IN THE LUNG AND PLEURA OF CHRYSOTILE IN COMBINATION WITH FINE PARTICLES COMPARED TO AMOSITE-ASBESTOS FOLLOWING SHORT-TERM INHALATION EXPOSURE

**STUDY No. 8**

# QUANTIFICATION OF THE PATHOLOGICAL RESPONSE AND FATE IN THE LUNG AND PLEURA OF CHRYSOTILE IN COMBINATION WITH FINE PARTICLES COMPARED TO AMOSITE-ASBESTOS FOLLOWING SHORT-TERM INHALATION EXPOSURE

---

**Inhalation Toxicology, 2011; 23(7); 372-391**

**D. M. Bernstein, R. A. Rogers, R. Sepulveda, K. Donaldson, D. Schuler, S. Gaering, P. Kunzendorf, J. Chevalier, and S. E. Holm**

---

The marked difference in biopersistence and pathological repsonse between chrysotile and amphibole asbestos has been well documented. This study is unique in that it has examined a commercial chrysotile product that was used as a joint compound. The pathological response was quantified in the lung and translocation of fibers to and pathological response in the pleural cavity determined. This paper presents the final results from the study. Rats were exposed by inhalation 6 h/day for 5 days to a well-defined fiber aerosol. Subgroups were examined through 1 year. The translocation to and pathological response in the pleura was examined by scanning electron microscopy and confocal microscopy (CM) using noninvasive methods. The number and size of fibers was quantified using transmission electron microscopy and CM. This is the first study to use such techniques to characterize fiber translocation to and the response of the pleural cavity. Amosite fibers were found to remain partly or fully imbedded in the interstitial space through 1 year and quickly produced granulomas (0 days) and interstitial fibrosis (28 days). Amosite fibers were observed penetrating the visceral pleural wall and were found on the parietal pleural within 7 days postexposure with a concomitant inflammatory response seen by 14 days. Pleural fibrin deposition, fibrosis, and adhesions were observed, similar to that reported in humans in response to amphibole asbestos. No cellular or inflammatory response was observed in the lung or the pleural cavity in response to the chrysotile and sanded particles (CSP) exposure. These results provide confirmation of the important differences between CSP and amphibole asbestos.

*Inhalation Toxicology*, 2011; 23(7): 372–391

ISSN 0895-8378 print/ISSN 1091-7691 online
DOI: 10.3109/08958378.2011.575413

informa
healthcare

**RESEARCH ARTICLE**

# Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure

D.M. Bernstein[1], R.A. Rogers[2], R. Sepulveda[2], K. Donaldson[3], D. Schuler[4], S. Gaering[4], P. Kunzendorf[5], J. Chevalier[6], and S.E. Holm[7]

*[1]Consultant in Toxicology, Geneva, Switzerland, [2]Rogers Imaging Corporation, Needham, Massachusetts, USA, [3]University of Edinburgh, ELEGI Colt Laboratory, Edinburgh, Scotland, [4]Harlan Laboratories Ltd., Füllinsdorf, Switzerland, [5]GSA Gesellschaft für Schadstoffanalytik mbH, Ratingen, Germany, [6]Experimental Pathology Services AG, Muttenz/Basel, Switzerland, and [7]Georgia-Pacific, LLC, Atlanta, Georgia, USA*



**Abstract**

The marked difference in biopersistence and pathological response between chrysotile and amphibole asbestos has been well documented. This study is unique in that it has examined a commercial chrysotile product that was used as a joint compound. The pathological response was quantified in the lung and translocation of fibers to and pathological response in the pleural cavity determined. This paper presents the final results from the study. Rats were exposed by inhalation 6 h/day for 5 days to a well-defined fiber aerosol. Subgroups were examined through 1 year. The translocation to and pathological response in the pleura was examined by scanning electron microscopy and confocal microscopy (CM) using noninvasive methods. The number and size of fibers was quantified using transmission electron microscopy and CM. This is the first study to use such techniques to characterize fiber translocation to and the response of the pleural cavity. Amosite fibers were found to remain partly or fully imbedded in the interstitial space through 1 year and quickly produced granulomas (0 days) and interstitial fibrosis (28 days). Amosite fibers were observed penetrating the visceral pleural wall and were found on the parietal pleural within 7 days postexposure with a concomitant inflammatory response seen by 14 days. Pleural fibrin deposition, fibrosis, and adhesions were observed, similar to that reported in humans in response to amphibole asbestos. No cellular or inflammatory response was observed in the lung or the pleural cavity in response to the chrysotile and sanded particles (CSP) exposure. These results provide confirmation of the important differences between CSP and amphibole asbestos.

**Keywords:** Chrysotile, amphibole, fine particles, inhalation, pathology, lung

## Introduction

The marked difference in biopersistence and pathological response between chrysotile and amphibole asbestos has been documented in studies from a range of sources (Bernstein & Hoskins, 2006). This study, however, is unique in that it has examined a commercial chrysotile product that was used as a joint compound. In addition, the pathological response was quantified by compartment in the lung and translocation of fibers to and pathological response in the pleural cavity determined. The interim results from the study were presented in Bernstein et al. (2010). This paper presents the final results from the study, including the details of the measurements in the pleural cavity.

This study was specifically designed to evaluate the pathological response and fiber distribution within the lung and pleural cavity. The difficulty of sampling the thin pleural surfaces has been well documented. As summarized

*Address for Correspondence:* D.M. Bernstein, Consultant in Toxicology, 40 chemin de la Petite-Boissière, 1208 Geneva, Switzerland. E-mail: davidb@itox.ch

*(Received 25 February 2011; revised 23 March 2011; accepted 23 March 2011)*

by Zocchi (2002) , direct *in vitro* measurements of the biophysical properties of the pleura appear unreliable, because the mesothelium is very labile (Fentie et al., 1986; Peng et al., 1994; Agostoni, 1998 ), and the procedures required to obtain specimens are likely to compromise it (Agostoni, 1998). In order to minimize pleural sampling artifacts, two independent methods were developed for examining the translocation of fibers to the pleural cavity and any associated inflammatory response following exposure either to the chrysotile and sanded particulate (CSP) or to the amosite asbestos. These methods included examination of the diaphragm as a parietal pleural tissue and the in situ examination of the lungs and pleural space obtained from freeze-substituted tissue in deep-frozen rats.

The diaphragm was chosen as the parietal pleural tissue for examination because it can be quickly removed at necropsy with minimal alteration of the visceral lung surface. An area which included an important lymphatic drainage site (stomata) on the diaphragmatic surface was selected for examination of possible inflammatory response using scanning electron microscopy (SEM) and for the presence of fibers using confocal microscopy (CM).

In order to examine the visceral pleura environment, including the subpleural lung, the visceral pleural itself, and the pleural space, a noninvasive method for determining fiber location, size, inflammatory, and fibrotic response was used on rats, which were deep frozen immediately after killing.

In this study, to simulate the exposures encountered during the use of the product, the joint compound was applied and then dust released during sanding was collected. Sanding of the joint compound resulted in concomitant exposure to both chrysotile fibers (inherent within the joint compound) and joint compound particles. However, few fibers >20 μm were present after sanding. Consequently, in order to fulfill the requirements of the protocol on which the exposure design was based (EUR 18748 EN, 1999; ILSI, 2005) (>100 f/cc longer than 20 μm; this length category being related to pathogenesis), chrysotile fibers were added to the sanded joint compound (CSP).

### CSP sample characteristics

The specification and preparation of the Ready-mix used to produce the sanded material has been described previously (Brorby et al., 2008; Bernstein et al., 2010). Extensive characterization including comparison of the bivariate size distribution was performed to confirm that the chrysotile used in the recreated formulation in this study (JM 7RF3 from the Jeffrey Mine in Quebec, Canada) closely matched that from a historical sample of the joint compound (Brorby et al., 2008). No historical Ready-mix formulations specified use of amphibole asbestos at any time.

To simulate typical usage of the joint compound, the recreated material was applied to pieces of drywall the ends of which were sealed with tape according to the instructions for the original material. The material was allowed to dry for at least 48 h and then sanded. Individual boards were sanded for 20–30 min. Four different boards were used to obtain a sufficient mass of material for these studies. The sanded material was collected in a large Ziploc bag and the bag was sent to the Research and Consulting Company Ltd. (RCC; currently known as Harlan Laboratories Ltd.), Füllinsdorf, Switzerland, where the inhalation exposures were performed.

Amosite used in this study was from the same batch as used in previous studies (Hesterberg et al., 1997, 1999a,b; McConnell et al., 1999; Rogers et al., 1999).

## Materials and methods

All studies were conducted by RCC Ltd. (Basel, Switzerland) according to the Swiss Ordinance relating to Good Laboratory Practice adopted 18 May 2005 [RS 813.112.1]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted 26 November 1997 by the OECD Council [C (97)186/Final].

A pilot-study using both chrysotile alone and CSP was performed to assess the feasibility of a mixed exposure study. A few animals were exposed to each test material for 5 days and then examined for up to 3 days postexposure. The results, reported by Bernstein et al. (2008), confirmed the feasibility of performing this mixed exposure study. Detailed description of the fiber exposure methods is presented in Bernstein et al. (2008).

The methodology used in the fiber exposure and the in-life phases of the study conforms to the guideline issued by the European Commission (ECB/TM/26 rev.7, 1999) with the following enhancements:

- The fiber evaluation was performed using an Analytical Scanning Transmission Electron Microscope with Energy Dispersive X-ray analysis (ASTEM-EDS) using an accelerating voltage of 80 kV and a magnification of at least 10,000×.
- The analytical part of the ISO 13794 method for the determination of asbestos in ambient air by the indirect-transfer transmission electron microscopy (TEM) procedure was used.
- The stopping rules for fiber counting included specific rules for four different length categories as follows: 100 fibers with a length < 5 μm, 200 fibers with a length between 5 and 20 μm, and 100 fibers with a length > 20 μm and 100 particles.

### Sample preparation

The JM 7RF3 grade 7 chrysotile sample was received by RCC Ltd. from Exponent, Inc. The fiber as received contained fiber bundles, which were too thick to be rat respirable. In order to separate the fiber bundles, the fiber was processed using a small-scale, table-top, Cyclotec 1093 Sample Mill (FOSS Tecator, Sweden). This

is a low volume device, which opens the fiber bundles while obviating thermal degradation or contamination. Samples of a few milligrams of chrysotile were placed into the Cyclotec for a period of 1 min. This procedure was repeated three times for each sample to effectively open the bundles. No further processing was performed on the chrysotile. As presented in the results, the length distribution of the processed fibers in the aerosol was consistent with that of the pre-Cyclotec processed chrysotile sample.

Amosite used in this study was from the same batch as used in previous studies (Hesterberg et al., 1997, 1999a,b; McConnell et al., 1999; Rogers et al., 1999).

### Animal exposure

Acclimation: All animals were acclimatized to the restraint tubes and the inhalation exposure conditions by sham dosing over a period of 4 days of ~ 1, 2, 4, and 6 h on each successive day, respectively.

Three groups of rats were exposed for 6 h per day for 5 days to:

- Group 1: Filtered air alone (negative control group).
- Group 2: A fixed exposure level of well-characterized CSP.
- Group 3: A fixed exposure level of well-characterized amosite asbestos fibers.

Groups of 93 weanling (8- to 10-week-old) male rats (HanRcc: WIST(SPF), Harlan Laboratories Ltd. Laboratory Animal Services, 4414 Füllinsdorf/ Switzerland) were exposed by inhalation in a flow-past nose-only exposure system for 6 h/day for a period of 5 consecutive days either to CSP (group 2) or to amosite asbestos (group 3). This system was derived from Cannon et al. (1983) and is different from conventional nose-only exposure systems in that fresh fiber aerosol is supplied to each animal individually and exhaled air is immediately exhausted. Schematic diagrams of each exposure systems used in the study were presented in Bernstein et al. (2008). In the negative control group (group 1), 56 rats were exposed in a similar fashion to filtered air passed through an unloaded aerosol generator. In group 2, additional commercial grade 7RF3 chrysotile (from the same batch used to reformulate the joint compound) was added to the sanded powder containing short fiber chrysotile to assure compliance with the fiber exposure specifications of the EC protocol (EUR 18748 EN., 1999). In groups 2 and 3, a fiber concentration higher than that required by the EC Protocol of 100 fibers with length L > 20 μm/cm$^3$ was used in order to assure there was sufficient long fiber exposure. No amphibole (tremolite) fibers were detected in any of the analytical TEM with energy dispersive x-ray analysis (EDS) examinations.

For group 2 (CSP), two aerosols were generated using individual rotating brush aerosol generators. A fiber aerosol was generated from chrysotile fiber 7RF3 and a separate dust/fiber aerosol was generated from sanded material. The chrysotile fiber aerosol was passed through a 500-ml Pyrex glass cyclone to assist in the elimination of fiber bundles. The sanded powder aerosol was passed through a micronizing jet mill to reduce the particle size to be rat respirable. In-line $^{63}$Ni charge neutralizers were used to reduce the electrostatic charge to Boltzmann equilibrium. Following the charge neutralizers, the fiber and powder aerosols were mixed through a stainless steel Y-connection and then delivered directly into the nose-only flow-past exposure chamber.

For group 3, the aerosol of the amosite fiber was generated using a rotating brush aerosol generator followed by a $^{63}$Ni charge neutralizer to reduce the electrostatic charge on fibers to Boltzmann equilibrium. The aerosol was then delivered directly into the nose-only flow-past exposure chamber.

Control animals were exposed to filtered air passed through a separate brush-feed generator.

The aerosol mass was sampled for ~ 5 h during each exposure. Aerosol samples were collected on the appropriate filters in the vicinity of the animal's snout. Likewise, the temperature, relative humidity, and oxygen concentration were measured on atmosphere/ aerosol samples collected directly from the delivery tube in the breathing zone of the animals. Also, in order to monitor and control the gravimetric concentration of the sanded powder aerosol alone, filter samples were also taken from a sampling outlet following the micronizing jet mill.

The methods for the gravimetric determination of aerosol concentrations; sampling of fiber number and size distribution of aerosol concentrations; particle size of dust aerosol; counting rules for the evaluation of aerosol and lung burden samples by TEM; and clinical examination and body weights have been presented in Bernstein et al. (2008 and 2010).

### Methods for determination of postexposure endpoints

Fiber lung burden and histopathology were initially analyzed immediately following the end of the 5$^{th}$ day of exposure. This was termed day 0 of the nontreatment postexposure period.

Postexposure endpoints were developed in order to best answer the questions posed by this study. In the lung, these included:

- determination of the size and number of fibers in the lung in order to determine the biopersistence of the fibers,
- pathological response to the presence of fibers using histological examination, and
- confocal microscopic examination in order to determine the lung compartments in which the fibers are located and to visualize the juxtaposition of the fibers within the lung and any associated cellular response.

In the pleura, endpoints included:

- determination of the size and number of fibers on the diaphragm as a representative parietal pleural tissue, and any associated pathological response as a function of time postexposure, and
- examination of the lung pleural/interface using frozen chest sections in order to examine noninvasively the translocation of fibers to the pleural cavity and any pathological response.

Table 1 summarizes the end points analyzed in subgroups of rats at each of the postexposure time points shown. The detailed specifications of these methods have been presented in Bernstein et al. (2010).

Two methods were used to perform this analysis.

## Diaphragm

A biopsy punch (10 mm diameter) was used to collect tissue discs of a uniform area of parietal pleural for microscopic analysis. Fields of view were randomly selected and stacks of 25 serial sections each were recorded. Five fields of view were recorded from each parietal pleural tissue specimen. The dimensions of voxels in the recorded volume were (x, y, and z dimensions, respectively) 0.17, 0.17, and 0.39 μm.

## SEM image collection and analysis

Parietal pleural tissue specimens prepared for SEM were brought into focus at 1000× magnification. Random fields of view were recorded from each tissue piece and SEM image data were analyzed for inflammatory cells and fiber profiles.

Reported are all fiber profiles in length classes beginning with ≥3 μm. The procedure was to locate parietal pleural areas observed on the diaphragm, collect a series of images, move at least two field widths, and repeat the process.

The number of fibers in each field of view was counted by a human operator who was looking for the characteristic bright points or lines, which indicated a reflective or refractile fiber. In instances where free ends of the fiber were observed, fiber length was recorded using three-dimensional measurement techniques. Fibers in the parietal pleura were categorized as occurring:

- in contact with the parietal surface of tissue, and
- within the parietal tissue.

## Inflammatory cells

Pleural macrophages are a normal constituent of the pleural space. Numerous free cells localized in pleural spaces would represent inflammatory cells at these time

Table 1. Postexposure end points analyzed in subgroups of rats at each time points shown.

| Days after cessation of the 5-day exposure | Air control | CSP | Amosite asbestos |
|---|---|---|---|
| 0 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Lung histopathology | Lung histopathology | Lung histopathology |
| | Lung confocal microscopy | Lung confocal microscopy | Lung confocal microscopy |
| | Pleura—diaphragm | Pleura—diaphragm | Pleura—diaphragm |
| 1 | — | Fiber lung burden | Fiber lung burden |
| 2 | — | Fiber lung burden | Fiber lung burden |
| 7 | — | Fiber lung burden | Fiber lung burden |
| | | Lung histopathology | Lung histopathology |
| | | Lung confocal microscopy | Lung confocal microscopy |
| | | Pleura—diaphragm | Pleura—diaphragm |
| 14 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Lung histopathology | Lung histopathology | Lung histopathology |
| | Lung confocal microscopy | Lung confocal microscopy | Lung confocal microscopy |
| | Pleura—diaphragm | Pleura—diaphragm | Pleura—diaphragm |
| 30 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Lung histopathology | Lung histopathology | Lung histopathology |
| | Lung confocal microscopy | Lung confocal microscopy | Lung confocal microscopy |
| | Pleura—diaphragm | Pleura—diaphragm | Pleura—diaphragm |
| 90 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Lung histopathology | Lung histopathology | Lung histopathology |
| | Lung confocal microscopy | Lung confocal microscopy | Lung confocal microscopy |
| | Pleura—diaphragm | Pleura—diaphragm | Pleura—diaphragm |
| 181 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Pleura—frozen chest sections | Pleura—frozen chest sections | Pleura—frozen chest sections |
| 272 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Pleura—frozen chest sections | Pleura—frozen chest sections | Pleura—frozen chest sections |
| 363 | Fiber lung burden | Fiber lung burden | Fiber lung burden |
| | Pleura—frozen chest sections | Pleura—frozen chest sections | Pleura—frozen chest sections |

points. However, we determined that counting the number of free cells adherent to the parietal pleural surface did not produce a measurable observation. Therefore, we report instances of presence or absence in areas in which adherent cells appear to be accumulated. Groupings of more than three cells were considered to represent an inflammatory response.

## Results

### Validation of aerosol generation procedure

In this study, as described in the methods section above, the Grade 7 chrysotile fiber was prepared by passing it through a small-scale, table-top, Cyclotec 1093 Sample Mill (FOSS Tecator, Sweden). This is a low volume device, which separates the fiber bundles obviating thermal degradation or contamination. Brorby et al. (2008) reported on the TEM size distribution of the primary fibers and bundles of the Grade 7 chrysotile sample used in this study. Figure 1 compares the size distribution as determined by TEM of the original chrysotile sample (Brorby, personal communication) with that of the chrysotile aerosol to which the animals were exposed in this study. The methods used in this study successfully separated the thicker fiber bundles, removed the thicker fibers that were not respirable by the rat, and resulted in an aerosol exposure that was representative of the original material.

### Validation of lung digestion procedure

Comparative CM was used to assure that the lung digestion and TEM procedures used in this study did not affect the fiber dimensions of the chrysotile present in the lung (Bernstein et al., 2004).

The results of this analysis confirmed that there is a very good correlation between the length distribution as measured by the lung digestion procedure/TEM and the confocal methodology with a correlation $r^2 = 0.9$. In addition, the TEM procedure does not reduce the length distribution of the fibers seen in confocal analysis. The mean number of fibers remaining at each time point for the chrysotile group showed a good correlation ($r^2 = 0.9$)

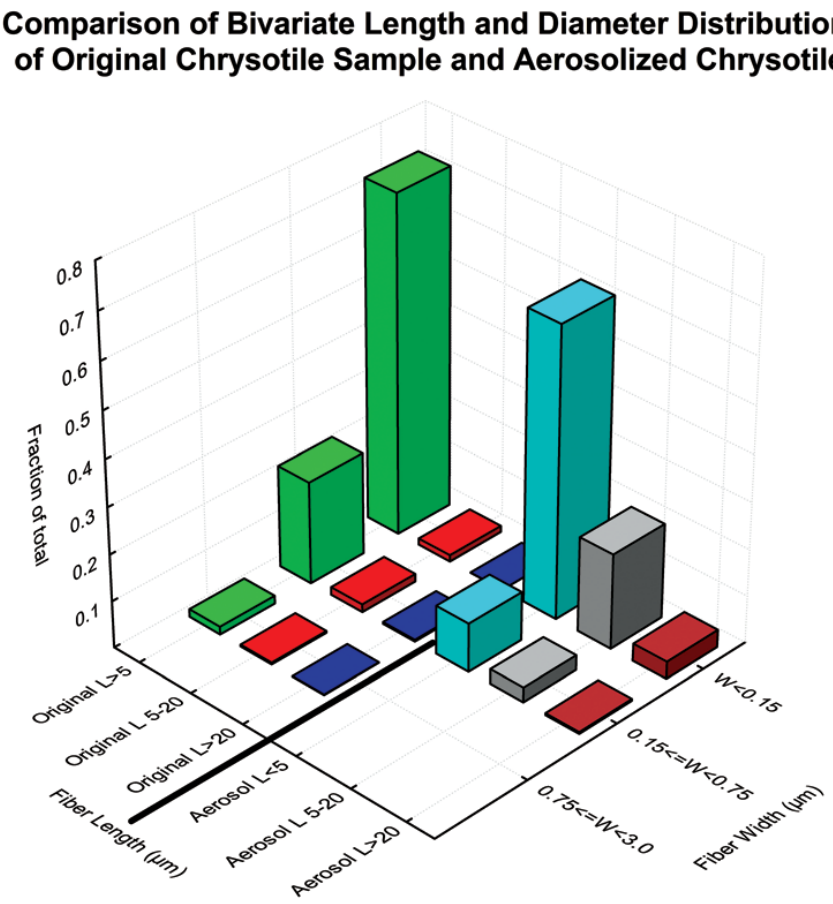



Figure 1. Comparison of the size distribution as determined by TEM of the original chrysotile sample (Brorby, personal communication) with that of the chrysotile aerosol to which the animals were exposed in this study.

between the TEM measurements from the lung digestion procedure and the measurements obtained by CM.

The analytical examination of both the aerosol and the lung samples using TEM included routinely chemical identification of fiber type using energy dispersive x-ray analysis (EDS), which allowed also the detection of the crystalline structure of the fibers by selected area electron diffraction. No amphibole (tremolite) fibers were detected in the chrysotile samples in any of the TEM/EDS examinations.

The aerosol concentration and size distribution of all groups are shown in Table 2. The fiber aerosol concentrations were chosen based upon the EC protocol, which specifies that the exposure atmospheres should have at least 100 fibers/cm$^3$ > 20 μm. In this study, additional chrysotile was added to the sanded material in order to achieve the mean number of fibers/cm$^3$ > 20 μm. The resulting mean number of fibers per cm$^3$ > 20 μm was 295 for chrysotile and 201 for amosite. Figure 2 shows the mean number of fibers in the exposure atmospheres in each of the three length categories < 5 μm, 5–20 μm, and > 20 μm for the chrysotile and amosite aerosols.

The mean number of WHO fibers (defined as fibers > 5 μm long, < 3 μm wide, and with length:width ratios > 3:1; WHO, 1985) in the CSP atmosphere was 1496 fibers/cm$^3$, which is > 10,000 times the OSHA occupational exposure limit of 0.1 fibers/cm$^3$. The amosite exposure atmosphere had fewer shorter fibers, resulting in a mean of 584 WHO fibers/cm$^3$. The mean total number of fibers of all sizes in the exposure atmosphere was 6543 fibers/cm$^3$ for chrysotile and 953 fibers/cm$^3$ for amosite.

The bivariate length and diameter size distributions of the CSP aerosol and the amosite asbestos aerosol have been presented in Bernstein et al. (2010).

### Fiber lung burdens

The mean concentrations and dimensions of the fibers recovered from the lungs at each time point for CSP and for amosite, respectively, are presented in Tables 3 and 4.

For the CSP-exposed rats (Table 3), the mean number of fibers longer than 20 μm decreased from 0.31 million fibers L > 20 μm per lung immediately following exposure (day 0) to 0 fibers at 90 days. At day 0, fibers up to 90 μm in length were observed. The maximum fiber length steadily decreased from 7 days onward and from 90 days through 365 days, with the maximum length in the range of 20–25 μm. (At 181 days, one fiber of 21 μm in length was observed on the filter aliquot analyzed from one animal and at 275 and 365 days one fiber of 25 μm in length was observed on the filter aliquot analyzed from one animal.) As these results were obtained through the lung digestion procedure, it was not possible to determine the association of these fibers with cells in particular macrophages. While in general the rat alveolar macrophage has been shown *in vitro* (Morimoto et al., 1994; Luoto et al., 1995; Zeidler-Erdely et al., 2006) to engulf fibers up to 20 μm in length, the results suggest that an occasional macrophage can engulf a slightly longer fiber.

For the amosite-exposed rats (Table 4), the number of fibers longer than 20 μm decreased slightly from 2.74 million fibers per lung immediately after exposure to 1.98 million fibers per lung at 7 days postexposure and 1.4 million fibers longer than 20 μm per lung observed at 365 days. The maximum fiber length observed 0 days postexposure was 110 μm and remained very similar throughout the 365 days postexposure period with a maximum length of 105 μm observed at 365 days.

Figures 3 and 4 show the bivariate length and diameter distribution of fibers in the CSP and the amosite-exposed lungs, respectively, immediately after cessation of exposure (day 0) and at 365 days after cessation of exposure. Only relatively short and thin fibers remain in the CSP-exposed lungs. For the amosite-exposed lungs, the thicker fibers, which likely deposited in the tracheobronchial tree, have been cleared by 365 days. The distribution of the thinner fibers remained remarkably similar to what was observed at 0 days postexposure.

The clearance of fibers from the lung through 365 days postexposure is shown in Figures 5 and 6 for the CSP-exposed animals and the amosite-exposed animals, respectively. Each figure shows the data and clearance curves for fibers < 5 μm in length, fibers 5–20 μm in length, and fibers > 20 μm in length. Individual values for each animal and each size fraction are shown as are the clearance curves and the clearance half-times. For the CSP-exposed animals, the clearance curves were best fit using nonlinear estimation to a single exponential. For the amosite-exposed animals, the clearance curves were best fit using nonlinear estimation to a double exponential; with the clearance half-times expressed as the weighted $T_{1/2}$ (EUR 18748 EN., 1999).

In the CSP-exposed animals (Figure 5), the fibers longer than 20 μm were rapidly cleared from the lung with a clearance half-time of 4.5 days. The fibers 5–20 μm in length were cleared with a half-time of 12.8 days, while the fibers < 5 μm in length cleared with a half-time of 27.8 days.

In the amosite-exposed animals (Figure 6), the number of fibers longer than 20 μm remaining in the lung showed a small reduction immediately following exposure with little subsequent clearance from the lung, with a weighted clearance half-time of > 1000 days. This initial reduction is likely due to fibers that deposited in the trachea-bronchial tree. The fibers 5–20 μm in length and the fibers < 5 μm in length also showed an initial reduction immediately following cessation of exposure. However, the strong inflammatory response created by the longer fibers appears to have locked-up the shorter fibers as well, with a weighted clearance half-time > 1000 days for these smaller length fractions.

### Histopathological results

The results from the histopathological examination of the lungs have been presented in detail in Bernstein

Table 2. Aerosol concentration and size distribution of the air control, CSP, and amosite inhalation exposure atmospheres.

| Exposure group | Gravimetric concentration mg/m³ (SD) | Density g/cm³ | Number of fibers evaluated | Number of total fibers/ cm³ | WHO fibers/ cm³ | Percent WHO fibers | Number of fibers ≥ 20 μm/ cm³ | Percent of all fibers ≥ 20 μm/ cm³ | Diameter range (μm) | Length range (μm) | GMD (μm) (SD) | GML (μm) (SD) | Mean diameter (μm) (SD) | Mean length (μm) (SD) | Length weighted Arthm. Dmeter (μm) | Length weighted Geom. Ddiameter (μm) | Aspect ratio | Equivalent fiber diameter (μm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (Group 1) Air control | 0 | — | 2.0 | 0.078 | 0.042 | 53.8% | 0 | 0% | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| (Group 2) CSP | 2.64 (0.097) | 2.6 | 2076 | 6543 | 1496 | 22.9% | 295 | 4.5% | 0.01–0.6 | 1.0–180 | 0.04 (2.26) | 3.48 (2.33) | 0.06 (0.07) | 5.43 (8.93) | 0.06 | 0.04 | 187.8 | 0.03 |
| Sanded material | 1.05 (0.085) | — | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 1.18 (MMAD) | n.a. | 2.75 | n.a. | n.a. | n.a. | n.a. | n.a. |
| (Group 3) Amosite | 6.39 (1.19) | 3.5 | 2059 | 953 | 584 | 61.2% | 201 | 21.1% | 0.03–1.2 | 0.8–150 | 0.26 (1.95) | 8.13 (2.91) | 0.32 (0.17) | 13.81 (16.99) | 0.39 | 0.29 | 50.9 | 0.17 |

The mean gravimetric concentration of the CSP exposure atmosphere was 2.64 mg/m³. The corresponding mean gravimetric concentration of the sanded material (including sanded 7RF3 chrysotile) was 1.05 mg/m³, with the sanded material having a mass median aerodynamic diameter (MMAD) of 1.18 μm. The corresponding mean gravimetric concentration of the amosite exposure atmosphere was 6.39 mg/m³. This gravimetric difference was due to the difference in the amosite fiber size distribution in comparison with the chrysotile.

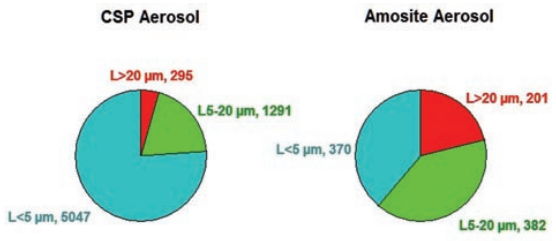



Figure 2. The mean number of fibers in the exposure atmospheres in each of the three length categories < 5 µm, 5–20 µm, and > 20 µm are illustrated for the CSP and amosite aerosols.

et al. (2010). These findings confirmed that animals exposed to CPP produced no sign of pulmonary inflammation, aside from a macrophage response, at any time point. In contrast, the animals exposed to amosite asbestos showed a marked inflammatory response starting immediately after cessation of exposure (day 0). By 28 days postexposure, the lungs exhibited interstitial fibrosis with a Wagner Grade 4. These lesions were observed both in the conventional histopathological micrographs and in the 3D confocal micrographs. Because CM permits noninvasive imaging of a cube of tissue, micrographs using this technique were especially useful in showing the juxtaposition of especially the long amosite fibers in the tissue and their relationship to the observed lesions.

### Translocation of fibers to the pleural cavity and subsequent response

One important objective of this study was to examine the translocation of fibers to the pleural cavity using nonevasive techniques and to evaluate the possible response to the fibers.

Two methods were used to perform this analysis. The examination of the diaphragm as a representative parietal pleural tissue was performed on the same animals that were examined for lung histopathology and CM. These animals were examined at time points starting at 0 days, immediately following cessation of exposure, through 90 days postexposure. The examination of the visceral pleural and the subvisceral pleural regions of the lung was performed on subgroups of animals starting at 181 days through 365 days postexposure. The visceral pleural examination method required immediate deep freezing of the rats following killing, which precluded other analyses and thereby accounted for the differential timing between the two methods.

### Diaphragm

The number of fibers in each field of view (average field of view was 0.03 $mm^2$) was counted by a human operator, who was looking for the characteristic bright points or lines, which indicate a reflective or refractile fiber. In instances where free ends of the fiber were observed, fiber length was recorded using three-dimensional measurement techniques. Fibers in the parietal pleura were categorized as occurring:

- in contact with parietal surface of tissue, or
- within the parietal tissue.

### Inflammatory response to fibers

While pleural macrophages are a normal constituent of the pleural fluid (Noppen et al., 2000), the likelihood of finding individual macrophages adherent to the parietal pleural surface is small. Instead, examination was directed in areas in which adherent cells appear to be accumulated. Groupings of more than three cells (macrophages, neutrophils, etc.) were considered as representing an inflammatory response.

In animals exposed to CSP, no parietal pleura lesions were observed at any time points studied. The parietal pleura surface from an animal exposed to CSP at 90 days postexposure is shown in Figure 7. An occasional macrophage is observed, however, there is no associated inflammatory response or lesions.

In animals exposed to amosite asbestos, when amosite fibers were observed on the parietal pleural surface, enlarged macrophages adherent to the parietal surface were also observed. Macrophages exhibited extended pseudopodia with Lamella project a, which is indicative of activated macrophages. Fibrotic lesions were also occasionally observed. As shown in Figure 8, a network of large activated macrophages and an associated fibrin matrix network was observed on the parietal pleural surface at 14 days postexposure. At 90 days postexposure, numerous macrophages were observed on the parietal pleural as shown in Figure 9 (The triangular indentation seen in the micrograph was likely due to the back of a forceps, which was used for straightening the diaphragm after removal from the animal). Confocal imaging of the diaphragm has indicated the presence of a number of amosite fibers in this region.

### Visceral pleural examination

While examination of the diagram provided a unique opportunity to examine the differential response on the parietal pleural surface, how fibers are transported to the pleural cavity and what impact they may have on the visceral pleural barrier have long been an open question.

To address this issue, the visceral pleural was systematically examined from cross-sections of rats that were frozen in liquid nitrogen immediately following sacrifice. This procedure was used in order to avoid possible artifacts that could stem from cross-contamination of fibers from the lung to the pleural cavity when tissues are manipulated at necropsy. The examination included a systematic survey using CM of the visceral pleural wall, the adjacent subpleural alveoli and the pleural space. The features of the tissues were evaluated

Table 3. CSP-exposed lungs—mean concentrations and dimensions of the fibers recovered from the lungs at each time point.

| | 0 Day | 1 Day | 2 Days | 7 Days | 14 Days | 30 Days | 90 Days | 181 Days | 275 Days | 365 Days |
|---|---|---|---|---|---|---|---|---|---|---|
| B07031 Lung samples group 2—CSP | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean |
| No. of fibers evaluated | 2568.5 | 2471.0 | 2461.0 | 2339.0 | 2216.0 | 2098.0 | 1382.0 | 1252.5 | 1348.0 | 1940.0 |
| No. of total fibers (millions/lung)/(standard deviation) | 39.57 (11.73) | 42.26 (9.64) | 30.44 (5.32) | 38.71 (3.21) | 22.27 (6.00) | 13.44 (3.11) | 6.13 (0.67) | 5.53 (0.42) | 3.15 (0.76) | 3.43 (0.79) |
| No. WHO fibers (millions/lung)/(standard deviation) | 9.78 (2.79) | 9.82 (2.02) | 7.91 (2.75) | 7.84 (3.21) | 3.91 (1.65) | 1.76 (0.54) | 0.53 (0.17) | 0.43 (0.10) | 0.51 (0.28) | 0.49 (0.03) |
| No. WHO fibers of total fibers (%) | 24.7% | 23.2% | 26.0% | 20.2% | 17.5% | 13.1% | 8.7% | 7.8% | 16.3% | 14.2% |
| No. of fibers L > 20 µm (millions/lung/(standard deviation) | 0.31 (0.11) | 0.24 (0.05) | 0.23 (0.03) | 0.11 (0.02) | 0.02 (0.00) | 0.01 (0.01) | 0.00-- | 0.001-* | 0.001--* | 0.001--* |
| Fibers L > 20 µm of total fibers (%) | 0.8% | 0.6% | 0.7% | 0.3% | 0.1% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% |
| No. of fibers L 5–20 µm (millions/lung)/(standard deviation) | 9.47 (2.70) | 9.58 (1.98) | 7.69 (2.74) | 7.72 (2.59) | 3.89 (1.65) | 1.75 (0.54) | 0.53 (0.17) | 0.43 (0.10) | 0.51 (0.28) | 0.49 (0.03) |
| Fibers L 5–20 µm of total fibers (%) | 23.9% | 22.7% | 25.2% | 20.0% | 17.4% | 13.0% | 8.7% | 7.8% | 16.2% | 14.2% |
| No. of fibers L ≤ 5 µm (millions/lung)/(standard deviation) | 29.79 (9.08) | 32.44 (7.81) | 22.53 (3.21) | 30.88 (7.29) | 18.37 (4.61) | 11.68 (2.72) | 5.60 (0.52) | 5.10 (0.45) | 2.64 (0.51) | 2.94 (0.80) |
| Fibers L ≤ 5 µm of total fibers (%) | 75.3% | 76.8% | 74.0% | 79.8% | 82.5% | 86.9% | 91.3% | 92.2% | 83.7% | 85.8% |
| Diameter range (µm) | 0.01–1.3 | 0.01–1.0 | 0.01–2.0 | 0.01–2.0 | 0.01–1.0 | 0.01–1.0 | 0.01–1.0 | 0.01–0.5 | 0.01–0.6 | 0.01–0.5 |
| Length range (µm) | 1.0–90 | 1.0–90 | 1.0–110 | 1.0–80 | 0.5–32 | 0.5–40 | 0.5–20 | 0.5–21.0 | 0.5–25.0 | 0.5–25.0 |
| Mean diameter (µm) | 0.11 | 0.09 | 0.10 | 0.10 | 0.09 | 0.07 | 0.06 | 0.06 | 0.05 | 0.04 |
| SD | 0.11 | 0.09 | 0.10 | 0.10 | 0.09 | 0.08 | 0.07 | 0.06 | 0.05 | 0.04 |
| Mean length (µm) | 4.60 | 4.45 | 4.66 | 4.02 | 3.55 | 3.19 | 2.83 | 2.95 | 3.69 | 3.59 |
| SD | 3.77 | 3.50 | 3.80 | 3.10 | 2.47 | 2.27 | 1.95 | 1.87 | 2.46 | 2.39 |
| GMD (µm) | 0.06 | 0.06 | 0.07 | 0.07 | 0.06 | 0.05 | 0.04 | 0.04 | 0.04 | 0.03 |
| GSD | 2.82 | 2.46 | 2.53 | 2.47 | 2.45 | 2.19 | 2.31 | 2.20 | 2.04 | 1.86 |
| GML (µm) | 3.72 | 3.64 | 3.82 | 3.30 | 2.95 | 2.59 | 2.31 | 2.49 | 3.07 | 2.99 |
| GSD | 1.88 | 1.85 | 1.84 | 1.84 | 1.93 | 1.98 | 1.93 | 1.80 | 1.90 | 1.85 |
| Length weighted arithmetic dia. (µm) | 0.13 | 0.11 | 0.12 | 0.12 | 0.10 | 0.09 | 0.08 | 0.07 | 0.07 | 0.05 |
| Length weighted geometric dia. (µm) | 0.07 | 0.07 | 0.07 | 0.07 | 0.06 | 0.06 | 0.05 | 0.05 | 0.05 | 0.04 |
| Mode diameter (µm) | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Mode length (µm) | 2.5 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.5 | 2.0 | 2.5 |
| Median diameter (µm) | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Median length (µm) | 4.0 | 4.0 | 4.0 | 3.5 | 3.0 | 2.5 | 2.5 | 2.5 | 3.0 | 3.0 |
| Aspect ratio mean | 101.4 | 92.6 | 93.2 | 75.7 | 76.0 | 76.4 | 80.5 | 84.3 | 101.3 | 117.9 |
| Total length of fibers per lung (m) | 182 | 188 | 142 | 156 | 79 | 43 | 17 | 16.3 | 11.6 | 12.3 |
| Mass of fibers per lung in milligrams (density 2.6 g/cm$^3$) | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| No. of particles evaluated | 705.0 | 702.0 | 704.0 | 705.0 | 702.0 | 708.0 | 287.0 | 365.5 | 255.0 | 363.0 |
| Mean no. of particles (millions/lung) | 2.47 | 2.20 | 2.08 | 2.59 | 1.79 | 1.48 | 0.23 | 0.294 | 0.205 | 0.146 |
| ≤ 1 µm particles (millions/lung lobes) | 2.06 | 2.08 | 1.98 | 2.46 | 1.75 | 1.48 | 0.23 | 0.292 | 0.205 | 0.145 |
| > 1 µm to ≤ 3 µm particles (millions/lung lobes) | 0.41 | 0.12 | 0.10 | 0.13 | 0.04 | 0.00 | 0.00 | 0.002 | 0.000 | 0.000 |
| > 3 µm particles (millions/lung lobes) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.000 | 0.000 | 0.000 |

*Standard deviation not presented as only one fiber was observed with length 21 µm—181 days and 25 µm—272 and 365 days.

Table 4. Amosite-exposed lungs—mean concentrations and dimensions of the fibers recovered from the lungs at each time point.

| | 0 Day | 1 Day | 2 Days | 7 Days | 14 Days | 30 Days | 90 Days | 181 Days | 275 Days | 365 Days |
|---|---|---|---|---|---|---|---|---|---|---|
| B07031 Lung samples group 3—amosite | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean |
| No. of fibers evaluated | 2923.0 | 2883.0 | 2902.5 | 2881.5 | 2866.5 | 2860.5 | 2793.5 | 2811.0 | 2857.0 | 2844.5 |
| No. of total fibers (millions/lung)/(standard deviation) | 23.30 (8.04) | 20.33 (2.79) | 21.85 (5.41) | 17.84 (4.62) | 18.17 (4.65) | 18.02 (3.13) | 9.56 (3.15) | 10.01 (1.99) | 9.56 (0.93) | 9.82 (1.25) |
| No. WHO fibers (millions/lung)/(standard deviation) | 12.91 (4.02) | 10.56 (1.48) | 12.77 (3.21) | 9.56 (3.00) | 8.38 (2.03) | 9.33 (1.54) | 4.98 (1.68) | 5.46 (1.68) | 5.76 (0.93) | 5.78 (0.96) |
| No. WHO fibers of total fibers (%) | 55.4% | 51.9% | 58.4% | 53.6% | 46.1% | 51.7% | 52.1% | 54.5% | 60.2% | 58.9% |
| No. of fibers L > 20 µm (millions/lung/(standard deviation) | 2.74 (0.81) | 2.24 (0.41) | 2.81 (0.56) | 1.98 (0.58) | 1.63 (0.37) | 1.98 (0.28) | 1.10 (0.56) | 1.11 (0.43) | 1.33 (0.23) | 1.39 (0.19) |
| Fibers L > 20 µm of total fibers (%) | 11.8% | 11.0% | 12.9% | 11.1% | 8.9% | 11.0% | 11.5% | 11.1% | 14.0% | 14.1% |
| No. of fibers L 5–20 µm (millions/lung)/(standard deviation) | 10.16 (3.40) | 8.32 (1.18) | 9.96 (2.72) | 7.58 (2.45) | 6.76 (1.71) | 7.34 (1.31) | 3.88 (1.14) | 4.35 (1.26) | 4.42 (0.72) | 4.39 (0.81) |
| Fibers L 5–20 µm of total fibers (%) | 43.6% | 40.9% | 45.6% | 42.5% | 37.2% | 40.7% | 40.6% | 43.4% | 46.3% | 44.7% |
| No. of fibers L ≤ 5 µm (millions/lung)/(standard deviation) | 10.40 (4.31) | 9.77 (2.24) | 9.08 (2.41) | 8.28 (2.90) | 9.79 (3.24) | 8.70 (2.33) | 4.58 (1.57) | 4.55 (0.42) | 3.80 (0.48) | 4.04 (0.55) |
| Fibers L ≤ 5 µm of total fibers (%) | 44.6% | 48.1% | 41.6% | 46.4% | 53.9% | 48.3% | 47.9% | 45.5% | 39.8% | 41.1% |
| Diameter range (µm) | 0.03–3.0 | 0.03–3.0 | 0.02–3.0 | 0.02–2.0 | 0.03–1.5 | 0.03–1.5 | 0.03–1.6 | 0.03–1.4 | 0.03–2.0 | 0.03–1.5 |
| Length range (µm) | 0.5–110 | 0.6–110 | 0.7–100 | 0.6–125 | 0.8–90.0 | 0.6–105 | 1.0–110 | 0.5–105.0 | 0.7–95.0 | 1.0–105.0 |
| Mean diameter (µm) | 0.35 | 0.34 | 0.37 | 0.34 | 0.33 | 0.34 | 0.36 | 0.36 | 0.33 | 0.30 |
| SD | 0.21 | 0.22 | 0.26 | 0.19 | 0.19 | 0.18 | 0.21 | 0.19 | 0.19 | 0.18 |
| Mean length (µm) | 9.54 | 9.23 | 10.16 | 9.22 | 8.03 | 9.18 | 8.95 | 8.85 | 10.66 | 10.78 |
| SD | 10.11 | 10.16 | 10.68 | 10.10 | 9.01 | 9.76 | 9.78 | 9.37 | 10.71 | 11.59 |
| GMD (µm) | 0.29 | 0.28 | 0.29 | 0.27 | 0.27 | 0.29 | 0.29 | 0.30 | 0.27 | 0.23 |
| GSD | 1.84 | 1.99 | 2.06 | 2.02 | 2.00 | 1.85 | 2.11 | 1.98 | 2.11 | 2.19 |
| GML (µm) | 6.23 | 5.87 | 6.50 | 5.87 | 5.05 | 5.92 | 5.65 | 5.75 | 6.94 | 6.81 |
| GSD | 2.65 | 2.65 | 2.72 | 2.64 | 2.72 | 2.63 | 2.68 | 2.64 | 2.59 | 2.71 |
| Length weighted arithmetic dia. (µm) | 0.43 | 0.43 | 0.47 | 0.41 | 0.42 | 0.41 | 0.46 | 0.44 | 0.42 | 0.39 |
| Length weighted geometric dia. (µm) | 0.33 | 0.32 | 0.34 | 0.32 | 0.32 | 0.33 | 0.35 | 0.33 | 0.31 | 0.28 |
| Mode diameter (µm) | 0.40 | 0.40 | 0.20 | 0.40 | 0.20 | 0.40 | 0.30 | 0.35 | 0.35 | 0.35 |
| Mode length (µm) | 2.0 | 2.0 | 2.0 | 1.5 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.5 |
| Median diameter (µm) | 0.33 | 0.30 | 0.30 | 0.30 | 0.30 | 0.33 | 0.35 | 0.35 | 0.30 | 0.30 |
| Median length (µm) | 6.0 | 6.0 | 7.0 | 6.0 | 4.5 | 6.0 | 5.5 | 6.0 | 7.0 | 7.0 |
| Aspect ratio mean | 32.19 | 32.51 | 34.51 | 33.43 | 28.76 | 30.70 | 29.57 | 30.6 | 37.6 | 42.1 |
| Total length of fibers per lung (m) | 224.7 | 189.6 | 223.3 | 168.8 | 147.7 | 166.8 | 88.1 | 92.6 | 103.2 | 106.5 |
| Mass of fibers per lung in milligrams (density 2.6 g/cm$^3$) | 0.15 | 0.13 | 0.19 | 0.10 | 0.09 | 0.10 | 0.06 | 0.06 | 0.06 | 0.06 |
| No. of particles evaluated | 357.0 | 297.5 | 201.5 | 317.5 | 379.0 | 317.0 | 465.5 | 287.0 | 201.0 | 163.0 |
| Mean no. of particles (millions/lung) | 1.38 | 0.93 | 0.79 | 0.87 | 0.85 | 0.87 | 0.72 | 0.440 | 0.369 | 0.316 |
| ≤ 1 µm particles (millions/lung lobes) | 1.14 | 0.72 | 0.60 | 0.83 | 0.82 | 0.84 | 0.68 | 0.375 | 0.340 | 0.305 |
| > 1 µm to ≤ 3 µm particles (millions/lung lobes) | 0.23 | 0.19 | 0.19 | 0.04 | 0.02 | 0.02 | 0.04 | 0.065 | 0.029 | 0.012 |
| > 3 µm particles (millions/lung lobes) | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.000 | 0.000 | 0.000 |

Clearance half-times of fibers.

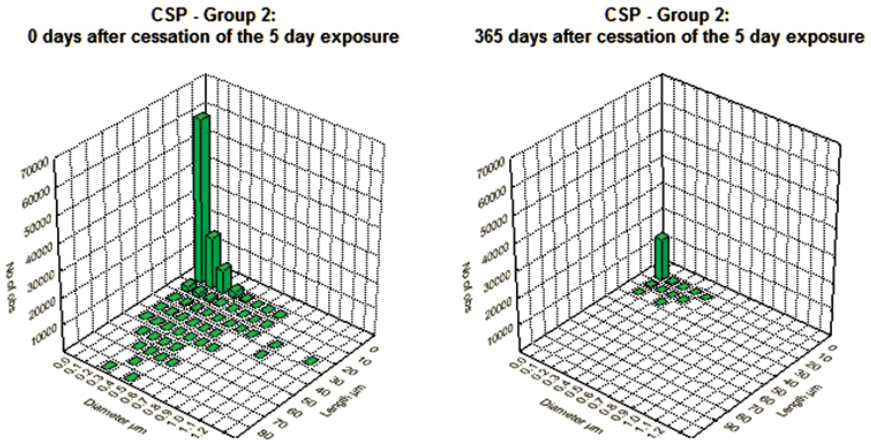



Figure 3. Bivariate length and diameter distribution of fibers in the CSP-exposed lungs, respectively, immediately after cessation of exposure (day 0) and at 365 days after cessation of exposure.

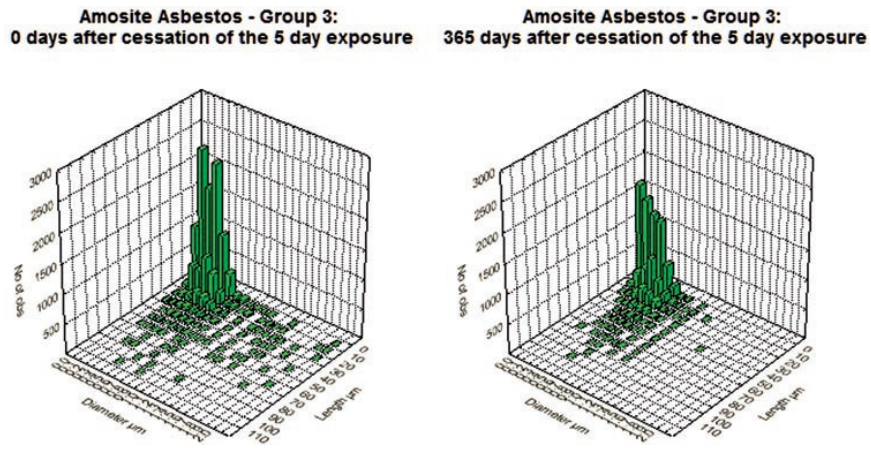



Figure 4. Bivariate length and diameter distribution of fibers in the amosite-exposed lungs, respectively, immediately after cessation of exposure (day 0) and at 365 days after cessation of exposure.

and the location and length of any fibers present were determined.

In addition, the thickness of the pleural wall was measured at between 5 and 10 points in each section examined. The results of this analysis are shown in Figure 10. The visceral pleural wall averaged ~ 2 µm in thickness in the air control group. The width ranged from < 1 µm to ~ 7 µm and remained relatively constant from 181 days through 365 days postexposure. The visceral pleural thickness in the CSP-exposed rats was nearly identical to that of the air control animals with no statistical difference at any time point.

In the amosite-exposed rats, the mean visceral pleural thickness was ~ 5 µm, more than double that of the air control and CPS exposure groups. This difference was statistically significant at all-time points from 181 to 365 days (Dunnett's t-test, $p < 0.01$). The thickness of the visceral pleural in the amosite-exposed animals ranged from ~ 1 µm to > 20 µm.

Another measure of effect was the determination of pleural defects. Pleural defects were defined as a change in the pleural surface (visceral or parietal) thickness or surface interface appearance as indicated by connective tissue increase, accumulation of cells, or, in the case of visceral pleural side, appearance of subpleural alveolar involvement by inflammatory cells or connective tissue. The average number of pleural defects per field of view (average field of view was 0.0075 $mm^2$) is shown in Figure 11. No pleural defects were observed in either the air control or the CSP-exposed animals at any time point. In the amosite-exposed rats, the number of pleural defects per field of view ranged from 40 at 181 days to > 15 at 365 days.

The number and length of fibers present at the visceral surface were also quantified as shown in Figure 12. No fibers were observed at the visceral pleural surface in the air control or the CSP-exposed animals at 181,

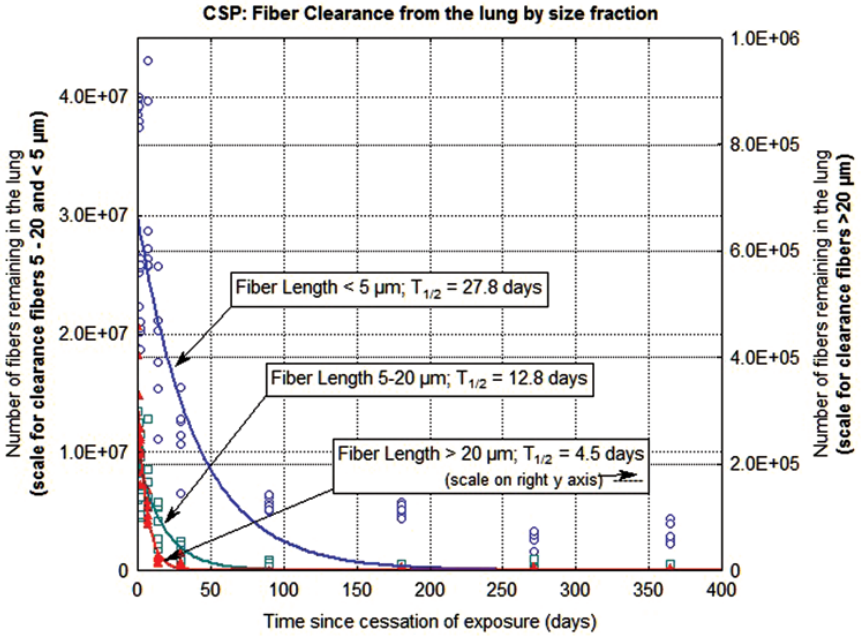



Figure 5. The clearance of fibers from the lung through 365 days postexposure is shown for CSP-exposed animals. The data and clearance curves for fibers < 5 µm in length, fibers 5–20 µm in length, and fibers > 20 µm in length are presented. (Note that the axis for the number of fibers remaining in the lung > 20 µm is on the right side of the graph.)

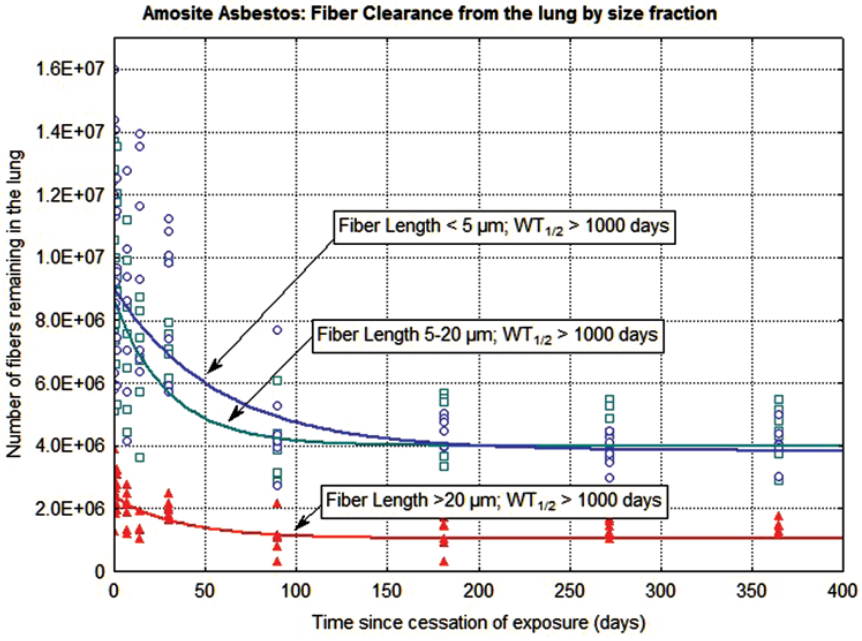



Figure 6. The clearance of fibers from the lung through 365 days postexposure is shown for amosite-exposed animals. The data and clearance curves for fibers < 5 µm in length, fibers 5–20 µm in length, and fibers > 20 µm in length are presented.

275, or 365 days postexposure. In the amosite-exposed animals, an average of 16 fibers were observed at 181 days, 10 fibers at 272 days, and 4 fibers at 365 days normalized to a mean observed parietal pleural area of 0.01 $mm^2$. The fiber length observed ranged from ~ 1 µm to > 12.5 µm.

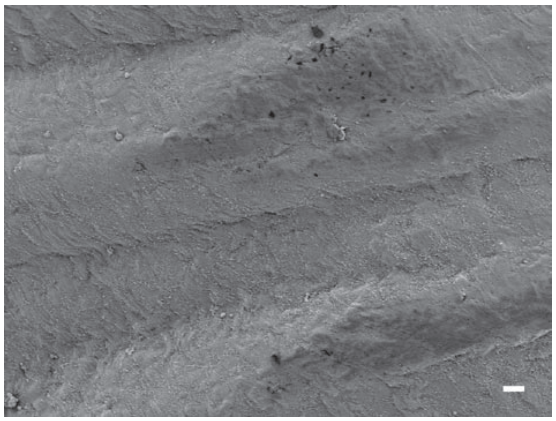


Figure 7. The parietal pleural surface from an animal exposed to CSP at 90 days postexposure. An occasional macrophage is observed; however, there is no associated inflammatory response or lesions.

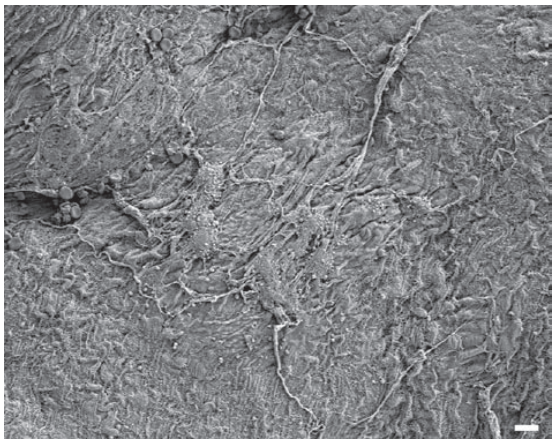


Figure 8. The parietal pleural surface from an animal exposed to amosite asbestos at 14 days postexposure. A network of large activated macrophages, which have laid down a fibrin matrix, is observed.

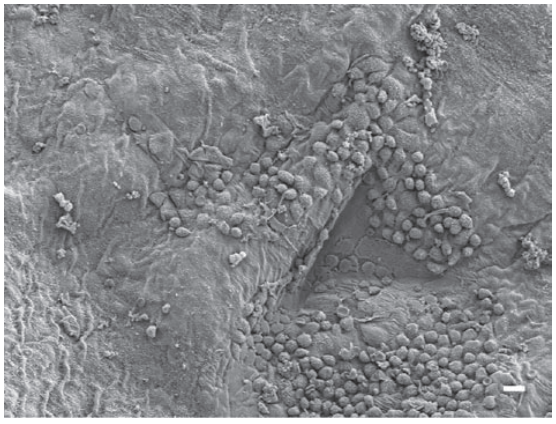


Figure 9. The parietal pleural surface from an animal exposed to amosite asbestos at 90 days postexposure. Numerous macrophages are observed on the parietal pleural (the triangular indentation seen in the micrograph was likely due to the back of a forceps, which was used for straightening the diaphragm after removal from the animal). Confocal imaging of the diaphragm indicated the presence of a number of amosite fibers in this region.



### Examination of the visceral pleura interface

#### *Air control*

A typical view of the pleural space at 181 days postexposure from a control animal is shown in Figure 13. The subpleural alveolar septa is seen on the left with the parietal pleura shown on the right. The brighter white is indicative of collagen in the visceral and parietal pleural walls. Free macrophages are present within the pleural space (bottom middle).

The pleural space at 272 days postexposure from a control animal is very similar as shown in the confocal micrograph in Figure 14. The subpleural alveolar septa adjacent to visceral pleura are seen on the left, pleural space in the middle, and parietal pleura with the chest on the right.

#### *CSP*

A typical field of view from an animal exposed to CSP mixture at 272 days postexposure is shown in Figure 15. This confocal micrograph is very similar to that seen for the air control (Figure 14).

#### *Amosite asbestos*

A confocal 3D micrograph of the visceral pleura and the adjacent subpleural alveoli from an animal exposed to amosite asbestos at 181 days postexposure is shown in Figure 16. Amosite fibers are seen in a subpleural granuloma with numerous alveolar macrophages in the subpleural alveoli.

Figure 17 shows an amosite fiber ~ 39 μm in length within a subpleural granuloma at 272 days postexposure. The right edge of this long fiber pierces the subpleural capsule. The thicker bright white matrix is indicative of a fibrotic thickening of the visceral pleura.

A typical field of view from an animal exposed to amosite asbestos at 272 days postexposure is shown in Figure 18. The subpleural alveolar septa seen in the left center of the image contains fibrotic lesions (thicker bright white matrix, which is indicative of enhanced collagen deposition). The parietal pleura and chest wall is shown on the right. Within the alveolus on the left, a number of subpleural macrophages can be seen.

Figure 19 shows an amosite fiber penetrating the visceral pleural wall into the pleural space at 365 days following cessation of exposure. On the lung side, a well-developed subpleural granuloma is seen with alveolar macrophages on the surface.

## Discussion

### Within the lung

As presented above, the exposure concentration of fibers longer than 20 μm/cm$^3$ was more than double in the

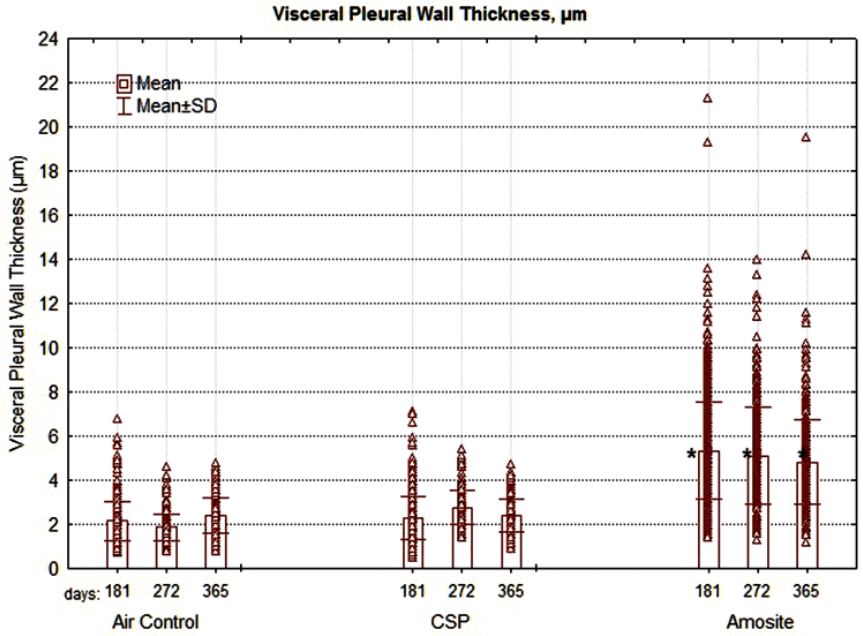



Figure 10. The thickness of the pleural wall measured at between 5 and 10 points in each section examined is shown for the air control, CSP, and amosite asbestos-exposed groups. The mean visceral wall thickness of the amosite-exposed group was statistically larger than the mean visceral wall thickness of the chrysotile or air-control groups (Dunnett's $T$-test, $P < 0.01$).

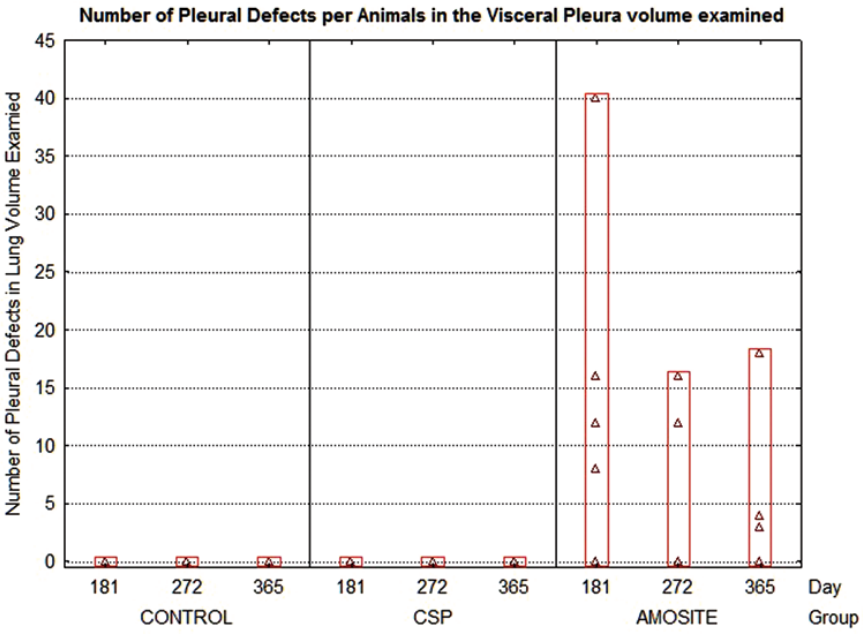



Figure 11. The average number of pleural defects per field of view (average field of view was 0.0075 mm$^2$) is shown for the air control, CSP, and amosite asbestos-exposed groups.

CSP-exposed rats as compared to the amosite-exposed rats and in both cases exceeded the number of fibers recommended in the protocol (ECB/TM/26 rev.7, 1999). Immediately following cessation of the 5-day exposure, the number of fibers > 20 µm in length remaining in the lung was 0.31 million for the CSP-exposed rats as compared to 2.74 million for the amosite-exposed rats. This is a result of the longer amosite fibers not dissolving or breaking apart in the lung in contrast to the chrysotile fibers, which rapidly break apart and are cleared.

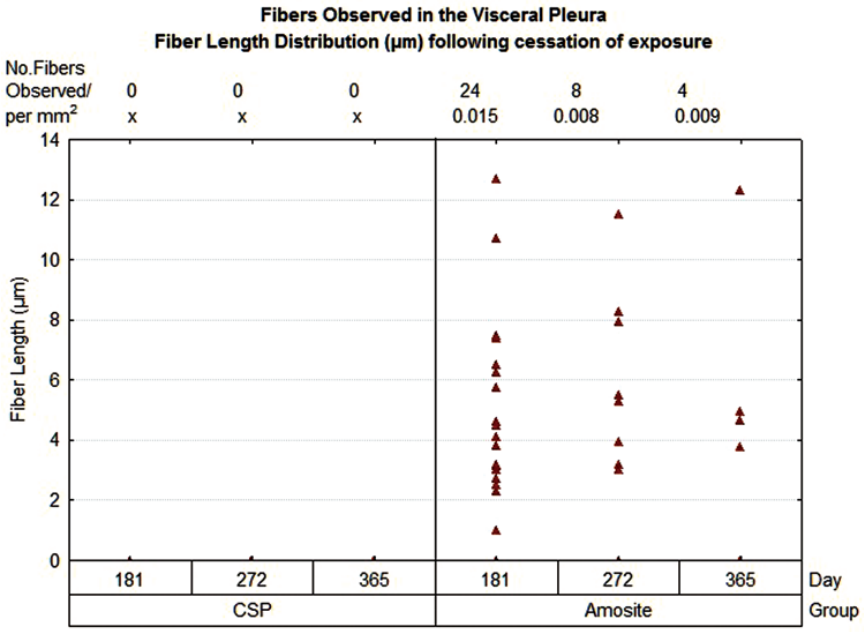



Figure 12. The number and length of fibers present at the visceral surface are shown.

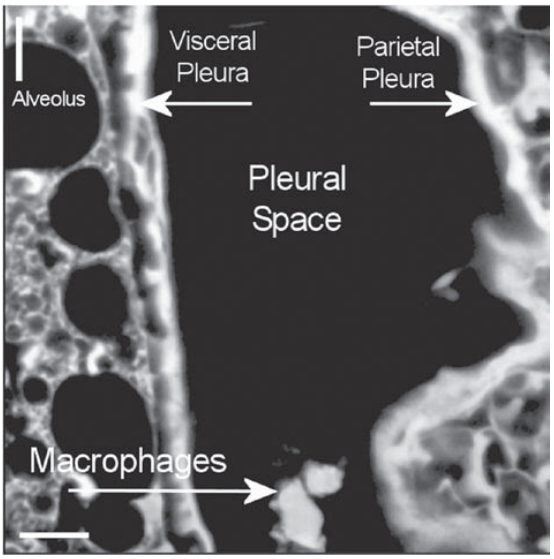



Figure 13. View of the pleural space at 181 days postexposure from a control animal is shown in the confocal image. The subpleural alveolar septa is seen on the left with the parietal pleura shown on the right. The brighter white is indicative of collagen in the visceral and parietal pleural walls. Free macrophages are present within the pleural space (bottom middle).

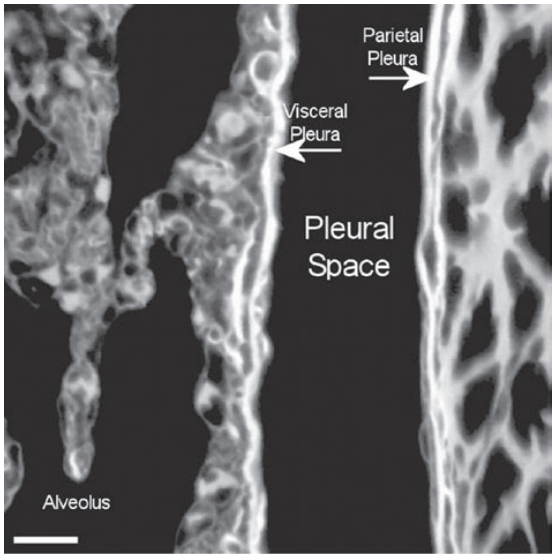



Figure 14. View of the pleural space at 272 days postexposure from a control animal is shown in the confocal image. The subpleural alveolar septa is seen on the left with the parietal pleura shown on the right. The brighter white is indicative of collagen in the visceral and parietal pleural walls.

## CSP

Chrysotile fibers are thin (0.8 angstroms) rolled sheets composed of magnesium silicate. The magnesium is on the outside of the sheet and can be solubilized in the alveolar surfactant. The crystalline structure of the silica matrix is attacked and broken apart by the acid environment of macrophages recruited in response to any inhaled particle (Pundsack, 1955; Wypych et al., 2005).

In this study, the CSP began clearing from the lung immediately following deposition, with a clearance half-time for fibers longer than 20 µm of 4.5 days. By the end of

the 5-day exposure, more than 90% of the inhaled fibers longer than 20 µm had already been cleared from the lung (as compared to the amosite exposure in which the longer fibers were not cleared).

Once deposited in the lung, the longer chrysotile fibers quickly broke apart into shorter pieces. As reported earlier (Bernstein et al., 2010), fibers were initially deposited on the bronchial and alveolar surfaces with very few fibers found in the interstitial space. Those that were observed in the interstitial space were quickly removed and by 90 days postexposure, the remaining fibers were found only in the macrophages. No inflammatory or pathological response was associated at any time point with exposure to CSP.

The fibers between 5 and 20 µm in length had a clearance half-time of 12.8 days, and those fibers < 5 µm in length had a clearance half-time of 27.8 days. These slightly longer clearance half-times for the shorter fibers are probably due to the large number of fibers being created as the longer fibers break apart. These clearance half-times are still considerably less than those found for insoluble particles (Stoeber et al., 1970; Muhle et al., 1987). These values are similar to those presented in the interim result publication based upon the data through 90 days postexposure (Bernstein et al., 2010).

These results are comparable to those reported previously for pure chrysotile exposures (Bernstein et al., 2004, 2005a,b) and are consistent with the pilot study on CSP (Bernstein et al., 2008).

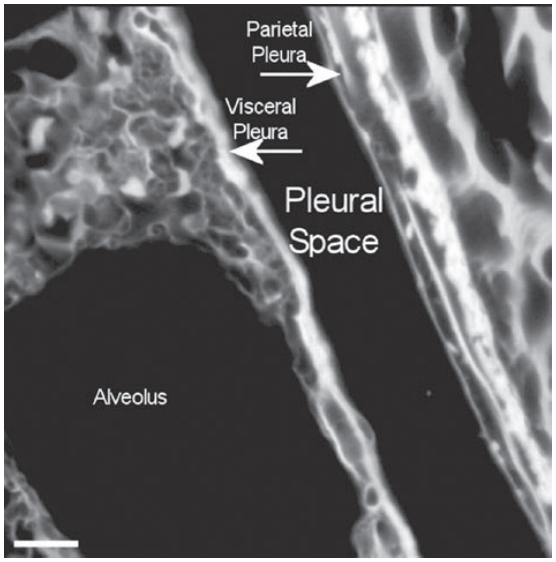



Figure 15. View of the pleural space from an animal exposed to CSP mixture at 272 days postexposure is shown in the confocal image. This confocal micrograph is very similar to that seen for the air control (Figure 14).

## Amosite asbestos

While CSP in the lung clears rapidly and produces no pathology, the amosite asbestos exposure group showed a markedly different response. Amosite has a notably different physical form than chrysotile. While chrysotile is a rolled thin sheet, amosite asbestos is a double-chain silicate formed as a solid cylinder of silica. Amosite has a very low dissolution coefficient even in an acid environment at environmental or human body temperatures. Amosite asbestos is biopersistent in both the lung and in the macrophage environments (Speil & Leineweber, 1969).

Following deposition in the lung, all fiber lengths of amosite persist with clearance half-times of > 1000 days, which is greater than the lifetime of the rat. For fiber lengths 5–20 µm and longer than 20 µm, these results are similar to the interim results presented in Bernstein et al. (2010) through 90 days postexposure. For the fibers < 5 µm in length, in the earlier publication, a clearance half-time of 90 days was estimated. However, incorporating the full set of data through 365 days, even the short fibers no longer clear after 90 days postexposure, most likely being locked up in the intense inflammatory response caused by the longer fibers.

Already by the end of the 5-day exposure, an intense inflammatory response to amosite was observed

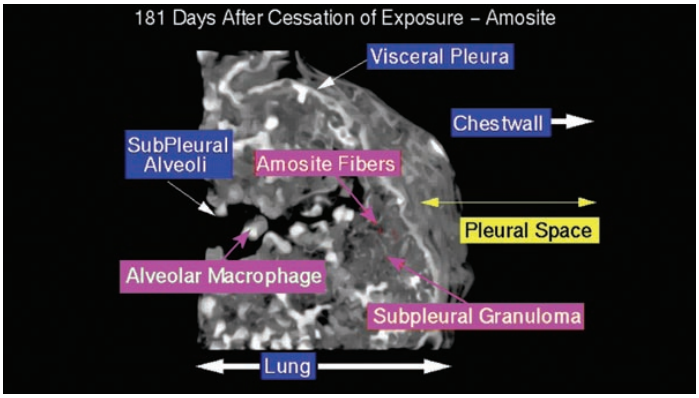



Figure 16. View of the pleural space from an animal exposed to amosite asbestos at 181 days postexposure. Amosite fibers are seen in a subpleural granuloma with numerous alveolar macrophages in the subpleural alveoli.

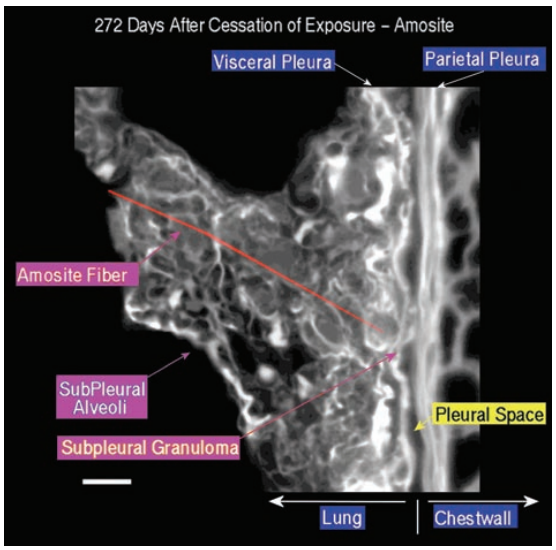



Figure 17. View of the pleural space from an animal exposed to amosite asbestos at 272 days postexposure. An amosite fiber ~ 39 µm in length is observed within a subpleural granuloma. The right edge of this long fiber pierces the subpleural capsule. The thicker bright white matrix is indicative of a fibrotic thickening of the visceral pleura.

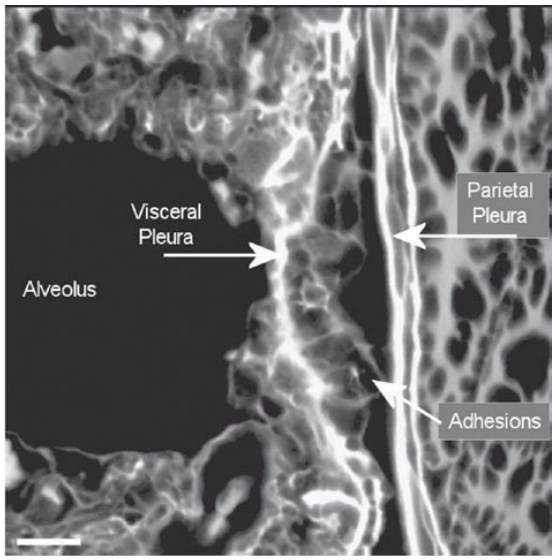



Figure 18. View of the pleural space from an animal exposed to amosite asbestos at 272 days postexposure. The subpleural alveolar septa seen in the left center of the image contains fibrotic lesions (thicker bright white matrix is indicative of enhanced collagen deposition). The parietal pleura and chest wall is shown on the right. Within the alveolus on the left, a number of subpleural macrophages can be seen.

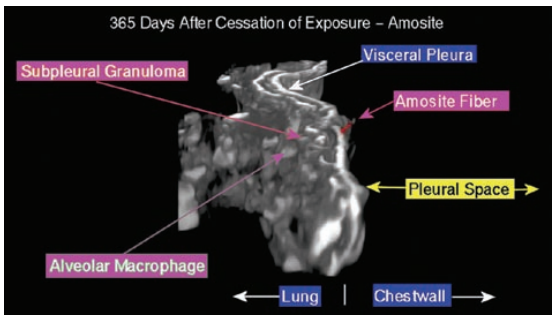



Figure 19. View of the pleural space from an animal exposed to amosite asbestos at 365 days postexposure. An amosite fiber penetrating the visceral pleural wall into the pleural space is seen. On the lung side, a well-developed subpleural granuloma is seen with alveolar macrophages on the surface.

including granuloma formation around the longer fibers, which the macrophages could not clear. By 28 days postexposure, the continued inflammation resulted in the formation of interstitial fibrosis. The response to amosite is similar to that of another amphibole asbestos, tremolite, that has been studied previously (Bernstein et al., 2005b).

Immediately after the end of exposure, amosite fibers were observed penetrating the airway wall, located completely under the airway wall, as well as within macrophages on the surface of the ciliated epithelium. In the lung parenchyma, a small number of fibers were observed partly or fully embedded into the interstitial space with fibers wholly or partly inside alveolar macrophages and touching alveoli, alveolar ducts, or respiratory bronchioles (Bernstein et al., 2010).

In contrast to the chrysotile fibers, at 90 days postexposure the amosite fibers were still observed penetrating the airway wall or located completely underneath the airway wall and on the surface of the ciliated epithelium. Even more important in terms of disease formation, substantial number of amosite fibers were found partly or fully embedded into the interstitial space with fibers observed wholly or partly inside alveolar macrophages and touching alveoli, alveolar ducts, or respiratory bronchioles (Bernstein et al., 2010).

The fibrotic response seen in this study following exposure to the amphibole amosite asbestos is similar to that reported in humans by Schneider et al. (2010). Schneider et al. (2010) reported that the fibrosis scores of the asbestosis cases correlated best with the number of uncoated commercial amphibole fibers.

## Translocation to the visceral pleura and then to the parietal pleura

### *CSP*

Systematic examination of the region of the lung immediately adjacent to the visceral pleural and the visceral pleural itself using samples taken from frozen rats demonstrates no chrysotile fibers at any time point. In addition, no inflammatory cells or increase in collagen formation are observed at any time point, that is the tissue appears normal as it does in the negative control group. The thickness of the visceral pleural wall in the CSP-exposed animals was the same as that in the air control group.

Similarly, examination of the diaphragm as a representative parietal pleural tissue shows no indication of

any inflammation at any time point in the CSP-exposed group. Unexpected interference was found in the signal from the confocal examination most likely due to protein coatings on the parietal pleural surface. As a result, the fiber examination can only be considered qualitative, not quantitative. In the CSP-exposed group, there was an indication of two possible shorter fibers present in one animal at 30 days postexposure. No other fibers were identified, and there was no cellular or inflammatory response associated with the two possible fibers.

***Amosite asbestos***

The range of pathological response observed in the amosite-exposed animals appears to mirror quite closely that which is observed in humans exposed to amphibole asbestos. Systematic examination of the region of the lung immediately adjacent to the visceral pleural and of the visceral pleural itself using samples taken in the frozen rat procedure has shown the presence of numerous amosite fibers as shown in Figures 16, 17, and 19. Figure 17 shows an amosite fiber ~ 40 μm in length in the lung directly adjacent to the visceral pleural surface with one end piercing the visceral pleural capsule. Figure 19 shows an amosite fiber penetrating the visceral wall from the lung into the pleural space. An important inflammatory response with numerous macrophages and possibly other cells present is associated with these amosite fibers.

The confocal methodology used in this study is quantitative in the assessment of excess collagen in the lung assayed by hydroxyproline (Antonini et al., 1999). The collagen is seen in the confocal micrographs as the bright white areas with the intensity of the brightness proportional to the amount of collagen. The normal lung as seen in the air controls in Figures 13 and 14 shows the normal collagen structure of the visceral and parietal walls as thin white lines on each side of the pleura. A nearly identical image seen in the CSP-exposed group is shown in Figure 15.

In the amosite-exposed group, as shown in Figure 17, both the visceral and the parietal pleural walls show a fibrotic response as indicated by the much brighter and thicker visceral and parietal surfaces on each side of the pleural space.

Adjacent to the end of the fiber (Figure 17), which is piercing the visceral pleural capsule, is a bridge to the parietal pleural surface, which is most likely an adhesion. In Figure 18, another example of adhesions between the visceral and parietal walls of the pleural cavity is seen in more detail with the folds of the adhesion appearing to extend out from the visceral surface to the parietal pleural surface.

The mean thickness of the visceral pleural wall in the amosite-exposed animals was more than twice that in the air control and the CSP. While the thickness of the air control and the CSP-exposed animals ranged up to 7 μm, that of the amosite-exposed animals ranged up to 21 μm. The greater thickness of the visceral wall in the amosite-exposed animals was associated with the presence of amosite fibers in these regions.

***Amosite diaphragm***

While the examination of the visceral pleural surface was performed in the frozen rat sections at intervals from 181 to 365 days postexposure, the examination of the parietal pleural surface (diaphragm) was performed at earlier intervals (between 0 and 90 days postexposure).

One of the most interesting results of this study is how quickly the amosite fibers reach the parietal pleural surface and initiate an inflammatory response. Within 7 days after the cessation of exposure, amosite fibers can be seen by CM on the diaphragm. By 14 days, an inflammatory response to these fibers was seen as shown in Figure 8. In addition, a fibrin matrix formed on the parietal pleural surface. Pleural injury and repair is characterized by disordered fibrin turnover, which contributes to the pathogenesis of pleural fibrosis. This is an early marker of pleural injury, and it has been proposed that disordered fibrin turnover plays a central role in the pathogenesis of pleural fibrosis. Fibrinogen is converted to fibrin forming the transitional intrapleural neomatrix when intrapleural coagulation is activated by chemical or inflammatory stimuli (Jantz & Antony 2008; Shetty et al., 2008).

The formation of fibrin has also been associated with the development of adhesions between the visceral and parietal pleural walls (Idell et al., 2001) as shown in Figure 18. Intrapleural coagulation is initiated by increased local expression of tissue factors in response to the local injury and fibrinolysis is concurrently downregulated, due to primarily increased expression of PAI-1 and downstream antiplasmins. Formation of adhesions between the visceral and parietal pleural surfaces occurs in association with intrapleural coagulation and downregulation of fibrinolysis (Shetty et al., 2008). Pleural adhesions and fusion of the visceral and parietal pleura commonly occur with the diffuse pleural thickening (Huggins & Sahn, 2004), which was observed for amosite asbestos in this study (Figure 10).

In the quantification of the number of fibers in the CM examination of the visceral pleura, the area of the visceral pleural surface examined was recorded for each animal individually and ranged from a mean per time point examined of 0.0038–0.0076 $mm^2$. The total pleural surface area of the rat has been reported as 2450 $mm^2$ (Bermudez et al., 2003), and approximately one-half of this would represent the visceral pleural surface. Based upon this and the number of fibers observed in these areas, and assuming that the distribution is uniform throughout the visceral pleura, the total fiber burden of the visceral pleura would be in the range of a few million fibers (all lengths).

## Conclusions

This study is unique in that it has examined a commercial joint compound product that historically used chrysotile and it quantified not only the pathological response and fiber distribution by compartment in the lung, but also

the translocation of fibers to and pathological response in the pleural cavity.

The translocation to and pathological response in the pleura was examined by SEM and CM using noninvasive methods. The number and size of fibers was quantified using TEM and CM. This is the first study to use such techniques to characterize fiber translocation to and the response of the pleural cavity.

Amosite fibers were found to remain partly or fully imbedded in the interstitial space through 1 year postexposure and quickly produced granulomas (0 days) and interstitial fibrosis (28 days). Amosite fibers were observed penetrating the visceral pleural wall and were found on the parietal pleural within 7 days postexposure with a concomitant inflammatory response seen by 14 days postexposure. Pleural fibrin deposition, fibrosis, and adhesions were also observed in response to the amosite and were similar to that observed in humans in response to amphibole asbestos.

No cellular or inflammatory response was observed in the lung or the pleural cavity in response to the CSP exposure.

These results provide confirmation of the important differences between CSP compound and amphibole asbestos.

## Declaration of interest

This work was supported by a grant from Georgia-Pacific, LLC.

## References

Agostoni E, Zocchi L. 1998. Mechanical coupling and liquid exchanges in the pleural space. Clin Chest Med 19:241–260.

Antonini JM, Charron TG, Roberts JR, Lai J, Blake TL, Rogers RA. 1999. Application of laser scanning confocal microscopy in the analysis of particle-induced pulmonary fibrosis. Toxicol Sci 51:126–134.

Bermudez E, Mangum JB, Moss OR, Wong BA, Everitt JI. 2003. Pleural dosimetry and pathobiological responses in rats and hamsters exposed subchronically to MMVF 10a fiberglass. Toxicol Sci 74:165–173.

Bernstein DM, Hoskins JA. 2006. The health effects of chrysotile: current perspective based upon recent data. Regul Toxicol Pharmacol 45:252–264.

Bernstein DM, Rogers R, Smith P. 2004. The biopersistence of brazilian chrysotile asbestos following inhalation. Inhal Toxicol 16:745–761.

Bernstein DM, Chevalier J, Smith P. 2005. Comparison of Calidria chrysotile asbestos to pure tremolite: final results of the inhalation biopersistence and histopathology examination following short-term exposure. Inhal Toxicol 17:427–449.

Bernstein DM, Donaldson K, Decker U, Gaering S, Kunzendorf P, Chevalier J, Holm SE. 2008. A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. Inhal Toxicol 20:1009–1028.

Bernstein D, Rogers R, Smith P. 2005. The biopersistence of Canadian chrysotile asbestos following inhalation: final results through 1 year after cessation of exposure. Inhal Toxicol 17:1–14.

Bernstein DM, Rogers RA, Sepulveda R, Donaldson K, Schuler D, Gaering S, Kunzendorf P, Chevalier J, Holm SE. 2010. The pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite asbestos following short-term inhalation exposure: interim results. Inhal Toxicol 22:937–962.

Brorby GP, Sheehan PJ, Berman DW, Greene JF, Holm SE. 2008. Re-creation of historical chrysotile-containing joint compounds. Inhal Toxicol 20:1043–1053.

Cannon WC, Blanton EF, McDonald KE. 1983. The flow-past chamber: an improved nose-only exposure system for rodents. Am Ind Hyg Assoc J 44:923–928.

European Commission. 1999. ECB/TM/26 rev.7. Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF). European Commission. Joint Research search Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau.

Fentie IH, Allen DJ, Schenck MH, Didio LJ. 1986. Comparative electron microscopic study of bovine, porcine and human parietal pericardium, as materials for cardiac valve bioprostheses. J Submicrosc Cytol 18:53–65.

Hesterberg TW, Chase G, Axten C, Miller WC, Musselman RP, Kamstrup O, Hadley J, Morscheidt C, Bernstein DM, Thevenaz P. 1998. Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. Toxicol Appl Pharmacol 151:262–275.

Hesterberg TW, Axten C, McConnell EE, Hart GA, Miiller W, Chevalier J, Everitt J, Thevenaz P, Oberdörster G. 1999. Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the syrian golden hamster. Part I. Results of a subchronic study and dose selection for a chronic study. Inhal Toxicol 11:747–784.

Hesterberg TW, Axten C, McConnell EE, Oberdörster G, Everitt J, Miiller WC, Chevalier J, Chase GR, Thevenaz P. 1997. Chronic inhalation study of fiber glass and amosite asbestos in hamsters: twelve-month preliminary results. Environ Health Perspect 105 Suppl 5:1223–1229.

Huggins JT, Sahn SA. 2004. Causes and management of pleural fibrosis. Respirology 9:441–447.

Idell S, Mazar AP, Bitterman P, Mohla S, Harabin AL. 2001. Fibrin turnover in lung inflammation and neoplasia. Am J Respir Crit Care Med 163:578–584.

Jantz MA, Antony VB. 2008. Pathophysiology of the pleura. Respiration 75:121–133.

Luoto K, Holopainen M, Kangas J, Kalliokoski P, Savolainen K. 1995. The effect of fiber length on the dissolution by macrophages of rockwool and glasswool fibers. Environ Res 70:51–61.

McConnell EE, Axten C, Hesterberg TW, Chevalier J, Miiller WC, Everitt J, Oberdörster G, Chase GR, Thevenaz P, Kotin P. 1999. Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the Syrian golden hamster. Part II. Results of chronic exposure. Inhal Toxicol 11:785–835.

Morimoto Y, Yamato H, Kido M, Tanaka I, Higashi T, Fujino A, Yokosaki Y. 1994. Effects of inhaled ceramic fibres on macrophage function of rat lungs. Occup Environ Med 51:62–67.

Muhle H, Pott F, Bellmann B, Takenaka S, Ziem U. 1987. Inhalation and injection experiments in rats to test the carcinogenicity of MMMF. Ann Occup Hyg 31:755–764.

Noppen M, De Waele M, Li R, Gucht KV, D'Haese J, Gerlo E, Vincken W. 2000. Volume and cellular content of normal pleural fluid in humans examined by pleural lavage. Am J Respir Crit Care Med 162:1023–1026.

Peng MJ, Wang NS, Vargas FS, Light RW. 1994. Subclinical surface alterations of human pleura. A scanning electron microscopic study. Chest 106:351–353.

Pundsack FL. 1955. The properties of asbestos. I. The colloidal and surface chemistry of chrysotile. J Phys Chem 59:892–895.

Rogers RA, Antonini JM, Brismar H, Lai J, Hesterberg TW, Oldmixon EH, Thevenaz P, Brain JD. 1999. In situ microscopic analysis of asbestos and synthetic vitreous fibers retained in hamster lungs following inhalation. Environ Health Perspect 107:367–375.

Schneider F, Sporn TA, Roggli VL. 2010. Asbestos fiber content of lungs with diffuse interstitial fibrosis: An analytical scanning electron microscopic analysis of 249 cases. Arch Pathol Lab Med 134:457–461.2nd edn. Hodder Education: Hachette UK Company, London.

Shetty S, John J, Idell S. 2008. In Light RW, Arnold H (eds). Pleural Fibrosis. In *Textbook of Pleural Diseases*.

Speil S, Leineweber JP. 1969. Asbestos minerals in modern technology. Environ Res 2:166–208.

Stoeber W, Flachsbart H, Hochrainer D. 1970. Der aerodynamische durchmesser von latexaggregaten und asbestfassern. Staub-Reinh Luft 30:277–285.

Wypych F, Adad LB, Mattoso N, Marangon AA, Schreiner WH. 2005. Synthesis and characterization of disordered layered silica obtained by selective leaching of octahedral sheets from chrysotile and phlogopite structures. J Colloid Interface Sci 283:107–112.

Zeidler-Erdely PC, Calhoun WJ, Ameredes BT, Clark MP, Deye GJ, Baron P, Jones W, Blake T, Castranova V. 2006. *In vitro* cytotoxicity of Manville Code 100 glass fibers: effect of fiber length on human alveolar macrophages. Part Fibre Toxicol 3:5.

Zocchi L. 2002. Physiology and pathophysiology of pleural fluid turnover. Eur Respir J 20:1545–155.

STUDY No. 9

# LUNG CANCER AND MESOTHELIOMA RISK ASSESSMENT FOR A POPULATION ENVIRONMENTALLY EXPOSED TO ASBESTOS

**STUDY No. 9**

# LUNG CANCER AND MESOTHELIOMA RISK ASSESSMENT FOR A POPULATION ENVIRONMENTALLY EXPOSED TO ASBESTOS

**International Journal of Hygiene and Environmental Health (2013)**

**Marie-Hélène Bourgault, Michelle Gagné, Mathieu Valcke**

Asbestos-related cancer risk is usually a concern restricted to occupational settings. However, recent published data on asbestos environmental concentrations in Thetford Mines, a mining city in Quebec, Canada, provided an opportunity to undertake a prospective cancer risk assessment in the general population exposed to these concentrations. Using an updated Berman and Crump dose-response model for asbestos exposure, we selected population-specific potency factors for lung cancer and mesothelioma. These factors were evaluated on the basis of population-specific cancer data attributed to the studied area's past environmental levels of asbestos. We also used more recent population-specific mortality data along with the validated potency factors to generate corresponding inhalation unit risks. These unit risks were then combined with recent environmental measurements made in the mining town to calculate estimated lifetime risk of asbestos-induced lung cancer and mesothelioma. Depending on the chosen potency factors, the lifetime mortality risks varied between 0.7 and 2.6 per 100,000 for lung cancer and between 0.7 and 2.3 per 100,000 for mesothelioma. In conclusion, the estimated lifetime cancer risk for both cancers combined is close to Health Canada's threshold for "negligible" lifetime cancer risks. However, the risks estimated are subject to several uncertainties and should be confirmed by future mortality rates attributed to present day asbestos exposure.

G Model
IJHEH-12712; No. of Pages 7

ARTICLE IN PRESS

International Journal of Hygiene and Environmental Health xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

# Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos

Marie-Hélène Bourgault[a,*], Michelle Gagné[a], Mathieu Valcke[a,b]

[a] Institut national de santé publique du Québec (INSPQ), Montréal, Québec, Canada
[b] Département de santé environnementale et de santé au travail, Université de Montréal, Montréal, Québec, Canada

ARTICLE INFO

*Article history:*
Received 11 September 2012
Received in revised form 12 July 2013
Accepted 14 July 2013

*Keywords:*
Asbestos
Risk assessment
Environmental exposure
Lung cancer
Mesothelioma
Province of Quebec

ABSTRACT

Asbestos-related cancer risk is usually a concern restricted to occupational settings. However, recent published data on asbestos environmental concentrations in Thetford Mines, a mining city in Quebec, Canada, provided an opportunity to undertake a prospective cancer risk assessment in the general population exposed to these concentrations. Using an updated Berman and Crump dose–response model for asbestos exposure, we selected population-specific potency factors for lung cancer and mesothelioma. These factors were evaluated on the basis of population-specific cancer data attributed to the studied area's past environmental levels of asbestos. We also used more recent population-specific mortality data along with the validated potency factors to generate corresponding inhalation unit risks. These unit risks were then combined with recent environmental measurements made in the mining town to calculate estimated lifetime risk of asbestos-induced lung cancer and mesothelioma. Depending on the chosen potency factors, the lifetime mortality risks varied between 0.7 and 2.6 per 100,000 for lung cancer and between 0.7 and 2.3 per 100,000 for mesothelioma. In conclusion, the estimated lifetime cancer risk for both cancers combined is close to Health Canada's threshold for "negligible" lifetime cancer risks. However, the risks estimated are subject to several uncertainties and should be confirmed by future mortality rates attributed to present day asbestos exposure.



## Introduction

Asbestos is a group of natural fibrous minerals composed of silicates that exhibit particularly interesting physicochemical properties such as flexibility and resistance to traction, heat, and chemical reactions (U.S. EPA, 1988). Because of these properties, asbestos is used commercially and incorporated into numerous products such as cement, asphalt, and brake pads (ATSDR, 2001; Lajoie et al., 2003). Asbestos fibers are divided into two large mineralogical groups: amphiboles, which include crocidolite, amosite, tremolite, actinolite and anthophyllite; and serpentines, which include only chrysotile (WHO, 2006).

In humans, inhalation is the predominant exposure route for asbestos, and the resulting adverse health effects are primarily associated with the respiratory system. These effects have been demonstrated mainly in workers and in laboratory animals (Berman and Crump, 2003; Lajoie et al., 2003; Nicholson, 1986), but they have also been reported in populations non-occupationally exposed to asbestos (Marinaccio et al., 2012; Rake et al., 2009; Vinikoor et al., 2010). Exposure to all types of asbestos fibers is associated with benign pleural diseases, asbestosis (in occupational settings only), lung, larynx and ovary cancer; and mesothelioma of the pleura and peritoneum (IARC, 2012; Roach et al., 2002).

For over 25 years, efforts have been made to provide valid and reliable information about asbestos-related lung cancer and mesothelioma risk in the general population exposed through outdoor and indoor air (Silverstein et al., 2009). A study relying mostly on indirect exposure measurements was carried out in Quebec's asbestos mining area (comprising the towns of Thetford Mines, Black Lake, and Asbestos) where the mortality by lung cancer and mesothelioma were estimated for women in the general population (Camus et al., 1998, 2002). To do so, these authors used an approach developed by Nicholson (1986) for the U.S. EPA. Briefly, Nicholson (1986) characterized the risks of these asbestos-related cancers from epidemiological studies done among workers. Then, a linear dose–response relationship was assumed for lung cancer and mesothelioma respectively, and the corresponding slopes were defined as potency factors: $K_L$ for lung cancer and $K_M$ for mesothelioma. These potency factors were estimated independently of the type of asbestos fiber and could be applied to assess risks in asbestos-exposed populations. Since no direct airborne ambient data were available for the time period of interest (1900–1975), Camus et al. (1998, 2002) estimated the

* Corresponding author at: INSPQ, 190 Boul. Crémazie Est, Montréal, Québec, Canada H2P 1E2. Tel.: +1 514 864 1600x3229; fax: +1 514 864 7646.
*E-mail address:* marie-helene.bourgault@inspq.qc.ca (M.-H. Bourgault).

1438-4639/$ – see front matter. 
http://dx.doi.org/10.1016/j.ijheh.2013.07.008

G Model
IJHEH-12712; No. of Pages 7
ARTICLE IN PRESS



past cumulative exposures based on past production volumes of asbestos in the area, aerosol dispersion modeling, recent ambient air data, visible pollution recounts, residential histories and an international exposure expert panel. Then, the predicted excess of mortality by lung cancer and mesothelioma were estimated using Nicholson's $K_L$ and $K_M$, and compared to the corresponding observed excess of mortality among women from the mining area. Their results suggested that the model overestimate the absolute risk of lung cancer (10-fold) (Camus et al., 1998) and mesothelioma (50-fold) (Camus et al., 2002).

Subsequently, Berman and Crump (2003, 2008a) updated Nicholson's model by integrating results from more recent epidemiological studies and updates of studies that were initially used by Nicholson. They also introduced an additional parameter in the exposure-risk model which accounts for the differences in background lung cancer rates between cases and controls. Moreover, their work enabled them to propose fiber-specific potency factors, i.e. for chrysotile and amphibole separately, while accounting for differences in fiber dimensions (Berman and Crump, 2003). This resulted in an improvement of Nicholson's model given the evidence suggesting differences in the magnitude of the cancer-related toxicity between chrysotile and amphibole, particularly for mesothelioma (Berman and Crump, 2008a,b).

The Canadian mining of chrysotile asbestos is concentrated in Thetford Mines and Asbestos regions, in the province of Quebec. It is assumed that populations living in these towns are exposed to asbestos fibers released in the environment from the mining site and tailings (Lajoie et al., 2003) and, in the case of Thetford Mines, from ore residues used throughout the town for landscaping purposes (Marier et al., 2007). In 2007, Bisson and Couture published data on concentrations of asbestos fibers in outdoor air in the town of Thetford Mines (Bisson and Couture, 2007), and Marier et al. (2007) published data on indoor asbestos levels in the same town.

The present study was prompted by the availability of recent asbestos exposure data in the town of Thetford Mines. The objectives of this study were to: (1) select and evaluate relevant potency factors from Berman and Crump (2008a); (2) estimate asbestos exposure for the general population of Thetford Mines; and (3) use the potency factors and Berman and Crump general dose–response model to assess the lifetime cancer risk for lung cancer and mesothelioma of the population of Thetford Mines.

## Methods

The general approach followed involved the determination of the relevant dose–response relationship. This required the selection and evaluation, for both lung cancer and mesothelioma, of potency factors that are applicable to an asbestos risk assessment in the town of Thetford Mines, as well as the determination of corresponding lifetime inhalation unit risk. We then estimated an average lifetime exposure concentration from published data on environmental exposure to asbestos. Finally, the calculated lifetime unit risk was combined with the average lifetime exposure concentration to estimate the population's mesothelioma and lung cancer risks.

### *Determination of the relevant dose–response relationship*

#### *Selection of specific potency factors*

When making population-specific asbestos risk assessments, an international expert panel recommended applying potency factor estimates specific to the target exposure setting, rather than general (chrysotile) potency factors estimated across several cohorts pooled together (Health Canada, 2008). Thus, the potency factors we selected are those specifically calculated by Berman and Crump (2008a) for the Quebec mining and milling cohort (Asbestos town and Thetford Mines).

For lung cancer, Berman and Crump (2008a) determined the $K_L$ for the Quebec cohort based on data collected in asbestos mining workers from both Asbestos and Thetford Mines. We retained the best estimate (BE) as well as the upper bound (UB) of the uncertainty interval on this $K_L$ (0.00029 and 0.0011 per unit exposure – i.e. $(\text{f/ml*year})^{-1}$). Since amphiboles have a potency to induce mesothelioma several hundred times greater than that of chrysotile (Berman and Crump, 2008a,b; Hodgson and Darnton, 2000), the potency factor for mesothelioma is very sensitive to the number of amphiboles in the total asbestos fibers counts, which is not the case for lung cancer. Since the contamination by tremolite (an amphibole) of the chrysotile ore is greater in Thetford Mines compared to Asbestos (Berman and Crump, 2008a), the BE and UB values of $K_M$ that we retained (respectively 0.021 and $0.065 \times 10^{-8}$ $(\text{f/ml*year})^{-1}$) reflect Berman and Crump (2008a)'s data for the Thetford Mines workers only.

#### *Predictive validity of the potency factors*

To evaluate the predictive validity of the selected potency factors, we followed the approach used by Camus et al. (1998, 2002) to evaluate Nicholson's $K_L$ and $K_M$. Thus, we relied on the model's predictions of the number of cancer-related deaths ($P$) presumably attributable to non-occupational asbestos exposure in the female population of Quebec's asbestos mining area (towns of Thetford Mines and Asbestos). To compute $P$, we used past cumulative exposure estimates developed by Camus et al. (1998) for best exposure estimate (25 f/ml*year), as well as subjective plausible upper range (125 f/ml*year) and lower range (5 f/ml*year) values. Indeed, Camus et al. (1998) proposed this subjective plausible range to take into account the possible errors in their estimation of past environmental and household exposure. We also used the observed ($O$) and expected ($E$) number of cancer-related deaths in that same population for the time period of interest (1970–1989) (Camus et al., 1998, 2002). In his study, Camus et al. (1998) derived the expected number of cancer-related deaths from two different indicators namely, the standardized mortality ratio (SMR) and the standardized proportionate mortality ratio (SPMR). For the purpose of the current study, the latter was selected because it generates a more conservative risk assessment.

Hence, for lung cancer, the predicted relative risk (RR) for women in the studied population was calculated as follows:

$$\text{RR} = 1 + K_L \times X \quad (1)$$

where $K_L$ is the retained potency factor for lung cancer and $X$ is the cumulative exposure estimated by Camus et al. (1998).

Similarly to Camus et al. (1998), the $K_L$ was multiplied by a factor of 4.2 to account for the difference in exposure duration between workers (40 h/week) and the general population (168 h/week). $P$ was then computed as the product of the resulting RR and $E$, i.e. 64.5 according to Camus et al. (1998). Finally, we compared the predicted excess of deaths ($P - E$) with the excess mortality observed ($O - E$) in that same population, i.e. 6.5 as reported by Camus et al. (1998).

For mesothelioma, the outcome considered in the dose–response model refers to an absolute number of deaths. Indeed, we assumed that every case of mesothelioma leads to death and that the background incidence in an unexposed population is nil. Thus, we compared $P$, herein being calculated as a predicted number of incident cases of mesothelioma ($I_M$), with $O$, where $O$ equals 10 incidental cases (Camus et al., 2002). All things being equal, we computed $I_M$ proportionally as a function of the $I_M$

Please cite this article in press as: Bourgault, M.-H., et al., Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos. Int. J. Hyg. Environ. Health (2013), http://dx.doi.org/10.1016/j.ijheh.2013.07.008

G Model
IJHEH-12712; No. of Pages 7
ARTICLE IN PRESS
M.-H. Bourgault et al. / International Journal of Hygiene and Environmental Health xxx (2013) xxx–xxx 3

**Table 1**
Asbestos fiber concentrations (f/ml) measured in outdoor and indoor air in the city of Thetford Mines.

| | Outdoor air | Indoor air |
|---|---|---|
| Reference study | Bisson and Couture (2007) | Marier et al. (2007) |
| Year of sampling | 2004 | 2003–2004 |
| Method of analysis | PCM | TEM |
| Protocol | IRSST-243-1[a] | Modified NIOSH 7402[b] |
| Types of fibers | Total | Asbestos |
| Number of sample | 125 | 26 |
| Range of concentrations (f/ml) | 0.0015–0.056 | 0.000553–0.01 |
| Limit of detection (LOD) (f/ml) | 0.0015 | 0.000553 |
| Proportion of samples below LOD | 8.1% | 35% |
| Arithmetic mean[c] (f/ml) | 0.0059 | 0.002 |

[a] $L > 5$ μm, $D \geq 0.25$ and <3 μm, ratio $L/D > 3{:}1$ where $L$ is the length and $D$ the diameter of the fiber. This is modified from the protocol NIOSH 7400 which included the fibers with diameter ≥3 μm, (NIOSH, 1994a).
[b] $L > 5$ μm, $D \geq 0.25$ μm and <3 μm, ratio $L/D > 3{:}1$. This is modified from the protocol NIOSH 7402 which included the fibers with diameter ≥3 μm (NIOSH, 1994b).
[c] The arithmetic means were calculated and used for the purpose of our study (see text). A value equal to the limit of detection was attributed to the undetected samples.

obtained by Camus et al. (2002) for Nicholson's $K_m$ ($I_{M\_Ca}$), and the ratio of our $K_M$ ($K_{M\_sel}$) over Nicholson's $K_M$ ($K_{M\_Nic}$), such as:

$$I_M = \frac{I_{M\_Ca} \times K_{M\_sel}}{K_{M\_Nic}} \quad (2)$$

*Risk characterization*

We assessed the excess risk of lung cancer and mesothelioma of the pleura and peritoneum due to continuous lifetime exposure to asbestos (80 years). Thus, for each type of cancer, we multiplied the average lifetime exposure concentration of asbestos ($C_{avg}$; f/ml) by the relevant lifetime unit risk (UR; $(f/ml)^{-1}$), to obtain the cumulative lifetime risk:

$$R = C_{avg} \times UR \quad (3)$$

*Determination of a lifetime unit risk*

For a specific population, the lifetime inhalation unit risk (UR) represents the lifetime mortality risk for lung cancer or for mesothelioma attributable to a continuous lifetime exposure to 1 asbestos fiber/ml.

Using the selected $K_M$ and $K_L$ values described above, we calculated URs for each cancer site using the approach proposed by Berman and Crump (2003). Briefly, age-specific cancer risk projections were obtained using the $K_L$ and $K_M$ values that were integrated over an individual's lifetime. This was done by weighting the aforementioned projections on the basis of surviving probabilities inferred from lifetable data and accounting for all-causes and lung cancer mortality rates. The detailed equations used are those provided in Appendix E of Berman and Crump (2003) to which we made a correction. Specifically, in equation E-9 of that appendix, we removed the constant exposure concentration ($f$) since it is already taken into account in the Qi term (cumulative exposure estimate) of that same equation. For the purpose of our study, we calculated URs by using the lifetable data for the Chaudière-Appalaches administrative area (district), where the town of Thetford Mines is located.

Given that our assessment focused on the future cancer risk resulting from nowadays exposure, we used more recent mortality data from the Ministry of Health and Social Services of the Province of Quebec, covering the 2000–2003 time period. Although URs should be computed separately for male and female populations as well as for smokers and non-smokers (Berman and Crump, 2003; Berman, 2011), data on smoking habits were not available for the investigated population. Therefore, URs were only computed for Thetford Mines men and women separately, which would however not change significantly the URs computed. Indeed, the lifetables we used to determine URs, which regrouped smokers and non-smokers together, somehow implicitly considers the impact of smoking. Indeed, mortality data on women include mortality due to smoking, as much as data on men include deaths due to tobacco use.

*Exposure assessment*

Environmental fibers counts are typically made by transmission electron microscopy (TEM). TEM allows the specific identification of asbestos from other types of fibers (e.g. cellulose) and identifies the different types of asbestos fibers (amphiboles and serpentines). In comparison, phase contrast microscopy (PCM), generally used in occupational settings, does not allow these differentiations. Moreover, the counts made by PCM are limited to fibers thicker than approximately 0.25 μm (NIOSH, 1994a), while fibers finer than this could be detectable by TEM. Thus, the risk could be estimated by TEM if asbestos counts are restricted to fibers thicker than 0.25 μm (Lorber et al., 2007; Nolan et al., 2005). This exposure metric is called PCM-equivalent or PCME (NIOSH, 1994b). Because the potency factors are derived from occupational exposure data based on fibers counts made by PCM, PCM or PCME exposure metrics are generally considered to be more accurate in reflecting the risk than other exposure metrics (U.S. EPA, 1988; Berman, 2010)

To assess indoor inhalation exposure to asbestos fibers, we used the Thetford Mines' data of Marier et al. (2007). For the outdoor air exposure in the same city, we used the Bisson and Couture (2007) data. The data used for exposure assessment are summarized in Table 1, along with the protocols and analytical methods.

Indoor and outdoor asbestos measurements and analysis have been previously described (Marier et al., 2007; Bisson and Couture, 2007). Briefly, Marier et al. (2007) evaluated the indoor asbestos air concentrations (by PCME) in 26 residences of Thetford Mines. Most of the sampled residences were situated downwind from prevailing winds. Twenty-four residences (92%) were located within a distance of 2 km or less of the mine tailings sites; 15 (58%) were within 1 km. According to the answers provided by residents to a questionnaire, asbestos containing materials were absent of most of the residences sampled (23 out of 26). The samplers were placed in the middle of the most often used room in the house, at approximately 1 m from the floor. The sampling lasted for 80 min using the "modified aggressive method". This consists into placing a fan on the floor in the middle of the room in order to simulate air movement generated by normal activity. Bisson and Couture (2007) analyzed outdoor air samples by PCM for total fibers ($n = 125$). Two sampling station were placed on building rooftops (about 9 m above the ground). They were both influenced by the prevailing winds: one was located near the mine and mill and the other was located further downwind. Comparable numbers of air samples were collected at each site (62 and 63, respectively).

Please cite this article in press as: Bourgault, M.-H., et al., Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos. Int. J. Hyg. Environ. Health (2013), http://dx.doi.org/10.1016/j.ijheh.2013.07.008

G Model
IJHEH-12712; No. of Pages 7
ARTICLE IN PRESS
4 M.-H. Bourgault et al. / International Journal of Hygiene and Environmental Health xxx (2013) xxx–xxx

For both indoor and outdoor exposure estimates, we calculated arithmetic mean values, as they better reflect the risk resulting from exposure to environmental contaminants than geometric means or medians. This is particularly true in the low exposure area of the dose–response relationship. Also, arithmetic means are considered as more conservative exposure estimates (Crump, 1998; Seixas et al., 1988).

Thus, the average lifetime exposure concentration ($C_{avg}$) was calculated as the sum of the average indoor ($C_{ind}$) and outdoor ($C_{out}$) exposures concentrations, weighted by the respective proportions of the time spent indoors ($P_{ind}$) and outdoors ($P_{out}$):

$$C_{avg} = (C_{ind} \times P_{ind}) + (C_{out} \times P_{out}) \quad (4)$$

Average annual values for $P_{ind}$ and $P_{out}$ in the Province of Quebec are 91.3% and 8.7%, respectively (MSSS, 2002).

## Results

### *Predictive validity of the selected potency factors*

Table 2 presents the predictive validity of the selected potency factors for lung cancer risk in the female population of Thetford Mines over the uncertainty range of the cumulative exposure estimate for this population. Specifically, the ratio $(P-E)/(O-E)$ quantifies the over (if > 1) or under-estimation (if < 1) of the predicted excess mortality over the observed excess mortality. For comparative purposes, the results obtained by Camus et al. (1998) with Nicholson's $K_L$ are also indicated. It can be seen that the $(P-E)/(O-E)$ ratios obtained over the uncertainty range of Camus et al. (1998) cumulative exposure estimate include 1 when the $K_L$ values we've selected are considered. This is true regardless of whether the BE (ratios of 0.1–1.5) or UB (0.2–5.7) values are considered for the $K_L$. By contrast, applying Nicholson's potency factor in the same dose–response model overestimates lung cancer risk by 2- to 52-fold across the whole uncertainty range of the cumulative exposure estimate (Camus et al., 1998). Overall, these results confirm the adequacy of both the UB and BE of our selected potency factor for estimating the risk of lung cancer caused by asbestos in the city of Thetford Mines.

Data in Table 3 give an estimation of the accuracy of the $K_M$ values selected with regards to their predictive capacity for mesothelioma risk in the studied population. Indeed, the $I_M$ values reported can be directly compared to the 10 observed incident cases of mesothelioma reported by Camus et al. (2002). It can be seen that the $I_M$s obtained over the uncertainty range of the cumulative exposure estimate include 10 incident cases when both the BE (2.1–52.5) and UB (6.5–162.5) values are considered for the $K_M$. In comparison, between 100 and 2500 cases are predicted using Nicholson's $K_M$ across the uncertainty range of the average cumulative exposure estimate. Again, these results point toward the adequacy of our selected potency factor for estimating the risk of mesothelioma caused by asbestos in the city of Thetford Mines.

### *Exposure assessment and risk characterization*

We calculated an average lifetime exposure concentration ($C_{avg}$) of 0.0023 f/ml from measurements of asbestos fibers in indoor and outdoor air. Also, as shown in Table 4, we calculated a lifetime unit risk for lung cancer of 0.0030 and 0.011 $(f/ml)^{-1}$ with the BE and UB of the relevant potency factor respectively. For mesothelioma, the corresponding numbers were 0.0032 and 0.0099 $(f/ml)^{-1}$. The computed unit risks for both cancers combined were 0.0061 and 0.021 $(f/ml)^{-1}$ (Table 4).

**Table 2**
Evaluation of the selected $K_L$ from Berman and Crump (2008a; B&C) and Nicholson (1986) with regards to their accuracy to predict the observed excess deaths from lung cancer, using the asbestos exposure assessment made by Camus et al. (1998).

| Camus et al. (1998) Cumulative exposure estimates $(X)_L$ | | $K_L \times 100$, from various sources $(f/ml*year)^{-1}$ | | |
|---|---|---|---|---|
| | | B&C (BE)[a] | B&C (UB)[a] | Nicholson (1986)[b] |
| | | 0.029 | 0.11 | 1 |
| $X$ = 5 f/ml*year | RR | 1.01 | 1.02 | 1.21 |
| | $P$ | 64.9 | 66 | 78 |
| | $P-E$ | 0.4 | 1.5 | 13.5 |
| | $(P-E)/(O-E)$ | 0.1 | 0.2 | 2.1 |
| $X$ = 25 f/ml*year | RR | 1.03 | 1.12 | 2.05 |
| | $P$ | 66.5 | 71.9 | 132.2 |
| | $P-E$ | 2 | 7.4 | 67.7 |
| | $(P-E)/(O-E)$ | 0.3 | 1.1 | 10.4 |
| $X$ = 125 f/ml*year | RR | 1.15 | 1.58 | 6.25 |
| | $P$ | 74.3 | 101.7 | 403.1 |
| | $P-E$ | 9.8 | 37.2 | 338.6 |
| | $(P-E)/(O-E)$ | 1.5 | 5.7 | 52.1 |

*Note*: Expected deaths ($E$ = 64.5 from Camus et al., 1998); $O-E$, Observed excess deaths (= 6.5 from Camus et al., 1998); $P-E$, Predicted excess deaths.
[a] $K_L$ estimated from Quebec chrysotile mines and mills only (Asbestos and Thetford Mines; Berman and Crump, 2008a).
[b] $K_L$ estimated from a series of $K_L$ of 14 different occupational environments (Nicholson, 1986).

**Table 3**
Incident cases of mesothelioma ($I_M$) predicted using the selected $K_M$ from Berman and Crump (2008a; B&C) and Nicholson (1986), using the asbestos exposure assessment made by Camus et al. (2002).

| Source of $K_M$ | $K_M \times 10^{-8}$ $(f/ml*year)^{-1}$ | $I_M$ for various cumulative exposure estimates ($X$) from Camus et al. (2002) | | |
|---|---|---|---|---|
| | | $X$ = 5 f/ml*year | $X$ = 25 f/ml*year | $X$ = 125 f/ml*year |
| B&C (BE)[a] | 0.021 | 2.1 | 10.5 | 52.5 |
| B&C (UB)[a] | 0.065 | 6.5 | 32.5 | 162.5 |
| Nicholson (1986) [b] | 1 | 100 | 500 | 2500 |

[a] $K_M$ estimated from Thetford Mines chrysotile mine (Berman and Crump, 2008a).
[b] Estimated from a series of $K_M$ of 4 different occupational environments (Nicholson, 1986).

Please cite this article in press as: Bourgault, M.-H., et al., Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos. Int. J. Hyg. Environ. Health (2013), http://dx.doi.org/10.1016/j.ijheh.2013.07.008

G Model
IJHEH-12712; No. of Pages 7
ARTICLE IN PRESS
M.-H. Bourgault et al. / International Journal of Hygiene and Environmental Health xxx (2013) xxx–xxx 

**Table 4**
Unit risks and corresponding lifetime mortality risk for lung cancer and mesothelioma resulting from environmental exposure to asbestos fibers in Thetford Mines (Quebec) on the basis of best estimate (BE) and upper bound (UB) values of the retained potency factors.

| Cancer type | Unit risk[a] $(f/ml)^{-1}$ | | Lifetime mortality risk[b] (per 100,000 persons) | |
|---|---|---|---|---|
| | BE | UB | BE | UB |
| Lung cancer | 0.0030 | 0.011 | 0.7 | 2.6 |
| Mesothelioma | 0.0032 | 0.0099 | 0.7 | 2.3 |
| Both cancers | 0.0061 | 0.021 | 1.4 | 4.9 |

[a] Men and women combined.
[b] Rounded values; for an average lifetime (80 years) exposure concentration ($C_{avg}$) of 0.0023 f/ml, computed as described in Methods.

The lifetime mortality risks for lung cancer, considering respectively the $K_L$'s BE and UB values, are 0.7 and 2.6 per 100,000 persons continuously exposed during their lifetime to asbestos concentrations described above. For mesothelioma, the corresponding numbers are 0.7 and 2.3 per 100,000 persons on the basis of the relevant $K_M$. The lifetime mortality risk for both cancer combined is 1.4 per 100,000 persons based on the BE value of the potency factors; and of 4.9 per 100,000 persons based on the UB value (Table 4).

## Discussion

The main objectives of this study was to assess the cancer risk for a general population environmentally exposed to asbestos, using potency factors taken from previously published studies on dose–response models.

The results of the present study showed that the lifetime mortality risk for lung cancer and mesothelioma combined varied between 1.4 and 4.9 per 100,000 persons continuously exposed to asbestos for 80 years, depending on the statistical descriptors considered (BE or UB) for the population-specific potency factors. These numbers slightly exceed Health's Canada threshold for considering a lifetime cancer risk as negligible (i.e. 1 per 100,000) (Health Canada, 2010). They do however result from an environmental exposure that is rather high as compared to levels measured in a limited number of other indoor and outdoor settings in Canada and the United States, a point that could be important for risk management and decision-making.

First, data published by Bisson and Couture (2007) showed that outdoor total fibers mean concentrations in Thetford Mines (0.0059 f/ml) are 3 times greater than the total fibers mean concentrations measured in the urban areas of Montreal and Quebec City (Canada) (0.0019 f/ml), and the difference is statistically significant ($p < 0.01$) according to both a Kolmogorov–Smirnov and a Mann–Whitney test that we performed on raw data (data not shown).

Regarding indoor air, the average concentration of asbestos fibers in the 26 Thetford Mines residences (0.0019 f/ml) (Marier et al., 2007) is 28 times greater than the average concentration found in 5 residences across the United States (0.00005 f/ml) (Lee and Van Orden, 2008). It was, however, slightly lower than the average concentration measured during a small-scale monitoring survey of two lower Manhattan residential buildings, one week after the World Trade Center terrorist attacks (0.0029 f/ml) (Chatfield and Kominsky, 2001). However, while Chatfield and Kominsky (2001) used the standard PCME counting criteria of inclusion to assess the relevant fiber exposure metric, Marier et al. (2007) excluded fibers $\geq 3$ μm in diameter (see Table 1); thus, if the same counting criteria were used, Marier et al. (2007) data may have been slightly higher, resulting in a smaller difference with Chatfield and Kominsky's values.

To date, the model developed by Nicholson (1986) has been the most often used for asbestos cancer risk assessment (Azuma et al., 2009; Camus et al., 1998, 2002; Lorber et al., 2007). However, an increasing number of studies are now using Berman and Crump potency factors or developing ones based on meta-analytic techniques (Hodgson and Darnton, 2010; Lenters et al., 2011; Loomis et al., 2009). In our evaluation exercise (see Methods), the population-specific potency factors taken from Berman and Crump (2008a) better reflected the past cancer risk in the mining area described by Camus et al. (1998, 2002), and more so for mesothelioma. Thus, these potency factors appeared more adequate for the purpose of our prospective cancer risk assessment as well.

In addition, the population-specific $K_M$ and $K_L$ factors account for the proportion of asbestos fibers types in the investigated environment. This was a significant advantage for us, as environmental data from both Bisson and Couture (2007) and Marier et al. (2007) suggest that amphibole fibers are also present in the air of Thetford Mines. Although their precise concentrations are not available, a proportion of 1% of total asbestos fibers was suggested by Berman and Crump (2003, 2008b) for asbestos dust from Quebec mines and mills. Along with updated epidemiological data on both exposure estimates and relevant health outcomes in the investigated cohorts, the Berman and Crump model also integrates the use of control populations that are more relevant to the exposed population of interest with regards to potential confounders such as age and smoking habits, as compared to the default control U.S. population used in Nicholson's model.

The available literature suggests a wide range of possible potency factors depending of the cohort considered. Thus, using a "composite" general potency factor may introduce further uncertainties to an assessment targeting a specific population (Lenters et al., 2011; Berman and Crump, 2008b). Therefore, applying potency factor as specific as possible to the target exposure setting is indicated in order to reduce the uncertainty on the resulting risk estimates (Health Canada, 2008). However, in the present case, this would not have changed the results significantly. In fact, should specific potency factors not have been available, a reasonable alternative for our assessment would have been using potency factors estimated from cohorts exposed mostly to chrysotile fibers (with values of respectively 0.0004 $(f/ml*year)^{-1}$ (Lenters et al., 2011) and $0.025 \times 10^{-8}$ $(f/ml*year)^{-1}$ (Berman and Crump, 2003), for lung cancer and mesothelioma). This would have resulted in a lifetime mortality risk almost identical to those obtained in Table 4. We may thus conclude that for a given statistical descriptor of $K_M$ or $K_L$, using population-specific potency factors, rather than general ones, did not reduce significantly the uncertainty in our assessment, despite their accurate predictive value (see Tables 2 and 3). This statement may not hold for other assessments, in particular in settings exhibiting higher amphiboles content than in ours.

It is worth mentioning that another model for asbestos cancer risk assessment has been published by Hodgson and Darnton (2000), but since it requires an adaptation to consider lifelong exposure, it was not retained for the purpose of the present assessment.

Our study contains several uncertainties, the first of which relates to the determination of the lifetime unit risks. Indeed, the data obtained among workers are limited notably by the following factors: the difficulty of characterizing past exposures, the variations in the sampling and analytical methods (e.g. fiber counting

Please cite this article in press as: Bourgault, M.-H., et al., Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos. Int. J. Hyg. Environ. Health (2013), http://dx.doi.org/10.1016/j.ijheh.2013.07.008

G Model
IJHEH-12712; No. of Pages 7
ARTICLE IN PRESS



criteria), the mismatch between the subjects in the cohort and in the selected control population and the inadequate description of confounding factors such as smoking habits. For instance, for our analysis to hold, it must be assumed that the control population retained in the determination of the lifetime unit risks has the same smoking habits as the exposed population (Camus et al., 1998).

Second, the predictive capacity strongly relies on the adequacy of the selected potency factors. Those were evaluated on the basis of cancer mortality data reflecting past exposure levels that were in fact much higher than the ones used in the present study. Thus, our risk estimates would be overestimated should (i) a threshold-like dose–response mechanism be involved in the asbestos-induced carcinogenesis and (ii) the present environmental concentrations we used corresponded to an exposure dose below this threshold. Conversely, the risk would be underestimated if the dose–response relationship was supralinear, as suggested for peritoneal mesothelioma and lung cancer for exposure to amphiboles (Hodgson and Darnton, 2000).

Other uncertainties relate to our exposure assessment. Indeed, its representativeness is hampered by the relatively limited number of available data on environmental asbestos exposure (outdoor and indoor). For example, the majority of air samples were taken under the prevailing winds; the houses where the indoor air sampling was done were mostly located within 2 km of the mine tailings (Marier et al., 2007); and the ambient air concentrations measured in Bisson and Couture (2007) came from samplers placed on the roofs of public buildings. These characteristics may not apply to the day-to-day exposure conditions of the general population in Thetford Mines. Moreover, our exposures estimates might be overestimated since they rely on total fiber counts in the general environment of Thetford Mines rather than asbestos fibers counts.

Finally, our exposure assessment presumes no change in asbestos exposure conditions over time. For example, our risk estimate does not take into consideration the possibility of an acute exposure caused by accessing the mine tailings area.

## Conclusion

The originality of this study resides in the fact that it consists of a "population-specific" asbestos risk assessment in which the predictive value of the risk estimates used was assessed rather satisfactorily. This allowed the determination of lung cancer and mesothelioma risk that is close to 1 per 100,000. Such population-specific analysis could facilitate risk management decisions by the relevant authorities in view of other risk management considerations that could be accounted for. Nevertheless, the validity of the potency factors retained, and thus the estimated risks that we have obtained, could warrant further studies. For example, the cancer mortality rates for the coming decades in Thetford Mines, which would reflect the true risk resulting from the actual environmental exposure to asbestos, could be compared with the predictions we made herein, as long as this exposure remains comparable through time.

## Conflict of interest

The views and opinions expressed in this article are those of the sole authors and do not necessarily reflect the position of the Institut national de santé publique du Québec. The authors declare they have no financial competing interests.

## Acknowledgments

The authors express their gratitude to Dr France Labrèche and Dr Michel Camus for proofreading the manuscript, as well as to Dr Denis Belleville, Dr Louise De Guire, Dr Gaétan Carrier, and M. George Adib, for their valuable and constructive comments provided during the course of this work. This work was funded as part of the general scientific activities carried out according to the mandates of the Institut national de santé publique du Québec, a governmental institution of the Province of Quebec, Canada.

## References

ATSDR, 2001. Toxicological profile for asbestos. Agency for toxic substances and disease registry. U.S. Department of Health and Human Services, Atlanta.
Azuma, K., Uchiyama, I., Chiba, Y., Okumura, J., 2009. Mesothelioma risk and environmental exposure to asbestos: past and future trends in Japan. Int. J. Occup. Environ. Health 15, 166–172.
Berman, D.W., 2010. Comparing milled fiber, Quebec ore, and textile factory dust: has another piece of the asbestos puzzle fallen into place? Crit. Rev. Toxicol. 40 (2), 151–188.
Berman, D.W., 2011. Apples to apples: the origin and magnitude of differences in asbestos cancer risk estimates derived using varying protocols. Risk Anal. 31, 1308–1326.
Berman, D.W., Crump, K.S., 2003. Technical support document for a protocol to assess asbestos-related risk. Final draft (EPA 9345. 4-06). U.S. Environmental Protection Agency, Washington, DC.
Berman, D.W., Crump, K.S., 2008a. Update of potency factors for asbestos-related lung cancer and mesothelioma. Crit. Rev. Toxicol. 38 (S1), 1–47.
Berman, D.W., Crump, K.S., 2008b. A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. Crit. Rev. Toxicol. 38 (S1), 49–73.
Bisson, M., Couture, Y., 2007. Les fibres d'amiante dans l'air ambiant au Québec: analyse des données disponibles. Ministère du Développement durable, de l'Environnement et des Parcs, Montréal, Canada [in French].
Camus, M., Siemiatycki, j., Case, B.W., Désy, M., Richardson, L., Campbell, S., 2002. Risk of mesothelioma among women living near chrysotile mines versus US EPA asbestos risk model: preliminary findings. Ann. Occup. Hyg. 46 (S1), 95–98.
Camus, M., Siemiatycki, J., Meek, B., 1998. Nonoccupational exposure to chrysotile asbestos and the risk of lung cancer. N. Engl. J. Med. 338, 1565–1571.
Chatfield, E.J., Kominsky, J.R., 2001. Summary report: characterization of particulate found in apartments after destruction of the World Trade Center. Requested by: Ground Zero Elected Officials Task Force.
Crump, K.S., 1998. On summarizing group exposures in risk assessment: is an arithmetic mean or a geometric mean more appropriate? Risk Anal. 18, 293–297.
Health Canada, 2008. Chrysotile Asbestos Consensus Statement and Summary – Chrysotile Asbestos Expert Panel. Montreal, Quebec. November 13–14, 2007. Ottawa, Canada.
Health Canada, 2010. Federal Contaminated Site Risk Assessment in Canada, Part V: Guidance on Human Health Detailed Quantitative Risk Assessment for Chemicals (DQRAchem). Ottawa, Canada.
Hodgson, J.T., Darnton, A., 2000. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. Ann. Occup. Hyg. 44, 565–601.
Hodgson, J.T., Darnton, A., 2010. Mesothelioma risk from chrysotile [Letter]. Occup. Env. Med. 67, 432.
IARC, 2012. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol, 100C. A Review of Human Carcinogens: Arsenic, Metals, Fibres, and Dusts, International Agency for Research on Cancer. World Health Organisation, Geneva, Switzerland.
Lajoie, P., Dion, C., Drouin, L., Dufresne, A., Lévesque, B., Perrault, G., et al., 2003. Asbestos Fibres in Indoor and Outdoor Air – the situation in Québec. Institut national de santé publique du Québec, Montréal, Canada.
Lee, R.J., Van Orden, D.R., 2008. Airborne asbestos in buildings. Regul. Toxicol. Pharmacol. 50, 218–225.
Lenters, V., Vermeulen, R., Dogger, S., Stayner, L., Portengen, L., Burdorf, A., Heederik, D., 2011. A meta-analysis of asbestos and lung cancer: is better quality exposure assessment associated with steeper slopes of the exposure-response relationships? Environ. Health Perspect. 119, 1547–1555.
Loomis, D., Dement, J.M., Wolf, S.H., Richardson, D.B., 2009. Lung cancer mortality and fibre exposures among North Carolina asbestos textile workers. Occup. Environ. Med. 66, 535–542.
Lorber, M., Gibb, H., Grant, L., Pinto, J., Pleil, J., Cleverly, D., 2007. Assessment of inhalation exposures and potential health risks to the general population that resulted from the collapse of the World Trade Center towers. Risk Anal. 27, 1203–1221.
Marinaccio, A., Binazzi, A., Marzio, D.D., Scarselli, A., Verardo, M., Mirabelli, D., et al., 2012. Pleural malignant mesothelioma epidemic: incidence, modalities of asbestos exposure and occupations involved from the Italian National Register. Int. J. Cancer 130, 2146–2154.
Marier, M., Charney, W., Rousseau, R., Lanthier, R., Van Raalte, J., 2007. Exploratory sampling of asbestos in residences near Thetford Mines: the public health threat in Quebec. Int. J. Occup. Environ. Health 13, 386–397.
MSSS, 2002. Lignes directrices pour la réalisation des évaluations du risque toxicologique pour la santé humaine dans le cadre de la procédure d'évaluation et d'examen des impacts sur l'environnement et de l'examen des projets de réhabilitation de terrains contaminés, Québec. Ministère de la Santé et des Services sociaux du Québec, Québec, Canada [in French].

Please cite this article in press as: Bourgault, M.-H., et al., Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos. Int. J. Hyg. Environ. Health (2013), http://dx.doi.org/10.1016/j.ijheh.2013.07.008

G Model
IJHEH-12712; No. of Pages 7
ARTICLE IN PRESS
M.-H. Bourgault et al. / International Journal of Hygiene and Environmental Health xxx (2013) xxx–xxx 7

NIOSH, 1994a. Asbestos and Other Fibers by PCM, Method 7400. In: NIOSH Manual of Analytical Methods, fourth ed. National Institute for Occupational Health and Safety, U.S. Department of Health and Human Services, Atlanta.

NIOSH, 1994b. Asbestos by TEM, Method 7402. In: NIOSH Manual of Analytical Methods, fourth ed. National Institute for Occupational Health and Safety, U.S. Department of Health and Human Services, Atlanta.

Nicholson, W.J., 1986. Airborne asbestos health assessment update. EPA/600/8-84/003F. U.S. Environmental Protection Agency, Washington, DC.

Nolan, R.P., Ross, M., Nord, G.L., Axten, C.W., Osleeb, J.P., Domnin, S.G., et al., 2005. Risk assessment for asbestos-related cancer from the 9/11 attack on the World Trade Center. J. Occup. Environ. Med. 47, 817–825.

Rake, C., Gilham, C., Hatch, J., Darnton, A., Hodgson, J., Peto, J., 2009. Occupational, domestic and environmental mesothelioma risks in the British population: a case-control study. Br. J. Cancer 100, 1175–1183.

Roach, H.D., Davies, G.J., Attanoos, R., Crane, M., Adams, H., Phillips, S., 2002. Asbestos: when the dust settles an imaging review of asbestos-related disease. Radiographics 22, S167–S184.

Seixas, N.S., Robins, T.G., Moulton, L.H., 1988. The use of geometric and arithmetic mean exposures in occupational epidemiology. Am. J. Ind. Med. 14, 465–477.

Silverstein, M.A., Welch, L.S., Lemen, R., 2009. Developments in asbestos cancer risk assessment. Am. J. Ind. Med. 52, 850–858.

U.S. EPA, 1988. Asbestos (CASRN 1332-21-4). Web page taken from the Integrated Risk Information System (IRIS). U.S. Environmental Protection Agency, Washington DC. Available at: http://www.epa.gov/iris/subst/0371.htm (last accessed on July 2012).

Vinikoor, L.C., Larson, T.C., Bateson, T.F., Birnbaum, L., 2010. Exposure to asbestos-containing vermiculite ore and respiratory symptoms among individuals who were children while the mine was active in Libby, Montana. Environ. Health Perspect. 118, 1033-1028.

WHO, 2006. Elimination of Asbestos-Related Diseases. WHO/SDE/OEH/06.03. World Health Organization, Geneva, Switzerland.

Please cite this article in press as: Bourgault, M.-H., et al., Lung cancer and mesothelioma risk assessment for a population environmentally exposed to asbestos. Int. J. Hyg. Environ. Health (2013), http://dx.doi.org/10.1016/j.ijheh.2013.07.008

STUDY No. 10

# ANALYSIS OF WORKPLACE COMPLIANCE MEASUREMENTS OF ASBESTOS BY THE U.S. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (1984 – 2011)

**STUDY No. 10**

# ANALYSIS OF WORKPLACE COMPLIANCE MEASUREMENTS OF ASBESTOS BY THE U.S. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (1984 – 2011)

**Regulatory Toxicology and Pharmacology 72 (2015) 615-629**

**Dallas M. Cowan, Thales J. Cheng, Matthew Ground, Jennifer Sahmel, Allysha Varughese, Amy K. Madl**

The United States Occupational Safety and Health Adminstration (OSHA) maintains the Chemical Exposure Health Data (CEHD) and the Integrated Management Information System (IMIS) databases, which contain quantitative and qualitative data resulting from compliance inspections conducted from 1984 to 2011. This analysis aimed to evaluate trends in workplace asbestos concentrations over time and across industries by combining the samples from these two databases. From 1984 to 2011, personal air samples ranged from 0.001 to 175 f/cc. Asbestos compliance sampling data associated with the construction, automotive repair, manufacturing, and chemical/petroleum/rubber industries included measurements in excess of 10 f/cc, and were above the permissible exposure limit from 2001 to 2011. The utility of combining the databases was limited by the completeness and accuracy of the data recorded. In this analysis, 40% of the data overlapped between the two databases. Other limitations included sampling bias associated with compliance sampling and errors occurring from user-entered data. A clear decreasing trend in both airborne fiber concentrations and the numbers of asbestos samples collected parallels historically decreasing trends in the consumption of asbestos, and declining mesothelioma incidence rates. Although air samping data indicated that airborne fiber exposure potential was high (>10 f/cc for short and long-term samples) in some industries (e.g., construction, manufacturing), airborne concentrations have significantly declined over the past 30 years. Recommendations for improving the existing exposure OSHA databases are provided.

Regulatory Toxicology and Pharmacology 72 (2015) 615–629

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

# Analysis of workplace compliance measurements of asbestos by the U.S. Occupational Safety and Health Administration (1984–2011)

Dallas M. Cowan [a,*], Thales J. Cheng [a], Matthew Ground [b], Jennifer Sahmel [b], Allysha Varughese [a], Amy K. Madl [a]

[a] Cardno ChemRisk, LLC, Aliso Viejo, CA, United States
[b] Cardno ChemRisk, LLC, Boulder, CO, United States

ARTICLE INFO

*Article history:*
Received 13 December 2014
Available online 16 May 2015

*Keywords:*
OSHA
Asbestos
Compliance sampling
Industry
IMIS
CEHD

ABSTRACT

The United States Occupational Safety and Health Administration (OSHA) maintains the Chemical Exposure Health Data (CEHD) and the Integrated Management Information System (IMIS) databases, which contain quantitative and qualitative data resulting from compliance inspections conducted from 1984 to 2011. This analysis aimed to evaluate trends in workplace asbestos concentrations over time and across industries by combining the samples from these two databases. From 1984 to 2011, personal air samples ranged from 0.001 to 175 f/cc. Asbestos compliance sampling data associated with the construction, automotive repair, manufacturing, and chemical/petroleum/rubber industries included measurements in excess of 10 f/cc, and were above the permissible exposure limit from 2001 to 2011. The utility of combining the databases was limited by the completeness and accuracy of the data recorded. In this analysis, 40% of the data overlapped between the two databases. Other limitations included sampling bias associated with compliance sampling and errors occurring from user-entered data. A clear decreasing trend in both airborne fiber concentrations and the numbers of asbestos samples collected parallels historically decreasing trends in the consumption of asbestos, and declining mesothelioma incidence rates. Although air sampling data indicated that airborne fiber exposure potential was high (>10 f/cc for short and long-term samples) in some industries (e.g., construction, manufacturing), airborne concentrations have significantly declined over the past 30 years. Recommendations for improving the existing exposure OSHA databases are provided.



## 1. Introduction

The Occupational Safety and Health Administration (OSHA) maintains two separate databases that include methodological and analytical data resulting from compliance inspections. Prior to 1979, inspectors recorded a severity index, which reflected the ratio of the workplace measurement to the permissible exposure limit (PEL). After 1979, the actual exposure measurements were added to a digital database known as the Integrated Management Information System (IMIS) (Lavoue et al., 2013). The data in IMIS recorded from 1979 to 2013 were primarily qualitative information collected during compliance air sampling events such as: industry type, engineering controls and personal protective equipment (PPE) utilized by workers at the job site, worker job classification, and inspection type. Sampling data collected by OSHA inspectors and analyzed by the Salt Lake Technical Center's Analytical Services are entered into the Chemical Exposure Health Data (CEHD) database established in 1984 (Lavoue et al., 2013). The data contained in the CEHD were specifically related to quantitative results of air sampling events (e.g., sampling duration, asbestos concentration, location, industry, etc.). As of 2013, the CEHD contained sample results collected between 1984 and 2011. When the IMIS and CEHD databases are joined together, they potentially provide additional insight into occupational exposures to chemicals, including asbestos, over time and across industry sectors.

Asbestos has been used throughout the last part of the 19th century and much of the 20th century in commercial, industrial, maritime, railroad, and other applications. Asbestos encompasses several different mineral types, and has been used in hundreds of commercial products manufactured domestically and internationally including insulation, gaskets, packing, brakes, clutches, and drywall joint compound. According to the U.S. Geological Survey (USGS), (Virta, 2011) the U.S. began importing and consuming

* Corresponding author at: Cardno ChemRisk, LLC, 130 Vantis Suite 170, Aliso Viejo, CA, United States. Fax: +1 949 616 3899.
*E-mail address:* dallas.cowan@cardno.com (D.M. Cowan).

http://dx.doi.org/10.1016/j.yrtph.2015.05.002
0273-2300/

asbestos in the early-1900s. Consumption quickly increased throughout World War I and World War II before peaking in the 1970s when between 700,000 and 800,000 metric tons of asbestos were consumed in the U.S. annually from 1970 to 1980 (Fig. 1). The USGS estimated that approximately 68 billion pounds of asbestos were consumed (i.e., production plus importation) in the U.S. from 1900 to 2011 (Virta, 2011).

Asbestos-containing insulation use began to be phased out in the 1970s, and was followed by aggressive efforts to remove or encapsulate asbestos in schools and industrial facilities. A decreasing trend in asbestos consumption is evident from the 1980s to the 2000s (Melville, 2001). Still, many industries in the U.S. continue to use asbestos either as a raw material in products manufacturing or as a manufacturing component (Virta, 2011). As of 2013, the chloralkali and roofing-products industries consumed 98% of the total 2.3 million pounds of asbestos imported into the U.S. (Giannasi, 2007; USGS (U.S. Geological Survey), 2013).

According to the National Cancer Institute, incidence of mesothelioma in the U.S. is declining after peaking in the early to mid-1990s (Howlader et al., 2013; Weil, 1996; Weill et al., 2004). According to Price and Ware (2004) a return to background levels is not expected to occur until 2055; just under 71,000 cases of male mesothelioma incidence have been projected from 2003 until 2054.

Most industries have phased out the use of asbestos for use in specific applications. For example, the use of asbestos in specific commercial products (e.g., commercial fireplace ash and consumer patching compounds) was banned in 1977 by the Consumer Product Safety Commission (CPSC) (CPSC (Consumer Product Safety Commission), 1977a; CPSC (Consumer Product Safety Commission), 1977b). Although asbestos is still imported and consumed for certain applications in the U.S., exposures in the workplace are believed to be significantly lower than previous decades when asbestos was commonly used in industrial applications (Virta, 2006). Thus, the purpose of this analysis was to describe and quantify historical industry-specific trends in asbestos compliance measurements (air and bulk samples) collected throughout the U.S. by OSHA between 1984 and 2011. This is believed to be the first analysis to combine quantitative analytical results with qualitative information from OSHA compliance inspections in order to examine trends in occupational exposure to asbestos. By connecting these two databases, it is possible to gain insight into asbestos exposure potentials within and between industries across four decades of exposure data.

The objectives of this analysis were to: (1) collectively analyze the two partially overlapping databases to produce a database that includes both quantitative (air and bulk sampling data) and qualitative (personal protective equipment, job category, task, engineering controls, etc.) information from industrial hygiene sampling events; (2) evaluate temporal trends related to workplace measurements of airborne asbestos; (3) characterize industries where asbestos sampling showed low and high exposures and describe industry-specific trends; and (4) identify industries where asbestos exposures (short-term and long-term samples) were historically problematic and may potentially remain a concern in the U.S. today; (5) provide recommendations for improvement of existing OSHA exposure databases.

## 2. Methods

The CEHD and IMIS databases were reviewed, analyzed, and combined in order to examine trends in asbestos compliance sampling over time. The CEHD included sampling data for all chemicals evaluated during OSHA inspections between 1984 and 2011, and was searchable or downloadable from the OSHA website. For the purposes of this study, the asbestos-specific sampling data were separated from other chemicals by the unique substance code (9020) and combined onto one spreadsheet that represented all sampling data points from 1984 to 2011. This dataset included air and bulk samples and field blanks. For the IMIS database, a Freedom of Information Act (FOIA) request was submitted to the U.S. Department of Labor requesting all available asbestos sampling information in the database. The specified dataset was received as a standard Excel file and contained samples from 1980 to 2013.

Although the two datasets contained overlapping information, each dataset also contained unique variables. Both datasets included quantitative and qualitative information. The CEHD contained primary information related to specific OSHA sampling events conducted at industrial facilities throughout the U.S. (e.g.,

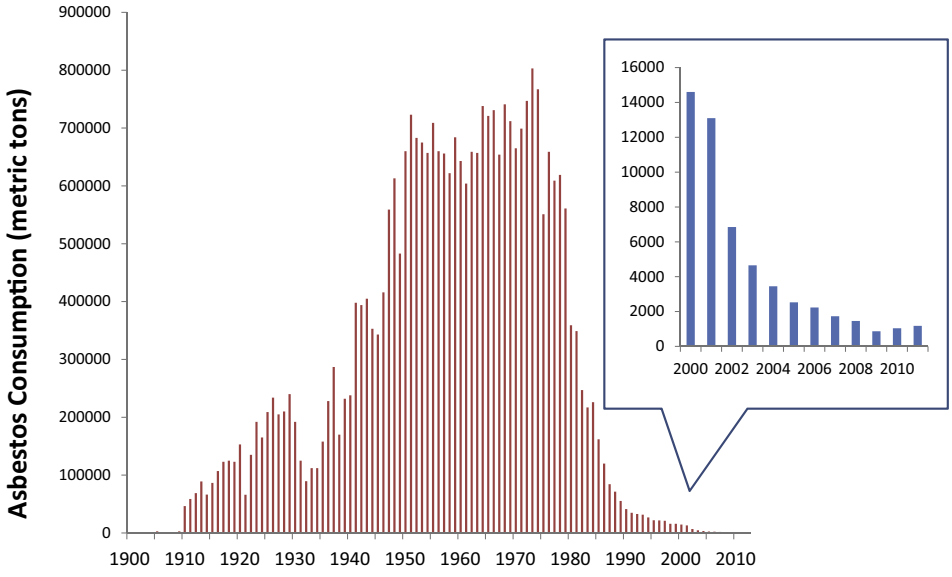



**Fig. 1.** Historical asbestos consumption in the United States (1900–2011). *Source:* U.S. Geological Survey (2012).

date, company, location, analytical results, etc.). The IMIS also contained extra details of these sampling events, such as the type of inspection performed, job title or description of the workers sampled, and whether those workers wore personal protection equipment (Table 1). Information shared between the two datasets included inspection number, sample number, NAICS (North American Industry Classification System) code, SIC (Standard Industrial Classification) code, and sampling location details. Unfortunately, neither database contained task-based or tool-specific information. Occasionally specific samples indicated the type of product that was bulk sampled, however, only 7 samples contained product-specific information and additional analysis was not conducted.

The datasets were combined using the "sampling number" variable because it was common to both CEHD and IMIS and was a unique number for each sample collected. To ensure that the databases were correctly combined, other variables were verified for consistency in the combined database (e.g., date, business location). Since a fewer number of samples were contained within IMIS, there were CEHD values that did not have corresponding IMIS values. Additionally, certain sampling data points in the IMIS corresponded to several CEHD data points because the IMIS data were applicable to an entire day of sampling data.

For the purposes of this study, the sample types were classified in the CEHD dataset as personal (P), area (A), or bulk (B). It should be noted that there were instances when information in one dataset contradicted the information in the other dataset. When individual data fields were contradictory or obvious data entry errors were discovered, they were excluded from the analysis.

Each individual sampling event was classified under a specific industry designation according to the SIC or NAICS code, which classifies entities by their primary type of business activity. Since the NAICS codes were established in 1997, the CEHD and IMIS databases did not have corresponding NAICS codes for every sample. Therefore the analysis relied on major SIC codes for stratifying the data for industry specific comparisons. SIC codes are 4-digit numerical codes designating a major industry in which a business entity is classified. The first two digits represent a major group classification and the second two digits represent more specific industry designation within a larger industry group. For the purposes of this analysis, we grouped industries by major SIC code and then further grouped into similar industries (e.g., agriculture, forestry, and fishing: 100–900) (Ambler and Kristoff, 1998). In order to analyze temporal trends, samples were stratified by decade and samples were divided into the following time periods: 1984–1989, 1990–1999, 2000–2009, and 2010–2011.

Personal air samples were stratified by duration (i.e.,<1 h, 1–4 h, >4 h) in order to compare sampling data among different durations and with contemporaneous occupational exposure limits. Specifically, each duration group was compared to the other two (<1 vs. 1–4 h, <1 vs. >4 h, and 1–4 vs. >4 h). ProUCL 4.1 was used to determine if the detected samples from each of the time ranges exhibited normal distributions. If the data were normally distributed, the student's *t*-test was used to compare concentrations. For non-parametric distributions, the Wilcoxon–Mann–Whitney test was available for concentration comparisons.

## 3. Results

Main findings were categorized in terms of the study objectives and included: (1) a summary of the main database sampling entries from the IMIS and CEHD databases; (2) an evaluation of temporal trends in asbestos compliance measurements; and (3) a comparison of trends in air sampling data across industry groups and a more detailed analysis of industries where air sampling results exceeded contemporaneous and current occupational exposure limits and those industries where samples recently exceeded such levels.

### *3.1. Summary of overlapping entries from the IMIS and CEHD databases*

The CEHD and IMIS databases represent the accumulation of asbestos sampling data collected by OSHA from 1984 to 2011 and together include both qualitative and quantitative variables that are important for understanding potential exposures in the workplace. The key variables considered for this analysis were sampling data and industry, sampling duration (min), asbestos bulk or air concentration (f/cc or % asbestos), PPE (respiratory protection Yes or No). Table 1 describes the sampling variables

**Table 1**
Comparison of OSHA databases (CEHD and IMIS) containing asbestos compliance sampling data.

| Data points* | CEHD<br>*n* = 70,901 | Overlapping variables<br>*n* = 29,212** | IMIS<br>*n* = 21,676 |
|---|---|---|---|
| Database variables | Office ID number | Inspection number | Street address |
| | Date reported | Establishment name | Job description |
| | 8-h TWA | City | Exposure level |
| | Instrument type | State | Exposure type |
| | Lab number | Zip code | Exposure frequency |
| | Field number | SIC code | PPE |
| | Blank used | NAICS code | Inspection type |
| | Time sampled | Sample date | PEL/adjusted PEL |
| | Air volume | Sample ID number | Establishment size |
| | Sample weight | Sample type | Employees exposed |
| | Qualifier | Substance code | |
| Years evaluated | 1984–2011 | N/A | 1984–2011 |
| Access | Publicly available | N/A | Available via Freedom of Information Act request |

PPE: personal protection equipment.
PEL: permissible exposure limit.
TWA: time-weighted average.
CEHD: Chemical Exposure Health Data.
IMIS: Integrated Management Information System.
* Represents individual sampling data points including blanks.
** Represents the number of CEHD data points that have qualitative information obtained from the IMIS database. On occasion, multiple CEHD data points corresponded to a single IMIS data point as they were from a single event.

discrete to each database, as well as the overlapping variables (e.g., inspection number, sampling number, establishment name).

When considering the databases separately, the CEHD contained 70,901 individual air and bulk samples and the IMIS contained 21,676 air and bulk samples (including field blanks). There were a total of 15,491 blank samples in the CEHD database and after removing these samples, 55,410 discrete samples remained. Combining the two datasets yielded a total of 29,212 sampling data points, which represented approximately 40% of samples from the CEHD database.

### 3.2. Temporal trends in asbestos compliance measurements

Asbestos consumption in the U.S. peaked in the 1970s and 1980s (approximately 800,000 metric tons per year) until consumption significantly decreased by a factor of approximately 350 times to current estimates of consumption of approximately 2000 tons in 2011 (Fig. 1) (USGS (U.S. Geological Survey), 2013). As expected, once asbestos began to be phased out from manufacturing processes, the number of compliance air samples taken by OSHA inspectors also began to decrease. In 1984, more than 200,000 metric tons of asbestos were consumed in the United States and approximately 3000 asbestos air samples were collected by OSHA. However, by 2011, less than 2000 metric tons of asbestos were imported and the number of asbestos air samples collected fell to less than 300. The parallel trend in decreasing consumption and sampling frequency is evident in Fig. 2.

A clear decline in the number of asbestos compliance samples (air and bulk) obtained by OSHA is apparent on a decade-by-decade basis (Fig. 3). For example, 10,779 personal air samples were collected in the 1980s, which declined throughout the 1990s ($n = 4771$) and 2000s ($n = 2034$). The number of bulk and area samples also decreased significantly over the same time period. Typically, comparing the number of personal and area samples collected over time, area samples represent approximately 50% of the personal samples collected.

Considering the entire dataset that contained air sampling data from 1984 to 2011, not accounting for outliers, personal asbestos

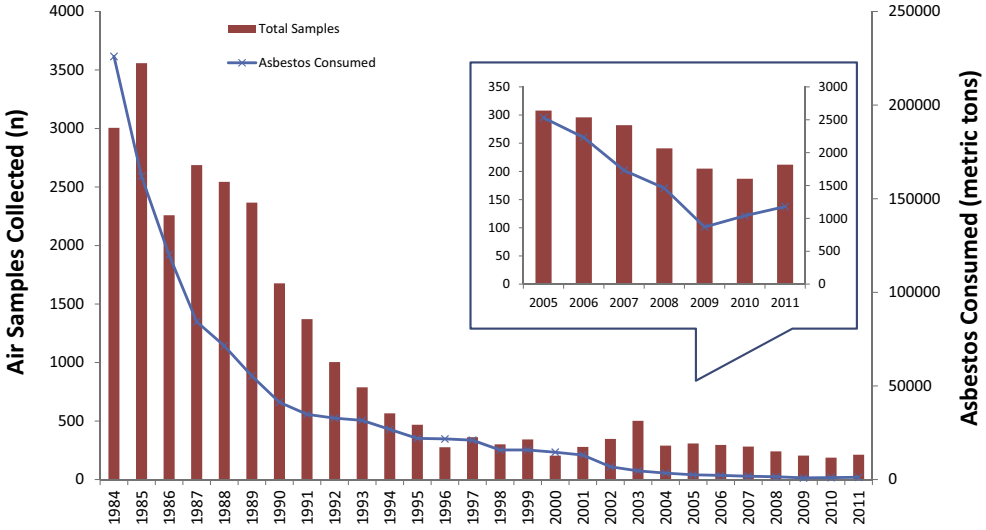



**Fig. 2.** Comparison of OSHA air sampling data with asbestos consumption in the United States (1984–2011).

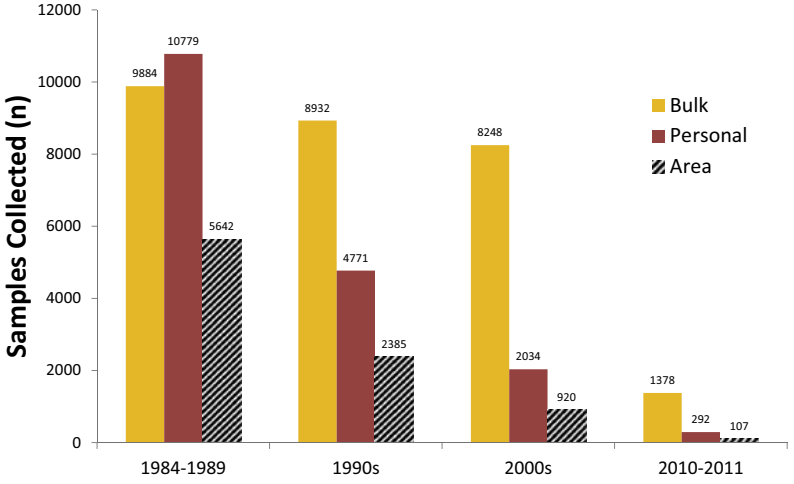



**Fig. 3.** Asbestos compliance samples collected by OSHA and contained within the CEHD (1984–2011).

**Table 2**
Results of OSHA asbestos compliance sampling across industry groups and decades.

| SIC group | Industry group | Personal | | | | | Area | | | | Bulk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Year | *n* | ND | *n*(p) | Range (f/cc) | *n* | ND | *n*(p) | Range (f/cc) | *n* | ND | *n*(p) | Range (%) |
| 100–900 | Agriculture, forestry, fishing | 84–89 | – | – | – | – | 24 | 23 | 1 | 0.03 | 37 | 8 | 29 | 0.001–96 |
| | | 90–99 | 9 | 9 | – | – | 5 | 5 | – | – | 32 | 14 | 18 | 35–85 |
| | | 00–09 | – | – | – | – | – | – | – | – | 10 | 5 | 5 | 2–100 |
| | | 10–11 | – | – | – | – | 2 | 2 | – | – | 6 | 6 | – | – |
| | | Total: | 9 | 9 | – | – | 31 | 30 | 1 | 0.03 | 85 | 33 | 52 | 0.001–100 |
| 1200–1400 | Mining | 84–89 | 2 | 2 | – | – | 3 | 3 | – | – | 10 | 2 | 8 | 7–80 |
| | | 90–99 | – | – | – | – | 10 | 10 | – | – | 2 | 1 | 1 | 50 |
| | | 00–09 | – | – | – | – | – | – | – | – | – | – | – | – |
| | | 10–11 | 3 | 3 | – | – | | | – | – | 5 | 4 | 1 | 3 |
| | | Total: | 5 | 5 | – | – | 13 | 13 | – | – | 17 | 7 | 10 | 3–80 |
| 1500 | Building construction-general contractors & operative builders | 84–89 | 630 | 365 | 265 | 0.001–16.1 | 201 | 160 | 41 | 0.001–17.07 | 752 | 179 | 573 | 0.001–90 |
| | | 90–99 | 235 | 172 | 63 | 0.003–13.2 | 150 | 146 | 4 | 0.02–2.5 | 865 | 378 | 487 | 0.001–90 |
| | | 00–09 | 95 | 85 | 10 | 0.024–0.32 | 70 | 69 | 1 | 0.027 | 1331 | 772 | 559 | 0.01–100 |
| | | 10–11 | 15 | 13 | 2 | 0.13–0.19 | 3 | 3 | – | – | 253 | 165 | 88 | 0.01–90 |
| | | Total: | 975 | 635 | 340 | 0.001–16.1 | 424 | 378 | 46 | 0.001–17.07 | 3201 | 1494 | 1707 | 0.001–100 |
| 1600 | Heavy construction, except building construction-contractors | 84–89 | 77 | 47 | 30 | 0.002–5.4 | 48 | 42 | 6 | 0.002–0.41 | 146 | 26 | 120 | 0.01–90 |
| | | 90–99 | 25 | 18 | 7 | 0.0078–5.34 | 18 | 17 | 1 | 0.02 | 155 | 93 | 62 | 0.001–85 |
| | | 00–09 | 26 | 26 | – | – | – | – | – | – | 44 | 19 | 25 | 1–90 |
| | | 10–11 | 2 | 2 | – | – | – | – | – | – | 13 | 7 | 6 | 10–30 |
| | | Total: | 130 | 93 | 37 | 0.002–5.4 | 66 | 59 | 7 | 0.002–0.41 | 358 | 145 | 213 | 0.001–90 |
| 1700 | Construction-special trade contractors | 84–89 | 3910 | 1687 | 2223 | 0.001–88.5 | 760 | 567 | 193 | 0.001–8 | 2187 | 407 | 1780 | 0.1–95 |
| | | 90–99 | 1958 | 1412 | 546 | 0.001–34.2 | 346 | 306 | 40 | 0.0042–1.04 | 1944 | 660 | 1284 | 0.0001–100 |
| | | 00–09 | 1231 | 1004 | 227 | 0.0023–34 | 168 | 158 | 10 | 0.0024–0.24 | 2068 | 1142 | 926 | 0.01–100 |
| | | 10–11 | 160 | 153 | 7 | 0.031–0.2 | 5 | 5 | | – | 353 | 192 | 161 | 0.01–75 |
| | | Total: | 7259 | 4256 | 3003 | 0.001–88.5 | 1279 | 1036 | 243 | 0.001–8 | 6552 | 2401 | 4151 | 0.1–100 |
| 2000–2600/3100–3900 | Manufacturing | 84–89 | 3364 | 1323 | 2041 | 0.001–54.5 | 823 | 660 | 163 | 0.002–77.02 | 2078 | 453 | 1625 | 0.0001–99.5 |
| | | 90–99 | 1225 | 703 | 522 | 0.001–54.4 | 394 | 353 | 41 | 0.01–3.8 | 1523 | 494 | 1029 | 0.001–99 |
| | | 00–09 | 197 | 186 | 11 | 0.058–0.59 | 91 | 90 | 1 | 0.0098 | 1019 | 617 | 402 | 0.01–100 |
| | | 10–11 | 23 | 22 | 1 | 1.1 | 4 | 4 | – | – | 87 | 55 | 32 | 0.8–66 |
| | | Total: | 4809 | 2234 | 2575 | 0.001–54.5 | 1312 | 1107 | 205 | 0.002–77.02 | 4707 | 1619 | 3088 | 0.0001–100 |
| 2800 | Chemicals and allied products | 84–89 | 121 | 91 | 30 | 0.003–12.82 | 67 | 64 | 3 | 0.01–0.12 | 230 | 59 | 171 | 0.001–99 |
| | | 90–99 | 88 | 58 | 30 | 0.006–5.3 | 10 | 9 | 1 | 0.17 | 205 | 80 | 125 | 0.001–100 |
| | | 00–09 | 7 | 7 | – | – | 2 | 2 | – | – | 100 | 56 | 44 | 1–98 |
| | | 10–11 | – | – | – | – | 2 | 2 | – | – | 22 | 18 | 4 | 0.6–10 |
| | | Total: | 216 | 156 | 60 | 00.003–12.82 | 81 | 77 | 4 | 0.01–0.17 | 557 | 213 | 344 | 0.001–100 |
| 2900 | Petroleum refining and related industries | 84–89 | 237 | 85 | 152 | 0.003–65.1 | 33 | 12 | 21 | 0.001–55 | 132 | 18 | 114 | 0.001–95 |
| | | 90–99 | 140 | 59 | 81 | 0.004–20.8 | 42 | 22 | 20 | 0.03–3.3 | 102 | 25 | 77 | 0.001–95 |
| | | 00–09 | 12 | 6 | 6 | 0.023–0.27 | 49 | 41 | 8 | 0.0034–0.01 | 84 | 45 | 39 | 0.01–100 |
| | | 10–11 | – | – | – | – | – | – | – | – | 23 | 10 | 13 | 4–30 |
| | | Total: | 389 | 150 | 239 | 0.003–65.1 | 124 | 75 | 49 | 0.001–55 | 341 | 98 | 243 | 0.001–100 |
| 3000 | Rubber and miscellaneous plastic products | 84–89 | 465 | 136 | 329 | 0.003–7 | 97 | 67 | 30 | 0.004–1.08 | 209 | 44 | 165 | 0.1–95 |
| | | 90–99 | 73 | 66 | 7 | 0.0045–0.09 | 33 | 32 | 1 | 0.0089 | 104 | 52 | 52 | 0.001–100 |
| | | 00–09 | 5 | 4 | 1 | 0.031 | 3 | 3 | – | – | 52 | 16 | 36 | 0.01–75 |
| | | 10–11 | 4 | 4 | – | – | 1 | 1 | – | – | 9 | 7 | 2 | 5–50 |
| | | Total: | 547 | 210 | 337 | 0.003–7 | 134 | 103 | 31 | 0.004–1.08 | 374 | 119 | 255 | 0.1–100 |

(*continued on next page*)

**Table 2** (*continued*)

| SIC group | Industry group | Personal | | | | | Area | | | | Bulk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Year | *n* | ND | *n*(p) | Range (f/cc) | *n* | ND | *n*(p) | Range (f/cc) | *n* | ND | *n*(p) | Range (%) |
| 4000 | Railroad transportation | 84–89 | 19 | 9 | 10 | 0.02–0.74 | 26 | 24 | 2 | 0.13–0.27 | 82 | 4 | 78 | 0.001–90 |
| | | 90–99 | 10 | 10 | – | – | 12 | 12 | – | – | 34 | 7 | 27 | 0.001–95 |
| | | 00–09 | 11 | 9 | 2 | 0.019–0.035 | 11 | 7 | 4 | 0.024–0.06 | 35 | 14 | 21 | 0.1–100 |
| | | 10–11 | – | – | – | – | 2 | 2 | – | – | – | – | – | – |
| | | Total: | 40 | 28 | 12 | 0.019–0.74 | 51 | 45 | 6 | 0.024–0.27 | 151 | 25 | 126 | 0.001–100 |
| 4200 | Motor freight transportation | 84–89 | 42 | 38 | 4 | 0.01–0.68 | 61 | 61 | – | – | 58 | 15 | 43 | 0.001–98 |
| | | 90–99 | 36 | 35 | 1 | 0.062 | 33 | 29 | 4 | 0.04–0.35 | 70 | 27 | 43 | 0.001–80 |
| | | 00–09 | 22 | 21 | 1 | 0.36 | 15 | 15 | – | – | 135 | 99 | 36 | 0.01–90 |
| | | 10–11 | 1 | 1 | – | – | 5 | 5 | – | – | 31 | 16 | 15 | 0.5–25 |
| | | Total: | 101 | 95 | 6 | 0.01–0.68 | 114 | 110 | 4 | 0.04–0.35 | 294 | 157 | 137 | 0.001–98 |
| 4400 | Water transportation | 84–89 | 39 | 21 | 18 | 0.03–1.42 | 14 | 13 | 1 | 0.04 | 60 | 7 | 53 | 0.001–90 |
| | | 90–99 | 27 | 9 | 18 | 0.027–3.71 | 4 | 4 | – | – | 45 | 20 | 25 | 1–85 |
| | | 00–09 | 45 | 15 | 30 | 0.013–4.7 | – | – | – | – | 34 | 20 | 14 | 0.01–90 |
| | | 10–11 | 8 | 8 | – | – | – | – | – | – | 2 | – | 2 | 1–10 |
| | | Total: | 119 | 53 | 66 | 0.013–4.7 | 18 | 17 | 1 | 0.04 | 141 | 47 | 94 | 0.001–90 |
| 4900 | Electrical, gas, and sanitary services | 84–89 | 214 | 147 | 67 | 0.001–4.74 | 173 | 151 | 22 | 0.0023–2.954 | 220 | 55 | 165 | 0.001–95 |
| | | 90–99 | 79 | 72 | 7 | 0.018–0.21 | 63 | 62 | 1 | 0.05 | 211 | 106 | 105 | 0.001–99 |
| | | 00–09 | 43 | 34 | 9 | 0.003–0.16 | 19 | 19 | – | – | 178 | 86 | 92 | 0.01–100 |
| | | 10–11 | 3 | 3 | – | – | 3 | 2 | 1 | 40 | 7 | 3 | 4 | 2–15 |
| | | Total: | 339 | 256 | 83 | 0.001–4.74 | 258 | 234 | 24 | 0.0023–40 | 616 | 250 | 366 | 0.001–100 |
| 4100, 4300, 4500–4800 | Transportation, communications, electric, gas & sanitary services | 84–89 | 288 | 266 | 22 | 0.003–15 | 481 | 454 | 27 | 0.003–39.2 | 446 | 140 | 306 | 0.001–90 |
| | | 90–99 | 130 | 129 | 1 | 0.08–0.08 | 208 | 207 | 1 | 0.14 | 321 | 180 | 141 | 0.001–97 |
| | | 00–09 | 63 | 63 | – | – | 85 | 85 | – | – | 374 | 274 | 100 | 0.01–100 |
| | | 10–11 | 13 | 13 | – | – | 24 | 24 | – | – | 88 | 64 | 24 | 0.2–70 |
| | | Total: | 494 | 471 | 23 | 0.003–15 | 798 | 770 | 28 | 0.003–39.2 | 1229 | 658 | 571 | 0.001–100 |
| 5000–5100 | Wholesale trade | 84–89 | 119 | 92 | 27 | 0.008–1.4 | 68 | 63 | 5 | 0.015–0.02 | 171 | 36 | 135 | 0.001–90 |
| | | 90–99 | 80 | 78 | 2 | 0.071–0.55 | 35 | 35 | – | – | 126 | 55 | 71 | 0.001–85 |
| | | 00–09 | 15 | 13 | 2 | 0.071–0.23 | 5 | 5 | – | – | 132 | 89 | 43 | 0.01–90 |
| | | 10–11 | 17 | 16 | 1 | 0.003 | – | – | – | – | 48 | 18 | 30 | 1–75 |
| | | Total: | 231 | 199 | 32 | 0.003–1.4 | 108 | 103 | 5 | 0.015–0.02 | 477 | 198 | 279 | 0.001–90 |
| 5200–5400, 5600–5900 | Retail trade | 84–89 | 92 | 76 | 16 | 0.003–33.2 | 139 | 128 | 11 | 0.002–22.3 | 204 | 54 | 150 | 0.001–90 |
| | | 90–99 | 58 | 50 | 8 | 0.003–0.21 | 92 | 92 | – | – | 223 | 85 | 138 | 0.001–80 |
| | | 00–09 | 22 | 20 | 2 | 0.0026–0.0044 | 45 | 45 | – | – | 286 | 167 | 119 | 0.01–100 |
| | | 10–11 | 2 | 2 | – | – | 5 | 5 | – | – | 23 | 13 | 10 | 0.05–10 |
| | | Total: | 174 | 148 | 26 | 0.0026–33.2 | 281 | 270 | 11 | 0.002–22.3 | 736 | 319 | 417 | 0.001–100 |
| 5500 | Automotive dealers and gasoline service stations | 84–89 | 78 | 72 | 6 | 0.03–0.62 | 18 | 18 | – | – | 43 | 13 | 30 | 0.001–80 |
| | | 90–99 | 61 | 59 | 2 | 0.03–0.056 | 21 | 21 | – | – | 82 | 55 | 27 | 0.002–85 |
| | | 00–09 | 12 | 12 | – | – | 3 | 3 | – | – | 65 | 39 | 26 | 0.05–100 |
| | | 10–11 | 1 | 1 | – | – | – | – | – | – | 13 | 7 | 6 | 0.04–70 |
| | | Total: | 152 | 144 | 8 | 0.03–0.62 | 42 | 42 | – | – | 202 | 113 | 89 | 0.001–100 |
| 6000–6400, 6700 | Finance, insurance, and real estate | 84–89 | 38 | 31 | 7 | 0.008–0.11 | 74 | 71 | 3 | 0.02–0.03 | 55 | 27 | 28 | 0.001–75 |
| | | 90–99 | 9 | 9 | – | – | 58 | 58 | – | – | 91 | 52 | 39 | 0.001–70 |
| | | 00–09 | 4 | 4 | – | – | 9 | 9 | – | – | 37 | 23 | 14 | 1.4–50 |
| | | 10–11 | – | – | – | – | 1 | 1 | – | – | 1 | 1 | – | – |
| | | Total: | 51 | 44 | 7 | 0.008–0.11 | 142 | 139 | 3 | 0.02–0.03 | 184 | 103 | 81 | 0.00175 |

**Table 2** (*continued*)

| SIC group | Industry group | Personal | | | | | Area | | | | Bulk | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Year | *n* | ND | *n*(p) | Range (f/cc) | *n* | ND | *n*(p) | Range (f/cc) | *n* | ND | *n*(p) | Range (%) |
| 6500 | Real estate | 84–89 | 61 | 43 | 18 | 0.02–0.83 | 104 | 100 | 4 | 0.005–0.02 | 208 | 44 | 164 | 0.001–85 |
| | | 90–99 | 45 | 39 | 6 | 0.007–0.3 | 84 | 67 | 17 | 0.0015–0.17 | 480 | 167 | 313 | 0.001–90 |
| | | 00–09 | 22 | 21 | 1 | 0.0064 | 33 | 32 | 1 | 0.14 | 446 | 214 | 232 | 0.04–100 |
| | | 10–11 | 4 | 4 | – | – | 7 | 6 | 1 | 0.006 | 80 | 48 | 32 | 1.1–85 |
| | | Total: | 132 | 107 | 25 | 0.0064–0.83 | 228 | 205 | 23 | 0.0015–0.17 | 1214 | 473 | 741 | 0.001–100 |
| 7000–7300, 7800–8900 | Services | 84–89 | 305 | 233 | 72 | 0.003–2.2 | 933 | 894 | 39 | 0.002–4.75 | 1313 | 358 | 955 | 0.1–96 |
| | | 90–99 | 194 | 178 | 16 | 0.001–0.71 | 391 | 387 | 4 | 0.0093–0.097 | 1555 | 644 | 911 | 0.001–100 |
| | | 00–09 | 131 | 128 | 3 | 0.0047–0.018 | 187 | 186 | 1 | 5 | 1357 | 792 | 565 | 0.01–100 |
| | | 10–11 | 10 | 10 | – | – | 27 | 25 | 2 | 0.007–0.008 | 229 | 137 | 92 | 0.01–90 |
| | | Total: | 640 | 549 | 91 | 0.001–2.2 | 1538 | 1492 | 46 | 0.002–5 | 4454 | 1931 | 2523 | 0.1–100 |
| 7500 | Automotive repair, services and parking | 84–89 | 274 | 241 | 33 | 0.0031–35.6 | 39 | 35 | 4 | 0.01–0.19 | 113 | 52 | 61 | 0.001–80 |
| | | 90–99 | 101 | 101 | – | – | 12 | 12 | – | – | 70 | 37 | 33 | 0.001–75 |
| | | 00–09 | 17 | 17 | – | – | 2 | 2 | – | – | 52 | 44 | 8 | 0.01–50 |
| | | 10–11 | 2 | 2 | – | – | 2 | 2 | – | – | 23 | 22 | 1 | 4 |
| | | Total: | 394 | 361 | 33 | 0.0031–35.6 | 55 | 51 | 4 | 0.01–0.19 | 258 | 155 | 103 | 0.001–80 |
| 7600 | Miscellaneous repair services | 84–89 | 56 | 25 | 31 | 0.006–175.44 | 34 | 30 | 4 | 0.01–0.32 | 46 | 13 | 33 | 0.001–85 |
| | | 90–99 | 22 | 18 | 4 | 0.089–1.3 | 9 | 9 | – | – | 70 | 36 | 34 | 0.001–95 |
| | | 00–09 | – | – | – | – | 1 | 1 | – | – | 16 | 15 | 1 | 1.06 |
| | | "10–11" | – | – | – | – | – | – | – | – | 5 | 4 | 1 | 90 |
| | | Total: | 78 | 43 | 35 | 0.006–175.44 | 44 | 40 | 4 | 0.01–0.32 | 136 | 66 | 68 | 0.001–95 |
| 9100–9900 | Public administration | 84–89 | 318 | 267 | 51 | 0.004–13 | 1379 | 1328 | 51 | 0.002–2.11 | 1036 | 307 | 729 | 0.001–95 |
| | | 90–99 | 160 | 159 | 1 | 0.44 | 341 | 337 | 4 | 0.001–0.029 | 584 | 329 | 255 | 0.001–90 |
| | | 00–09 | 51 | 49 | 2 | 0.0055–0.052 | 118 | 118 | – | – | 326 | 249 | 77 | 0.01–100 |
| | | "10–11" | 21 | 19 | 2 | 0.003 | 12 | 12 | – | – | 45 | 35 | 10 | 1–20 |
| | | Total: | 550 | 494 | 56 | 0.003–13 | 1850 | 1795 | 55 | 0.001–2.11 | 1991 | 920 | 1071 | 0.001–100 |

ND: non-detects.
*n*(p): number of samples above the detection limit.

concentrations ranged from non-detectable (ND) to a maximum of 175 f/cc (Table 2). From 1984 to 1989, personal air samples with detectable asbestos concentrations ranged from 0.001 to 175 f/cc. Although maximum concentrations continued to exceed 10 f/cc, asbestos concentrations in personal air samples continued to decrease in the 1990s and 2000s. From 1990 to 1999, concentrations ranged from 0.001 to 54.4 f/cc and 0.0023 to 34 f/cc in the 1990s and 2000s, respectively. Finally, from 2010 to 2011, personal air samples ranged in concentrations from 0.003 to 1.1 f/cc. Airborne concentrations associated with personal samples collected from 1984 to 1989 were significantly higher when compared with samples collected in the 1990s, 2000s, and the period from 2010 to 2011 ($p < 0.05$). Additional comparisons between decades (e.g., 1990s vs. 2000s) were not significantly different.

### 3.3. Trends in the data across industry groups

OSHA collected data from certain industry sectors more frequently than others. The top 40 "most-sampled" industry groups are listed in Table 3. The construction industry as a whole (SIC code 1500–1700) was the most sampled industry ($n = 20,248$), representing approximately 37% of the all samples included in the CEHD database (excluding field blanks). The construction and manufacturing industries represented the largest numbers of OSHA compliance samples collected, although fewer than 200 personal air samples were collected from 2010 to 2011 in these industries overall. In particular, special trade contractors had the highest number of personal samples collected by OSHA during from 1984 to 2011 ($n = 7259$). The greatest number of samples (personal, area, and bulk) were collected in the special trade construction industry ($n = 15,090$); followed by building construction ($n = 4602$); industries that manufacture or utilize stone, clay, glass, and concrete products ($n = 2658$); health services ($n = 2242$); and transportation equipment ($n = 2324$). Other major industries that were highly sampled over the last 30 years included manufacturing, services, public administration, transportation and utilities; however, these major industries comprised less than half of the construction industry as a whole (Fig. 4).

Individual samples taken by OSHA inspectors varied widely in duration from less than 20 min to more than 8 h. To determine the best method for grouping personal air samples by duration, statistical analyses were performed to compare the similarity or dissimilarity of airborne asbestos concentrations sampled at varying durations. Personal air samples collected in the manufacturing, construction and chemical/petroleum major industries were grouped by duration and were found to be significantly different across three different sample durations: <1 h (short-term), 1–4 h (medium), and >4 h (long-term) ($p < 0.05$). Specifically, samples that were less than 1 h in duration had concentrations that were significantly greater when compared to samples with durations between 1 and 4 h, and they were also significantly greater when compared to samples with durations greater than 4 h. Likewise, samples with durations between 1 and 4 h were significantly greater than the samples with durations greater than 4 h. This

**Table 3**
Total number of personal, area, and bulk samples collected in major industries by SIC code.

| Rank | Major SIC code | Industry | Personal | Area | Bulk | Total |
|---|---|---|---|---|---|---|
| 1 | 1700 | Construction-special trade contractors | 7259 | 1279 | 6552 | 15,090 |
| 2 | 1500 | Building construction-general contractors & operative builders | 977 | 424 | 3201 | 4602 |
| 3 | 3200 | Stone, clay, glass, and concrete products | 2050 | 184 | 424 | 2658 |
| 4 | 8000 | Health services | 190 | 524 | 1528 | 2242 |
| 5 | 3700 | Transportation equipment | 1289 | 235 | 800 | 2324 |
| 6 | 6500 | Real estate | 132 | 228 | 1214 | 1574 |
| 7 | 4300 | U.S. postal service | 250 | 413 | 683 | 1346 |
| 8 | 2600 | Paper and allied products | 334 | 201 | 594 | 1129 |
| 9 | 4900 | Electrical, gas, and sanitary services | 339 | 258 | 616 | 1213 |
| 10 | 9700 | National security and international affairs | 194 | 302 | 584 | 1080 |
| 11 | 8200 | Educational services | 70 | 240 | 738 | 1048 |
| 12 | 3000 | Rubber and miscellaneous plastic products | 547 | 134 | 374 | 1055 |
| 13 | 3400 | Fabricated metal products, except machinery & transport equipment | 212 | 133 | 602 | 947 |
| 14 | 2800 | Chemicals and allied products | 216 | 81 | 557 | 854 |
| 15 | 2900 | Petroleum refining and related industries | 389 | 124 | 341 | 854 |
| 16 | 7500 | Automotive repair, services and parking | 394 | 55 | 258 | 707 |
| 17 | 3300 | Primary metal industries | 186 | 103 | 479 | 768 |
| 18 | 7300 | Business services | 121 | 171 | 429 | 721 |
| 19 | 3500 | Industrial and commercial machinery & transport equipment | 242 | 95 | 334 | 671 |
| 20 | 9100 | Executive, legislative & general government, except finance | 48 | 331 | 263 | 642 |
| 21 | 2000 | Food and kindred products | 88 | 107 | 446 | 641 |
| 22 | 9500 | Administration of environmental quality and housing programs | 36 | 194 | 380 | 610 |
| 23 | 7000 | Hotels, rooming houses, camps, and other lodging places | 37 | 88 | 524 | 649 |
| 24 | 5000 | Wholesale trade-durable goods | 196 | 62 | 291 | 549 |
| 25 | 9600 | Administration of economic programs | 75 | 250 | 226 | 551 |
| 26 | 9200 | Justice, public order and safety | 104 | 208 | 227 | 539 |
| 27 | 1600 | Heavy construction, except building construction-contractors | 130 | 66 | 360 | 556 |
| 28 | 4500 | Transportation by air | 123 | 157 | 278 | 558 |
| 29 | 4200 | Motor freight transportation | 101 | 114 | 294 | 509 |
| 30 | 9400 | Administration of human resources programs | 34 | 346 | 142 | 522 |
| 31 | 5300 | General merchandise stores | 97 | 134 | 236 | 467 |
| 32 | 4800 | Communications | 78 | 188 | 201 | 467 |
| 33 | 5500 | Automotive dealers and gasoline service stations | 152 | 42 | 203 | 397 |
| 34 | 3600 | Electronic, electrical equipment & components, except computer equipment | 150 | 72 | 236 | 458 |
| 35 | 8300 | Social services | 49 | 72 | 246 | 367 |
| 36 | 9300 | Public finance. taxation and monetary supply | 37 | 188 | 95 | 320 |
| 37 | 8700 | Engineering, accounting, research, management & related services | 68 | 55 | 204 | 327 |
| 38 | 8800/8900 | Services, not elsewhere classified | 22 | 129 | 145 | 296 |
| 39 | 7900 | Amusement and recreation services | 19 | 67 | 212 | 298 |
| 40 | 7600 | Miscellaneous repair services | 79 | 44 | 137 | 260 |

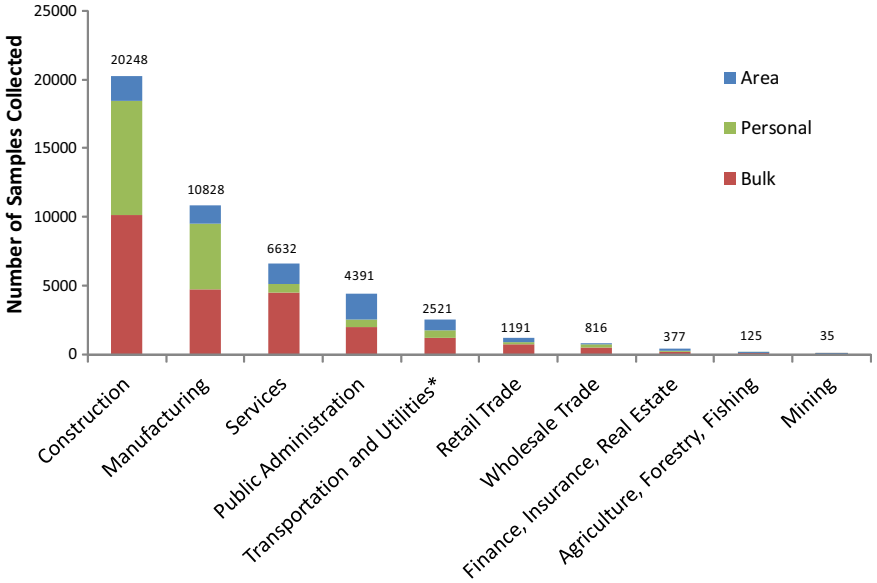



**Fig. 4.** Frequency among the ten most sampled industry groups (1984–2011). *Represent: Transportation, Communications, Electric, Gas & Sanitary Service Industries.

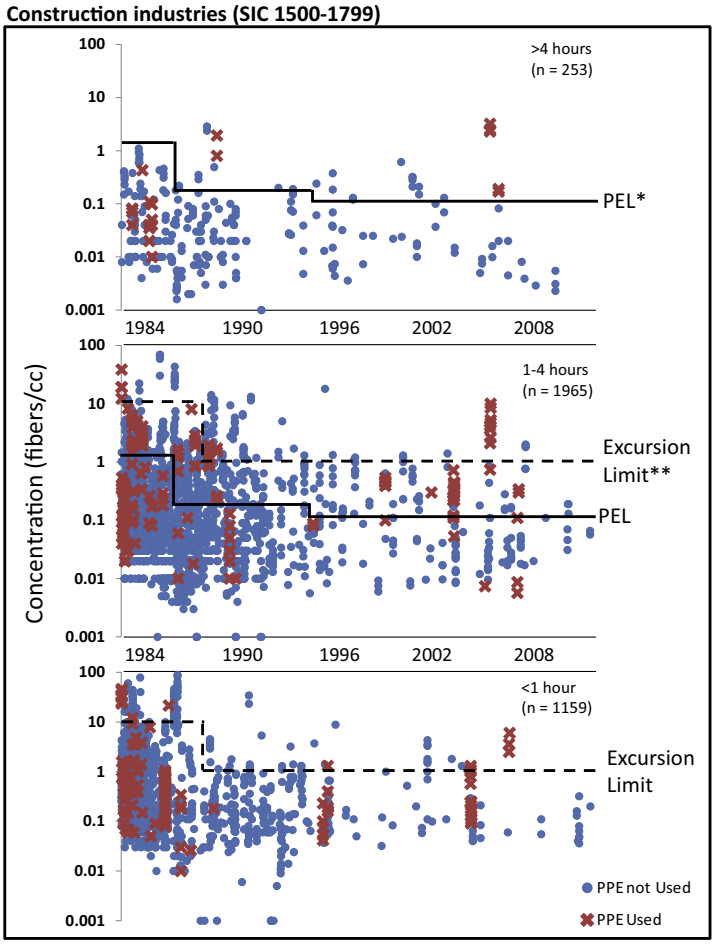



**Fig. 5.** Airborne fiber concentrations for <1, 1–4, and >4 h with and without information related to PPE compared with exposure guidelines over time. *PEL: permissible exposure limit based on 8-h time-weighted average. **Excursion limit: short-term permissible exposure limit based on average over 30 min.

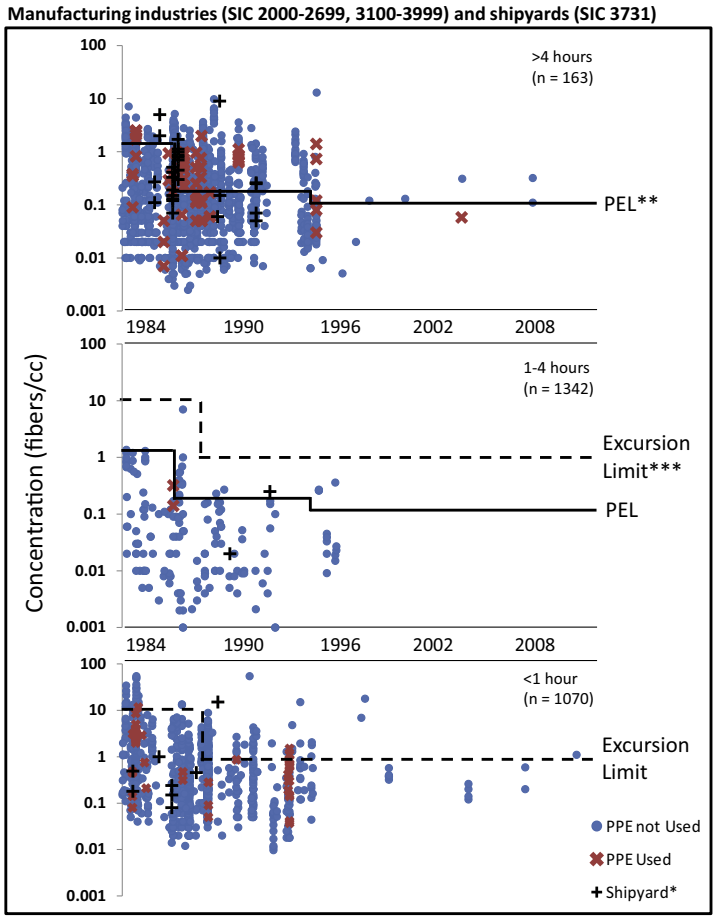



**Fig. 6.** Airborne fiber concentrations for <1, 1–4, and >4 h with and without information related to PPE compared with exposure guidelines over time. *There were no detected samples with reported PPE use from shipyards). **PEL: permissible exposure limit based on 8-h time-weighted average. ***Excursion limit: short-term permissible exposure limit based on average over 30 min.

pattern was consistent for all three industries. Therefore, airborne asbestos measurements for these three industries were evaluated according to these sample duration categories. Personal air samples collected in the automobile sales and service industries were not significantly different between <1 h (short-term) and >1 h (long-term) and therefore was not further divided into additional duration categories (e.g., <1, 4–8, >8 h).

Short-term airborne asbestos concentrations exceeding the 10 f/cc OSHA excursion limit were found in the construction, manufacturing, and chemical/petroleum industries (Figs. 5–8). With the exception of the automotive repair, three of the industry groups had a history of asbestos compliance sampling measurements in excess of 10 f/cc, as well as measurable asbestos exposures above the current PEL in the last ten years for which data were available (2001–2011). The retail trade and public administration industries also had short-term asbestos personal exposure air samples exceeding 10 f/cc; however, those samples were limited to the 1984–1989 time frame. Three industries had particularly notable results worthy of additional evaluation: construction, automotive, and shipbuilding (within the manufacturing industry).

Short- and long-term airborne fiber concentrations obtained in the construction industries (SIC codes 1500s–1700s), including considerations when PPE was used, were compared to occupational exposures standards, (Fig. 5). In the construction industries, 1100 of the sample results that were greater than one hour in duration exceeded the contemporaneous PELs [49.6% of all samples with durations greater than one hour (1100 *over PEL*/2218 *total*)] from 1984 to 2011. Of these, only 11.4 % (125/1100) samples were documented to have been taken when the worker was using PPE (for other samples, PPE information was not logged). The majority of these samples ($n = 1033$) had durations between one and four hours. For samples that were between one and four hours in duration ($n = 1965$), a total of 7.7% ($n = 152$) contained information related to PPE. For samples with durations greater than four hours ($n = 253$), a total of 8.3% ($n = 21$) contained information related to PPE. Considering those samples that had durations of less than one hour ($n = 1159$), 9.9% ($n = 115$) contained information related to PPE. These findings were similar in other industries (Figs. 6–8).

The shipbuilding industry (SIC code 3731) was classified within the manufacturing major SIC code, and has historically been associated with high concentrations of asbestos (Balzer and Cooper, 1968; Cooper and Balzer, 1968; Reitze et al., 1972; Sprince et al., 1985). Over the past 30 years a total of 137 personal, 40 area, and 251 bulk samples were collected in the shipbuilding industry. From 1984 to 1989, personal air samples with detectable asbestos concentrations (i.e., excluding ND) ranged from 0.01 to 8.99 f/cc ($n = 25$) and in the 1990s, personal air samples ranged from 0.05 to 0.26 f/cc ($n = 5$). Individual samples collected in the shipbuilding industry are identified in Fig. 6. None of the air sampling data

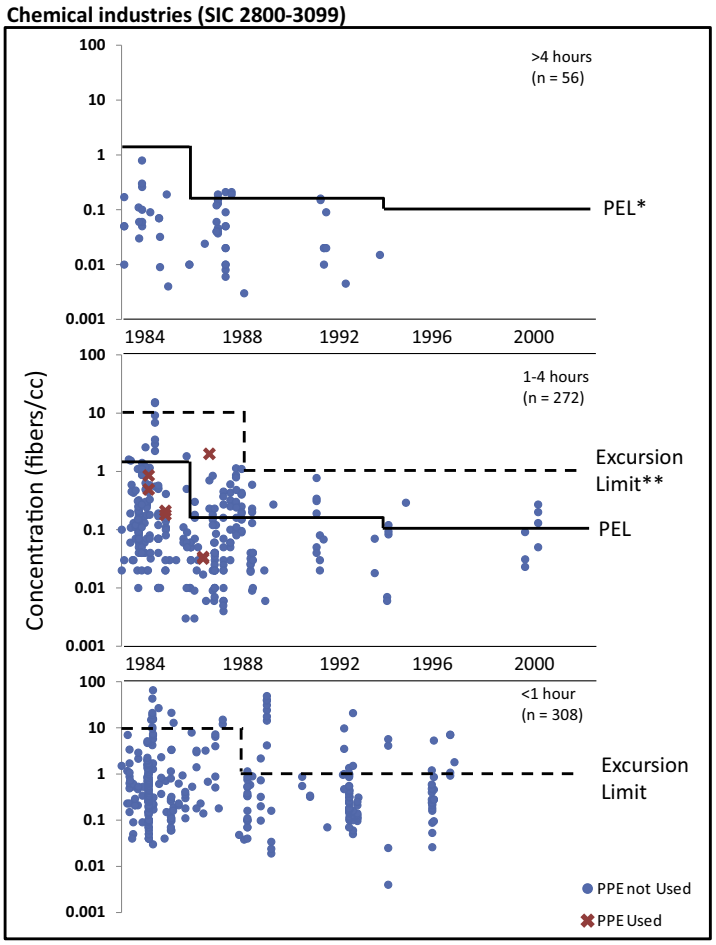



**Fig. 7.** Airborne fiber concentrations for <1, 1–4, and >4 h with and without information related to PPE compared with exposure guidelines over time. *PEL: permissible exposure limit based on 8-h time-weighted average. **Excursion limit: short-term permissible exposure limit based on average over 30 min.

($n = 137$) contained corresponding information related to PPE or respiratory protection.

Samples collected in the automobile industry (sales, service, and repair) decreased throughout the 1980s until 1994, when the last OSHA compliance sample was collected. In the 1990s, a total of 172 personal air samples were collected, and of those 170 samples were non-detectable for asbestos. The two personal samples, 0.03 and 0.056 f/cc, collected in the 1990s were well below the contemporaneous occupational exposure limits (Table 2, Fig. 8). Bulk sampling analysis in this industry yielded asbestos concentrations ranging from 0% to 100%; however, information related to the specific product type that was analyzed for asbestos content was rarely provided in the database (i.e., 7 samples included information on product type). A decrease in the number of samples collected in the combined databases was observed for the chemicals, petroleum, and rubber industries, where the last samples were collected in 1996. These findings are consistent with other research that noted significant decreases in asbestos concentrations in the U.S. automobile repair, pulp and paper, and petroleum industries from the 1970s to the 1980s (Coble et al., 2001; Creely et al., 2007; Paustenbach et al., 2003; Williams et al., 2007). Similar results have been noted in German workplaces where occupational exposures to asbestos have decreased from the 1950s to 1990s, due to rapidly declining industrial use of asbestos and additional regulatory oversight (Hagemeyer et al., 2006).

## 4. Discussion

OSHA sampling data have been used in the past to evaluate historical occupational exposures to various regulated physical and chemical agents (Froines et al., 1989; Froines et al., 1986b; Henneberger et al., 2004; Melville, 2001; Middendorf, 2004). Only recently has the data from both CEHD and IMIS been evaluated by linking the two databases using common variables (Finger and Gamper-Rabindran, 2013; Lavoue et al., 2013). Specifically, Lavoue et al. (Lavoue et al., 2013) noted the value of a comprehensive resource for occupational exposure data created by joining the two complementary datasets, while simultaneously highlighting the challenges posed by inherent bias potential associated with OSHA compliance data. This evaluation expanded on the analysis performed by Lavoue et al. to critically evaluate historical occupational asbestos exposure data across industries and time periods, with emphasis on sample duration and compliance with OSHA regulatory guidelines.

Based on this analysis, it is clear that the combined data from CEHD and IMIS can be useful for retrospective exposure

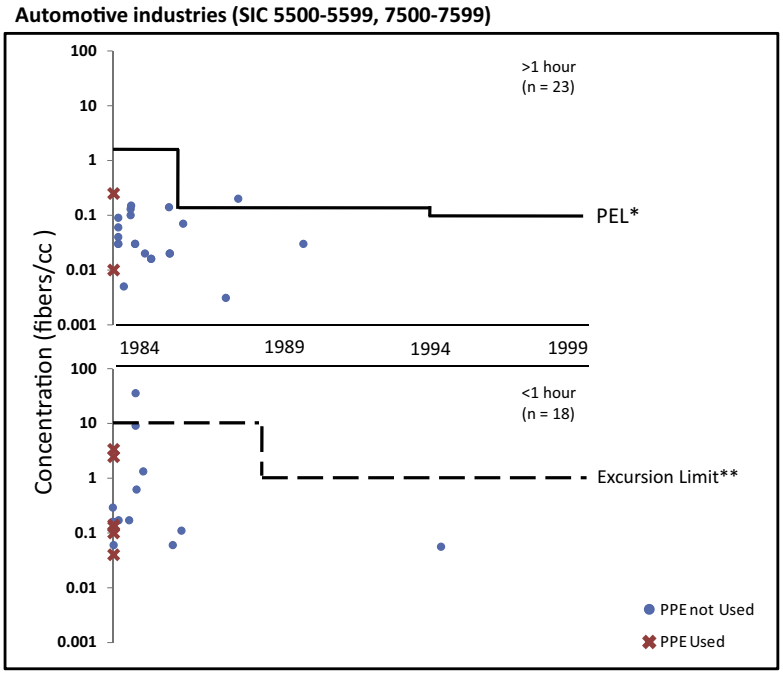



**Fig. 8.** Short- and long-term airborne fiber concentrations with and without information related to PPE compared with exposure guidelines over time. *PEL: permissible exposure limit based on 8-h time-weighted average. **Excursion limit: short-term permissible exposure limit based on average over 30 min.

assessments and for identifying temporal and specific industry trends. The CEHD accounts for a significant source of sampling data collected by OSHA. However, it is unclear how many of those samples would overlap with those from IMIS for the other substances evaluated by OSHA inspectors. Therefore, the value of combining the two datasets for the purpose of retrospective exposure assessment depends greatly on the target substance and information available in the IMIS and CEHD databases. Initially, this analysis aimed to combine the datasets to obtain more information about historical asbestos exposures, but since approximately 40% of the CEHD was represented in the IMIS, there was limited ability to accomplish that objective. Those interested in utilizing these two databases should consider their individual objectives before determining whether the combined dataset is worthwhile; in some cases the CEHD database alone may be sufficient. Also, it is unclear whether other chemicals contain more IMIS samples with more information than was available for asbestos. A recent analysis of OSHA sampling data related to lead found that only 39% of IMIS samples corresponded with CEHD samples, which was similar to the finding in this analysis (Lavoue et al., 2013).

U.S. government data clearly indicate that asbestos consumption, and the number of compliance samples obtained by OSHA decreased from 1984 to 2011 (USGS (U.S. Geological Survey), 2013). The corresponding decreasing trend in the number of samples collected by OSHA may reflect (1) a decrease in consumption of asbestos by manufacturing entities, (2) decreased removal of existing asbestos-containing materials; or (3) safer methods (e.g., wet or vacuuming methods) for removal of existing asbestos-containing material yielded less airborne asbestos fibers. When considered in combination with the declining incidence of mesothelioma across the U.S., the data appear to indicate that asbestos exposures have decreased over time and that the decreasing number of samples collected is a reflection of reduced exposure potential and increased control of workplace asbestos exposures overall (Weill et al., 2004).

Unfortunately, the relationship between PPE use and airborne asbestos concentrations as measured by OSHA from the combined dataset is unclear. According to the CEHD and IMIS data, airborne fiber concentrations exceeded contemporaneous exposure guidelines in certain industries by several orders of magnitude in the 1980s and 1990s; whether PPE was worn systematically in these environments is unknown and is a critical piece of information to properly evaluate exposure potential. Despite the fact that airborne concentrations as measured by OSHA decreased significantly after 1985, as of 2010, the OSHA asbestos sampling data indicated that exposures in certain industries (e.g., construction) still continue to exceed current occupational exposure guidelines. Therefore, exposures in excess of the OSHA regulatory limits may still be present in major industries such as construction and manufacturing and may present a public health challenge for those working in those industries when proper PPE is not implemented, as required by Federal Regulations (Maylie et al., 2004). According to NIOSH, asbestosis-related mortality has continued to increase from 77 in 1968 to 1265 in 1999 suggesting that asbestos exposure continues to be an occupational health concern (NIOSH (National Institute for Occupational Safety and Health), 2004).

Sampling duration was highly variable in this database and in many cases it was difficult to compare historical asbestos concentrations with the contemporaneous occupational exposure limits. However, Williams and colleagues (2007) noted that rough approximations could be made and compared with exposure limits as if the samples were representative of 8-h time weighted averages. An analysis using this methodology showed that, during the 1980s and 1990s, asbestos concentrations exceeded 10 f/cc and in some cases exceeded 50 f/cc (sample duration <4 h) in the construction and manufacturing industries (Table 2). An analysis of sample duration indicated that concentration was significantly associated with sample duration, suggesting that task-based samples were obtained by OSHA inspectors. However, in some cases, high concentrations were also observed in long-term samples

(>1 h); indeed, concentrations approaching 10 f/cc were observed as late as 2005. Additionally, there appeared to be very high exposure potential in the 1980s and 1990s in environments where workers were exposed to short- and long-term asbestos exposures exceeding 10 f/cc though there was limited or no PPE usage information.

There are limitations to using OSHA compliance sampling data to evaluate the true exposure potential of workers to asbestos or other chronic disease agents over time. First, the OSHA compliance measurements do not represent random sampling of workplace conditions, nor do they represent an industrial hygiene risk management strategy, limiting the generalizability of these data (Coble et al., 2001; Gomez, 1993; Henneberger et al., 2004). OSHA compliance samples are collected for different purposes than those samples collected for the purposes of exposure and risk management. Hewett (2001) pointed out that these two different goals (compliance vs. risk management) are often misinterpreted or misused. The OSHA compliance sample is simply a snapshot in time, providing a yes or no answer to the question of whether a specific worker exposure scenario is in compliance with the regulatory standards over an 8-h period of time (Leidel, 1977; Tuggle, 1981). The proper interpretation of these single-shift sampling data points is very important in the context of evaluating true exposures to workers. For example, a number of researchers over time have misinterpreted the OSHA PELs to be representative of upper exposure limits that can be applied using a long-term average, such as weeks, months or years, when in fact, they refer very specifically to daily occupational exposures only (Corn et al., 1994; Hewett, 2001; Rappaport, 1984). Use of a long-term averaging approach (i.e., allowing for occasional daily exposures above the PEL as long as the long-term average exposure remains below the PEL) can be problematic because it can have the effect of allowing the PEL to drift upwards from its original established value and therefore provide less protection for workers (Hewett, 2001). Such statistical manipulations of the sampling data should not be considered reliable for accurately characterizing worker exposures and compliance over time.

A valid exposure assessment and risk management sampling program for asbestos or other chronic disease agents should consider bias and variability in the samples collected. The potential for sampling bias in the OSHA data is strong, since the data were often obtained in response to safety referrals and complaints. These data can therefore disproportionately represent high exposure scenarios (Froines et al., 1986a). Such a sampling strategy, while valid from a compliance standpoint, limits the representativeness of exposure estimates derived using these data (Coble et al., 2001; Henneberger et al., 2004; Lavoue et al., 2013). Further, the known variability in occupational exposures (believed by the AIHA to be in the range of 2–3 standard deviations for moderate variability in typical occupational exposure samples) is not considered in the OSHA sampling strategy, meaning that exposures can also be underestimated by an unknown amount using this strategy (Ignacio and Bullock, 2006; Tuggle, 1981). However, as Gomez (1993) noted, the compliance sampling strategy can still be of value for its emphasis on "groups of similarly exposed workers at the high end of the exposure distribution, in contrast to randomly collected samples, which would also measure the exposure of many job titles with little exposure potential" (Olsen et al., 1991). Despite the potential limitations of interpreting OSHA compliance data, these asbestos-specific samples had never been previously compiled and mined to examine industry trends. This information is valuable for examining time and industry trends, in addition to noting specific industries where asbestos exposure may be an ongoing concern.

A second important limitation of this data set is the error rate of data entry. Occasionally, it was necessary during this analysis to decipher data based on other entered variables including units and sample type. It has been previously noted that OSHA compliance officers are extremely variable in terms of sampling methodology, which potentially inserts another source of bias (Froines et al., 1989; Gomez, 1993). A shortcoming of the IMIS database is that job titles are entered in free-text form instead of relying on a more uniform standard such as codes. Although additional details including task or method descriptions are noted in the original inspection records, this information is not reflected in the IMIS database (Gomez, 1993).

A third limitation of the OSHA dataset is that asbestos air samples were analyzed by PCM and therefore fiber type cannot be determined from these samples alone. Using PCM data as a proxy for asbestos fiber exposure can overestimate asbestos-specific exposure potential, at times substantially (Sahmel et al., 2014). Fiber type analyses are not considered when determining compliance with OSHA asbestos standards because the regulatory guidelines do not differentiate between fibers. It is also believed that the occupational environments sampled by OSHA did contain high levels of asbestos, and in most cases bulk sampling was conducted concomitantly with air sampling to verify the presence of asbestos in the work environment (Howitt et al., 1993; OSHA, 2012a). It appears that often times bulk samples were also collected during compliance inspections; however, fiber type was not included in either the CEHD or IMIS database. Although this information is not available in OSHA databases, additional insight can be gleaned from an analysis of the use of different fiber types in the various industry groups.

A fourth limitation of the dataset is that the SIC codes described job industries, not actual job type. For example, if a worker performed asbestos abatement work at a construction site, that work would get categorized as construction and not asbestos abatement. This omission makes separating general construction work from asbestos abatement tasks difficult. Also, SIC code designations were not originally designed to classify industries with similar occupational exposure potential (Linch et al., 1998). By the 1990s, most users of SIC codes believed the system was outdated and not reflective of industry in the U.S., which led to the development of NAICS codes (Ambler and Kristoff, 1998). Unfortunately, not all samples included in our combined dataset included both NAICS and SIC codes, and it was therefore not possible to further evaluate NAICS codes. However, for our purposes, the SIC codes appeared to adequately describe the industries evaluated.

A fifth limitation of the present study was the data lacking limits of detection for non-detected samples. There are multiple methods that could have been performed to address non-detected samples if limits of detection were reported, but the exclusion of this parameter forced us to analyze only the trends with the detected samples. We decided that this course would provide more useful information than what would have been provided if we assumed that non-detected samples had concentrations of zero. As such, readers should understand that the reported concentrations are only representative of detected samples.

A sixth and final limitation of the dataset was the lack of information available on worker PPE use. Although some sampling points included information related to PPE, many did not. This information is critical to understanding whether workers in the construction industry were indeed exposed, for example, to concentrations exceeding 50 f/cc in the 1980s. Unfortunately, only 15% of samples collected in the construction industries indicated whether or not PPE was used.

The primary objective of this study was to combine the CEHD and IMIS databases in order to create a comprehensive database of OSHA asbestos sampling data, with the goal of more accurately understanding historical trends in OSHA sampling events and their results over time and across industries. A secondary goal of the

study was to qualitatively compare airborne fiber concentrations with information related to products, administrative controls, PPE, and task descriptions. Unfortunately, the two databases only partially overlapped and for data that were overlapping, incomplete data entry often made any use of important qualitative data (i.e., task, PPE, engineering controls) relatively unhelpful. When examining the combined data, only 397 samples out of 29,212 (1.4%) included a qualifier indicating that abatement activities were performed during sampling. This lack of corresponding information between the two databases significantly affected the ability to evaluate the available sample results in terms of risk to workers because an analysis of PPE use could not be coupled with airborne concentrations.

A combined and comprehensive repository of historical occupational exposure data collected by OSHA currently does not exist. Such a comprehensive combined dataset could potentially be used to identify historical trends of occupational exposure among and between industries and sources over time, and potentially lead to a better understanding of how industries may improve similar exposure scenarios. Again, less than 50% of the quantitative and qualitative data contained within the IMIS and CEHD databases overlap, though the dataset is often incomplete and wrought with errors and omissions. Therefore, to fully maximize the utility and value of information offered by combining the databases, we present the following recommendations to enhance future use of newly collected data for OSHA to consider:

- The CEHD and IMIS databases are compiled and maintained by separate entities and are not directly linked or accessible on the OSHA website. In fact, the IMIS is only available through a FOIA request, whereas the CEHD is downloadable from the OSHA website. We recommend that these databases be linked in a more streamlined fashion or be integrated into one single database containing both quantitative and qualitative data.
- Provide a more standardized data entry process for inspectors with the use of drop-down menus and pop-up windows to ensure that all necessary fields are available. This addition would be valuable to prevent data entry errors, as well as data omissions (e.g., missing PPE information).
- As with any data set, a comprehensive quality control procedure should be integrated to prevent errors and omissions.
- Because SIC and NAICS codes describe particular industries rather than task or job type, a more standardized categorization method should be developed to capture specific job duties and tasks. For example, an administrative professional employed by a specific industry would certainly have different tasks than a skilled craftsman: this distinction is not currently captured in either the IMIS or CEHD data.
- As a standard methodology, the limits of detection for each laboratory analytical procedure should be integrated into the data so that censored data analysis is possible.
- A key variable for any exposure assessment is the use of respiratory protection or personal protective equipment. Currently, the IMIS dataset has a column for PPE, which is either affirmative (e.g., x for affirmative) or left blank, which the data user is left to interpret as either no PPE or no data. OSHA should consider incorporating a drop down menu indicating whether respiratory protection is used by the worker and the type of respirator donned by the worker.

## 5. Conclusions

A decreasing trend over the past 30 years in both airborne fiber concentrations and numbers of asbestos samples collected parallels available data regarding reductions in the importation and consumption of asbestos.

Airborne fiber exposure potential was very high (>10 f/cc for short and long-term samples) in some industries (e.g., construction, manufacturing). Despite that airborne concentrations have significantly declined over the past 30 years, for some industries, airborne fiber samples collected by OSHA exceeded the current PEL on occasion as late as 2011.

The CEHD and IMIS datasets, while not complete and not fully overlapping, provide insight into the characteristics of historical exposure sampling for asbestos, and this exercise may be valuable for those interested in historical exposure assessment, including questions specific to time period and industry.

A more comprehensive OSHA database combining CEHD and IMIS data would be useful for retrospective occupational exposure reconstructions, as well as for identification of trends to prevent future occupational exposures.

## Conflict of interest statement

The authors report no conflicts of interest. Funding for this manuscript was provided entirely by Cardno ChemRisk, a consulting firm that provides scientific advice to the government, corporations, law firms, and various scientific/professional organizations. Two of the authors (AKM, JS) have served as expert witnesses in litigation regarding historical exposures of various tradesmen to asbestos.

## Transparency Document

The Transparency document associated with this article can be found in the online version.

## References

Ambler, C.A., Kristoff, J.E., 1998. Introducing the North American industry classification system. Government Inf. Q. 15, 263–273.
Balzer, J.L., Cooper, W.C., 1968. The work environment of insulating workers. Am. Ind. Hyg. Assoc. J. 29, 222–227.
Coble, J.B. et al., 2001. Time trends in exposure measurements from OSHA compliance inspections of the pulp and paper industry. Appl. Occup. Environ. Hyg. 16, 263–270.
Cooper, W., Balzer, J. 1968. Evaluation and control of asbestos exposures in the insulating trade. 2nd International Conference on Biological Effects of Asbestos, Dresden.
Corn, M., McArthur, Bill, Dellarco, Michael, 1994. Asbestos exposures of building maintenance personnel. Appl. Occup. Environ. Hyg. 9, 845–852.
CPSC (Consumer Product Safety Commission). 1977a, Ban of Artifical Emberizing Materials (Ash and Embers) Containing Respirable Free-Form Asbestos, Code of Federal Regulations Title 16, Part 1305. 1977. pp. 428–430.
CPSC (Consumer Product Safety Commission). 1977b. Ban of Consumer Patching Compounds Containing Respirable Free-Form Asbestos, *Code of Federal Regulations* Title 16, Part 1304. 1977. pp. 425–428.
Creely, K.S. et al., 2007. Trends in inhalation exposure–a review of the data in the published scientific literature. Ann. Occup. Hyg. 51, 665–678.
Finger, S.R., Gamper-Rabindran, S., 2013. Mandatory disclosure of plant emissions into the environment and worker chemical exposure inside plants. Ecol. Econ. 87, 124–136.
Froines, J.R. et al., 1986a. Occupational health surveillance: a means to identify work-related risks. Am. J. Public Health 76, 1089–1096.
Froines, J.R. et al., 1986b. An approach to the characterization of silica exposure in U.S. industry. Am. J. Ind. Med. 10, 345–361.
Froines, J. et al., 1989. Hazard surveillance in occupational disease. Am. J. Public Health 79 (Suppl), 26–31.
Giannasi, F., 2007. Ban on asbestos diaphragms in the chlorine-related chemical industry and efforts toward a worldwide ban. Int. J. Occup. Environ. Health 13, 80–84.
Gomez, M.R., 1993. A proposal to develop a national occupational exposure databank. Appl. Occup. Environ. Hyg., 768–774
Hagemeyer, O. et al., 2006. Asbestos consumption, asbestos exposure and asbestos-related occupational diseases in Germany. Int. Arch. Occup. Environ. Health 79, 613–620.
Henneberger, P.K. et al., 2004. Industries in the United States with airborne beryllium exposure and estimates of the number of current workers potentially exposed. J. Occup. Environ. Hyg. 1, 648–659.

Hewett, P., 2001. Misinterpretation and misuse of exposure limits. Appl. Occup. Environ. Hyg. 16, 251–256.
Howitt, D.G. et al., 1993. The difficulties with low-level asbestos exposure assessments in public, commercial, and industrial buildings. Am. Ind. Hyg. Assoc. J. 54, 267–271.
Howlader, N., Noone A.M., Krapcho, M., Garshell, J., Neyman, N., Altekruse, S.F., Kosary, C.L., Yu, M., Ruhl, J., Tatalovich, Z., Cho, H., Mariotto, A., Lewis, D.R., Chen, H.S., Feuer, E.J., Cronin, K.A. 2013. SEER Cancer Statistics Review 1975–2010. In: Institute, N. C., (Ed.), Bethesda.
Ignacio, J.S., Bullock, W.H., 2006. A strategy for assessing and managing occupational exposures. American Industrial Hygiene Association Press, Fairfax, VA.
Lavoue, J. et al., 2013. Workplace measurements by the U.S. Occupational Safety and Health Administration since 1979: descriptive analysis and potential uses for exposure assessment. Ann. Occup. Hyg. 57, 681–683.
Leidel, N.A., Kenneth, A. Busch, Jeremiah, R. 1977. Lynch Occupational Exposure Sampling Strategy Manual. In: U.S. Department of Health, E., and Welfare (Ed.). National Institute for Occupational Safety and Health.
Linch, K.D. et al., 1998. Surveillance of respirable crystalline silica dust using OSHA compliance data (1979–1995). Am. J. Ind. Med. 34, 547–558.
Maylie, J. et al., 2004. Small conductance $Ca^{2+}$-activated $K^{+}$ channels and calmodulin. J. Physiol. 554, 255–261.
Melville, R.a.M.L., 2001. Influence of data elements in OSHA air sampling database on occupational exposure levels. Appl. Occup. Environ. Hyg. 16, 884–899.
Middendorf, P.J., 2004. Surveillance of occupational noise exposures using OSHA's Integrated Management Information System. Am. J. Ind. Med. 46, 492–504.
NIOSH (National Institute for Occupational Safety and Health), Worker Health Charbook. 2004. NIOSH, Cincinnati, OH, 2004.
Olsen, E. et al., 1991. Bias and random errors in historical data of exposure to organic solvents. Am. Ind. Hyg. Assoc. J. 52, 204–211.
OSHA. 2012a. Chemical Exposure Health Data, Occupational Safety and Health Administration.
Paustenbach, D.J. et al., 2003. An evaluation of the historical exposures of mechanics to asbestos in brake dust. Appl. Occup. Environ. Hyg. 18, 786–804.
Price, B., Ware, A., 2004. Mesothelioma trends in the United States: an update based on surveillance, epidemiology, and end results program data for 1973 through 2003. Am. J. Epidemiol. 159, 107–112.
Rappaport, S.M., 1984. The rules of the game: an analysis of OSHA's enforcement strategy. Am. J. Ind. Med. 6, 291–303.
Reitze, W.B. et al., 1972. Application of sprayed inorganic fiber containing asbestos: occupational health hazards. Am. Ind. Hyg. Assoc. J. 33, 178–191.
Sahmel, J., Barlow, C.A., Simmons, B., Gaffney, S.H., Avens, H.J., Madl, A.K., Henshaw, J., Lee, R.J., Van Orden, D., Sanchez, M., Zock, M., Paustenbach, D.J., 2014. Evaluation of take-home exposure and risk associated with the handling of clothing contaminated with chrysotile asbestos. Risk Anal. 34 (8), 1448–1468.
Sprince, N.L. et al., 1985. Asbestos-related disease in plumbers and pipefitters employed in building construction. J. Occup. Med. 27, 771–775.
Tuggle, R.M., 1981. The NIOSH decision scheme. Am. Ind. Hyg. Assoc. 42, 493–498.
USGS (U.S. Geological Survey). 2013. Mineral Commodity Summaries, 2013. U.S. Geological Survey, Reston, VA.
Virta, R. L. 2006. Worldwide Asbestos Supply and Consumption Trends from 1900 through 2003, U.S. Geological Survey, Reston, VA.
Virta, R. L. 2011. Asbestos. In: USGS (U.S. Geological Survey), (Ed.), 2011 Minerals Yearbook: Asbestos [Advance Release], August 2012. U.S. Geological Survey, Reston, VA, pp. 8.1–8.6.
Weil, D., 1996. If OSHA is so bad, why is compliance so good? RAND J. Econ. 27, 618–640.
Weill, H. et al., 2004. Changing trends in US mesothelioma incidence. Occup. Environ. Med. 61, 438–441.
Williams, P. et al., 2007. Retrospective exposure assessment of airborne asbestos related to skilled craftsmen at a petroleum refinery in Beaumont, Texas (1940–2006). J. Toxicol. Environ. Health A 70, 1076–1107.

STUDY No. 11

# DOMESTIC ASBESTOS EXPOSURE: A REVIEW OF EPIDEMIOLOGIC AND EXPOSURE DATA

**STUDY No. 11**

# DOMESTIC ASBESTOS EXPOSURE: A REVIEW OF EPIDEMIOLOGIC AND EXPOSURE DATA

**International Journal of Environmental Research and Public Health – November 2013**

**Emily Goswami, Valerie Craven, David L. Dahlstrom, Dominik Alexander, and Fionna Mowat**

Inhalation of asbestos resulting from living with and handling the clothing of workers directly exposed to asbestos has been established as a possible contributor to disease. This review evaluates epidemiologic studies of asbestos-related disease or conditions (mesothelioma, lung cancer, and pleural and interstitial abnormalities) among domestically exposed individuals and exposure studies that provide either direct exposure measurements or surrogate measures of asbestos exposure. A meta-analysis of studies providing relative risk estimates (n = 12) of mesothelioma was performed, resulting in a summary relative risk estimate (SRRE) of 5.02 (95% confidence interval [CI]: 2.48 – 10.13). This SRRE pertains to persons domestically exposed via workers involved in occupations with a traditionally high risk of disease from exposure to asbestos (i.e., asbestos product manufacturing workers, insulators, shipyard workers, and asbestos miners). The epidemiologic studies also show an elevated risk of interstitial, but more likely pleural, abnormalities (n = 6), though only half accounted for confounding exposures. The studies are limited with regard to lung cancer (n = 2). Several exposure-related studies describe results from airborne samples collected within the home (n = 3), during laundering of contaminated clothing (n = 1) or controlled exposure simulations (n = 5) of domestic exposures, the latter of which were generally associated with low-level chrysotile-exposed workers. Lung burden studies (n = 6) were also evaluated as a surrogate of exposure. In general, available results for domestic exposures are lower than the workers' exposures. Recent simulations of low-level chrysotile-exposed workers indicate asbestos levels commensurate with background concentrations in those exposed domestically.

# Domestic Asbestos Exposure: A Review of Epidemiologic and Exposure Data

**ARTICLE** *in* INTERNATIONAL JOURNAL OF ENVIRONMENTAL RESEARCH AND PUBLIC HEALTH · NOVEMBER 2013

Impact Factor: 2.06 · DOI: 10.3390/ijerph10115629 · Source: PubMed

*Int. J. Environ. Res. Public Health* **2013**, *10*, 5629-5670; doi:10.3390/ijerph10115629

OPEN ACCESS

International Journal of
**Environmental Research and Public Health**
**ISSN 1660-4601**
www.mdpi.com/journal/ijerph

*Review*

# Domestic Asbestos Exposure: A Review of Epidemiologic and Exposure Data

**Emily Goswami [1,*], Valerie Craven [1,†], David L. Dahlstrom [2,†], Dominik Alexander [3,†] and Fionna Mowat [4,†]**

[1] Exponent, 475 14th Street, Suite 400, Oakland, CA 94612, USA; E-Mail: cravenv@exponent.com
[2] New Era Sciences, LLC, Issaquah, WA 98027, USA; E-Mail: dldahlstrom@nes2013.com
[3] Exponent, 2595 Canyon Boulevard, Suite 440, Boulder, CO 80303, USA; E-Mail: dalexander@exponent.com
[4] Exponent, 149 Commonwealth Drive, Menlo Park, CA 94025, USA; E-Mail: fmowat@exponent.com

† These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: egoswami@exponent.com; Tel.: +1-510-268-5032; Fax: +1-510-268-5099.

*Received: 17 August 2013; in revised form: 18 October 2013 / Accepted: 22 October 2013 / Published: 31 October 2013*

---

**Abstract:** Inhalation of asbestos resulting from living with and handling the clothing of workers directly exposed to asbestos has been established as a possible contributor to disease. This review evaluates epidemiologic studies of asbestos-related disease or conditions (mesothelioma, lung cancer, and pleural and interstitial abnormalities) among domestically exposed individuals and exposure studies that provide either direct exposure measurements or surrogate measures of asbestos exposure. A meta-analysis of studies providing relative risk estimates (n = 12) of mesothelioma was performed, resulting in a summary relative risk estimate (SRRE) of 5.02 (95% confidence interval [CI]: 2.48–10.13). This SRRE pertains to persons domestically exposed via workers involved in occupations with a traditionally high risk of disease from exposure to asbestos (*i.e.*, asbestos product manufacturing workers, insulators, shipyard workers, and asbestos miners). The epidemiologic studies also show an elevated risk of interstitial, but more likely pleural, abnormalities (n = 6), though only half accounted for confounding exposures. The studies are limited with regard to lung cancer (n = 2). Several exposure-related

*Int. J. Environ. Res. Public Health* **2013**, *10* **5630**

studies describe results from airborne samples collected within the home (n = 3), during laundering of contaminated clothing (n = 1) or in controlled exposure simulations (n = 5) of domestic exposures, the latter of which were generally associated with low-level chrysotile-exposed workers. Lung burden studies (n = 6) were also evaluated as a surrogate of exposure. In general, available results for domestic exposures are lower than the workers' exposures. Recent simulations of low-level chrysotile-exposed workers indicate asbestos levels commensurate with background concentrations in those exposed domestically.

**Keywords:** domestic; exposure; epidemiology; asbestos fibers; take-home

## 1. Introduction

The potential exposure scenarios for individuals who are non-occupationally exposed to asbestos vary, but may include: (1) neighborhood exposure due to asbestos emissions from nearby asbestos-product manufacturing facilities, asbestos mines, construction work involving asbestos, or naturally occurring asbestos; (2) household exposure from the use of asbestos-containing materials (e.g., use of tremolite/erionite whitewash on the exterior of homes); and (3) household contamination resulting from asbestos fibers brought into the home on workers' clothing or bodies, and domestic activities such as handling or laundering workers' contaminated clothing. In this review, we discuss the third scenario, which can be referred to as secondary, para-occupational or take-home exposure, and herein is termed "domestic exposure".

*Early Reports of Domestic Exposure*

In 1960, a seminal case series reported by Wagner [1] was not only one of the first to associate asbestos exposure, specifically to crocidolite, with the development of malignant pleural mesothelioma in 33 persons, but was also the first to identify exposure pathways via non-occupational domestic and neighborhood asbestos exposure. Wagner's study was followed shortly by a case-control study by Newhouse and Thompson [2,3], which identified seven cases of pleural mesothelioma and two cases of peritoneal mesothelioma in patients whose relatives worked with asbestos, including chrysotile, amosite, and crocidolite. These workers' occupations included spinners, an engine-room worker, a boiler coverer, an asbestos factory foreman, a docker handling asbestos cargo, a railway carriage builder, and an asbestos factory worker. Later studies involving domestically exposed persons followed (e.g., [4,5]).

The review of indirect exposures in bystanders in the workplace and at home by Grandjean and Bach [6] provided a relatively early evaluation of indirect exposures to lead, beryllium, asbestos, and other substances, including bystander exposures and exposure to substances carried home from work by family members. The authors addressed early case reports, case series, and cross-sectional studies that documented cases of mesothelioma, lung cancer, asbestosis, and pleural plaques in persons believed to be exposed domestically through family members who worked mostly in shipyards or asbestos factories. No specific data on the number of persons included in their evaluation were provided, nor was information on fiber type provided.

In conjunction with the Workers' Family Protection Act of 1992, the U.S. National Institute for Occupational Safety and Health (NIOSH) produced the Report to Congress on Workers' Home Contamination Study Conducted under the Workers' Family Protection Act [7]. The authors of this report evaluated "the potential for, prevalence of, and issues related to the contamination of workers' homes with hazardous chemicals and substances...transported from the workplaces of such workers". In their report, NIOSH indicated that they included four cohort studies, one community study, seven case-control studies, and "numerous" case reports and case series. This report is the most comprehensive by NIOSH to date on this topic—it provides a summary of cohort studies, case-control studies, case reports, and case series, as well as an overview of studies that describe contaminated clothing. The report concluded that domestic asbestos exposures may pose an increased risk of disease, but did not provide analyses regarding the type of exposure (including fiber type), level, frequency, or duration needed to produce disease. As a follow-up to this report, NIOSH published a research agenda focused on protecting workers' families [8]. This agenda included characterizing the extent of home contamination, identifying populations at greatest risk of known and suspected take-home exposures, assessing the adverse health effects from take-home exposures, and assessing the effectiveness of prevention and remediation methods. To date, NIOSH has not published any results from this agenda.

An often-cited French report by the National Institute of Health and Medical Research (INSERM) [9] concluded that the risk of mesothelioma in persons exposed in a non-occupational and domestic setting was "established" in the literature, indicating that the source of the asbestos was typically dirty work clothes; however, this report does not provide any quantitative domestic exposure estimates, and it specifically states that good exposure data do not exist in the literature to feasibly evaluate the extent of domestic exposures. In addition, the authors lump non-occupational (termed "para-occupational" by the original authors) exposures together, which include domestic and direct exposures from the use of home products potentially containing asbestos (e.g., ironing boards and insulating gloves), thereby making it difficult to understand those exposures that resulted solely from domestic exposure. No specific data on the number of persons included in their evaluation were provided.

In 2000, Bourdès *et al.* [10] conducted a study focused solely on pleural mesothelioma based on five published studies, and the reported meta-relative risk included a study of household use of asbestos (e.g., whitewash, stucco) in Turkey in which 23 mesotheliomas were reported; however, a number of studies have been published since its culmination date of 1998. Three of the remaining four included studies provided information on the number of domestically-exposed persons with mesothelioma, reporting on a combined 21 cases of mesothelioma; the fifth included study did not provide information by exposure type, but noted that 17 persons (9%) likely or possibly had para-occupational exposure. In all but one of the included studies, the exposure was to amosite or mixed fibers (*i.e.*, amphibole and chrysotile fibers). Bourdès *et al.* also presented a review of non-occupational exposure measurements, which largely included environmental exposures (ambient exposures due to nearby sources) and indoor exposures due to specific asbestos-containing products used in the home or business (e.g., schools with sprayed asbestos, use of asbestos-containing whitewash in the home).

The purpose of our current paper has two specific aims: (1) to provide an up-to-date and comprehensive review of epidemiologic (cohort, case-control, and case reports and series) and exposure data regarding domestic exposure and mesothelioma, lung cancer, and interstitial and pleural

abnormalities and (2) to conduct a quantitative assessment using a meta-analysis approach to estimate the risk of mesothelioma among individuals domestically exposed *vs.* those not exposed. The issue of domestic exposures remains an important question because of potential ongoing uses of potentially hazardous materials. For asbestos, the issue becomes important because of ongoing litigation matters and the need to understand historical exposures to asbestos and the associations with asbestos-related diseases. During the time of writing this paper, another paper has been published which also provides a review of epidemiologic and exposure data regarding domestic exposures [11]; however, this paper does not provide a meta-analysis or quantitative evaluation of risk, excludes several studies that are included in the present paper, and uses different methods of evaluation.

## 2. Methods

The published literature from the 1960s to 2012 was searched using MEDLINE, accessed via PubMed (the U.S. National Library of Medicine). Key words included domestic, household, laundry, para-occupational, or take-home and asbestos (and specific fiber types, including crocidolite, amosite, and chrysotile), mesothelioma, lung cancer, asbestosis, or pleural changes. No specific restrictions were imposed on the literature search, although the review was restricted to the most recent update of a study population. The reference lists of articles were reviewed to identify studies that might not have been detected in the literature search. Each article was reviewed by at least two scientists for inclusion. In an attempt to be as comprehensive as possible, all studies that provided some primary epidemiologic or exposure information were included. Some studies were written in a foreign language; for these, the English abstract was relied upon for relevant information.

### *2.1. Epidemiology Review and Analysis*

Analytical and descriptive epidemiologic studies were considered in the qualitative review, including cohort, case-control, cross-sectional studies, case reports, and case series. The medical conditions of interest were mesothelioma, lung cancer, and interstitial and pleural abnormalities.

In addition to a qualitative review of the published epidemiologic studies, we also performed a quantitative meta-analysis of the studies reporting mesothelioma in domestically exposed persons. Only mesothelioma studies were included in the meta-analysis, because there were too few studies of lung cancer and interstitial and pleural abnormalities to perform meta-analyses for those endpoints. Epidemiologic studies were included in the meta-analysis if the original study reported relative risk estimates, or provided the information necessary to calculate a relative risk estimate, and a measure of variance (e.g., confidence intervals). Random-effects meta-analysis models were used to calculate summary relative risk estimates (SRREs), 95% confidence intervals (CIs), and corresponding *p*-values for heterogeneity (p-H). Statistical significance was identified when the 95% CI did not include 1.0. The random-effects model assumes that the study-specific effect sizes come from a random distribution of effect sizes according to a specific mean and variance. A p-H < 0.1 suggests significant "between-study" statistical variability in a meta-analysis model [12]. The relative risk estimates of the individual studies were weighted based on the inverse of the variance, which is related to the sizes of the study populations. Tests for heterogeneity were conducted, and subgroup analyses (specifically, case-control *vs.* cohort, modification by occupational exposure) were performed to discern any

potential sources of between-study variability. "One study removed" sensitivity analyses were conducted to evaluate the relative influence of each study on the model-specific SRRE. This was performed by generating an SRRE based on all studies in a particular model, followed by the removal of one study at a time to compare the overall SRRE with SRREs from models that had one study removed. Separate models were created to estimate the effects of occupational *vs.* neighborhood exposures. Potential confounding from occupational or neighborhood exposures was assessed by the methods described in each paper, as well as suggestions from the original authors' discussion of limitations. Analyses were conducted using Comprehensive Meta-Analysis (version 2.2.045; Biostat, Englewood, NJ, USA), STATA (version 1.0; StataCorp, College Station, TX, USA), and Episheet [13].

### *2.2. Exposure Review*

The exposure studies reviewed included a variety of study types that provided some direct asbestos exposure data or surrogate of asbestos exposure, and were categorized into four distinct groups: (1) studies describing results of airborne or settled dust samples collected within the homes of domestically exposed persons, (2) studies describing exposures during laundering or other handling of contaminated clothing, (3) studies describing controlled simulations of take-home exposures, and (4) lung burden studies. Due to the different in potency among asbestos fiber types, wherever possible, the type of asbestos fiber from which the exposure occurred is noted.

## 3. Results

In total 143 published articles were identified for inclusion in the review, and of these, 108 were evaluated for relevant information. Many of the studies were subsequently excluded after initial review. Specific reasons for exclusion included lack of quantitative data regarding risk and/or exposure (e.g., review articles with no original data) and studies that did not report specifically on domestically-exposed persons or that lumped those domestically-exposed with other types of asbestos exposures. The remaining articles are discussed below. Wherever possible, the asbestos fiber type to which the population was exposed was reported (Tables 1, 2 and 3).

### *3.1. Review of Domestic Epidemiologic Studies*

Studies of mesothelioma, lung cancer, and pleural and interstitial abnormalities with information regarding domestic exposure are discussed below by disease type.

#### 3.1.1. Mesothelioma

Table 1 provides a list of 32 case reports and case series of mesothelioma in asbestos-exposed domestic populations, beginning with Wagner and colleagues' case series of pleural mesothelioma in 1960. The case reports and series are provided for comprehensiveness, not to address the question of whether or not there is an association between domestic exposure to asbestos and mesothelioma, or the magnitude of that association.

**Table 1.** Case reports and series of mesothelioma in domestically exposed populations.

| Author and Year | Population Studied | Occupation of Worker(s) | Results | Exposure Information |
|---|---|---|---|---|
| Wagner *et al.* 1960 [1] | 33 Pleural mesothelioma cases in Northwest Cape Province (South Africa) | Crocidolite miners | 25/33 cases had non-occupational exposure (76%). | Nearly exclusively neighborhood exposure |
| Lieben & Pistawka 1967 [14] | 42 Pleural and peritoneal mesothelioma cases in southeast Pennsylvania | Insulation plant workers | 3/42 (7%) cases had domestic exposure: 2 were daughters of insulation plant workers; 1 mother of two insulation plant workers | Amosite and chrysotile |
| Rusby 1968 [15] | Pleural mesothelioma in mother of factory workers | Asbestos factory workers | Mother of 3 daughters who worked in an asbestos factory | Laundered clothing for 1–2 years, 26 years prior; no other asbestos contact |
| Heller *et al.* 1970 [16] | 10 Pleural mesothelioma cases at Massachusetts General Hospital 1960–1967 | Pipefitter | 1 woman (10%) washed her pipefitter husband's dusty work clothes; husband had asbestosis | Clothes washing |
| Bittersohl and Ose 1971 [17] (as cited in NIOSH 1995 [7]) | Wife of a chemical plant worker | Chemical plant worker | 1 woman with pleural mesothelioma whose husband was exposed to asbestos insulation at a chemical plant | Clothes washing |
| Champion 1971 [18] | Son of lagger | Lagger | Patient was never occupationally exposed to asbestos; father was a lagger who wore work overalls home; emphysematous changes seen in mother; sister had pleural plaques. | --- |
| Knappmann 1972 [19] (as cited in NIOSH 1995 [7]) | Brother of asbestos worker | Asbestos factory worker | Case report of mesothelioma in a man who lived for several years with his sister who was an asbestos worker | --- |
| Greenberg & Davies 1974 [20] | 246 Pleural and peritoneal mesothelioma cases in England, Wales, Scotland (1967–1968) | Asbestos factory workers | 2/246 (0.8%) with potential domestic exposures: 1 case had husband who worked in asbestos factory; 1 case lived near asbestos factory; parents worked at factory | Cases had 2 and 14 years of exposure, respectively |

**Table 1.** *Cont.*

| Author and Year | Population Studied | Occupation of Worker(s) | Results | Exposure Information |
|---|---|---|---|---|
| Lillington *et al.* 1974 [21] | Mesothelioma in husband and wife | Industrial exposure to asbestos | Husband had "industrial exposure", wife washed his clothes; both were diagnosed with pleural mesothelioma | Clothes washing |
| Milne 1976 [22] | 32 Pleural mesothelioma cases in Victoria, Australia | Asbestos cement factory | 1/32 cases (3%) had domestic exposure; father worked in asbestos cement plant. | --- |
| Edge & Chaudhury 1978 [23] | 50 Mesothelioma cases from Barrow in Furness (British shipbuilding town; 1966–1976) | Shipyard plumber | 1/50 (2%) was married to a shipyard plumber. | Crocidolite |
| Li *et al.* 1978 [24] | Family in which father was pipe insulator in a shipyard. | Shipyard insulator | Father had asbestosis and lung cancer; wife washed his clothes and had mesothelioma; daughter had mesothelioma. | Clothes washing |
| Epler *et al.* 1980 [25] | 2 wives of asbestos workers | Asbestos factory workers | Mesothelioma in 2 wives of asbestos workers:<br>1 husband worked in an asbestos product factory for 23 years;<br>1 husband worked in an asbestos product factory and had asbestosis and mesothelioma. | --- |
| Vianna *et al.* 1981 [26] | 288 pleural and peritoneal mesothelioma cases in NY state (1973–1978) | Farmers, fireman | 7/288 (2.4%) cases with potential indirect exposure (1 male, 6 females);<br>5 females lived with a farmer;<br>1 lived with a fireman. | --- |
| Martensson *et al.* 1984 [27] | Two children of an asbestos worker | Foundry worker | Female with no occupational exposure;<br>Father worked at foundry with insulation and hung his clothes where children played;<br>Male, brother of female, grew up in same house and worked as a storekeeper for company supplying shipyard electrical equipment. | Exposure referred to as "slight household asbestos exposure during childhood". |

**Table 1.** *Cont.*

| Author and Year | Population Studied | Occupation of Worker(s) | Results | Exposure Information |
|---|---|---|---|---|
| Krousel *et al.* 1986 [28] | Mother, daughter, and son with pleural mesothelioma | Factory workers | Mother worked as clothing sales person and candle-maker. First husband and second husband worked at lumber/shingle company. Family lived within a mile of lumber/shingle company that used asbestos wrap on pipes. Daughter worked as phone operator, husband was electrician. Son worked in submarine, shipyard, cement pipe maker, power company, and carpenter. | No microscopic evidence of asbestos fibers in son and daughter |
| Li *et al.* 1989 [29] | Family of asbestos worker | Insulator | Wife of insulator washed worker's laundry, used cloth sacks that were used to transport insulation as child's diapers. Child died of mesothelioma at age 32; mother died at age 49. Uncle who lived with family as teen and was briefly an insulator, developed mesothelioma at age 43. Father died of asbestosis at age 53. | Clothes washing and insulation cloth sacks as diapers. |
| Kane *et al.* 1990 [30] | 10 Cases of mesothelioma in patients 40 years old and under | Asbestos factory worker, shipyard insulator | Of 10 cases, 5 had household exposure (50%):<br>*Case 1:* Father delivered asbestos products;<br>*Case 2:* Father worked at glass factory that made asbestos products;<br>*Case 3:* Father worked as shipyard pipe insulator; lived 6 km from shipyard; mother had mesothelioma; father had adenocarcinoma;<br>*Case 5:* Brother-in-law worked in asbestos plant; lived 2 km from asbestos factory;<br>*Case 6:* Exposed to father's dusty work clothing for one year; older sister developed lung cancer with same exposure. | 1–18 years of exposure |

**Table 1.** *Cont.*

| Author and Year | Population Studied | Occupation of Worker(s) | Results | Exposure Information |
|---|---|---|---|---|
| Konetzke *et al.* 1990 (German) [31] | 48 Cases of mesothelioma from the National Cancer Register in East Germany and 19 cases of pleural plaques were investigated for non-occupational exposure to asbestos | --- | 22/48 (46%) cases caused by cleaning by members of the family of working clothes contaminated with asbestos. | Clothes washing |
| Oern *et al.* 1991 [32] (Norwegian; as cited in NIOSH 1995 [7]) | Sister and husband of asbestos workers | Insulators | Family had 2 brothers, a sister and her husband. All males were insulators; 1 brother had asbestosis, other brother and sister had mesothelioma; woman who cleaned work clothes developed mesothelioma at age 79. | Clothes washing |
| Chellini *et al.* 1992 [33] | 100 Cases of pleural mesothelioma in Tuscany, Italy (1970–1988) | Construction, plumber in chemical manufacturing | 4/100 (4%) cases identified with "possible domestic" exposure—women whose husbands or members of the family were occupationally exposed (3 in construction and one as a plumber in chemical manufacturing) and who used to wash their spouses' work clothes; same data also reported by Seniori-Constantini & Chellini 1997 [34]. | Clothes washing |
| Dodoli *et al.* 1992 [35] | 262 Cases of pleural mesothelioma in Leghorn and La Spezia, Italy (1958–1988) | Shipyard workers, oil refinery worker | 10 (3.8%) women washed their relatives' work clothes (9 shipyard workers, 1 oil refinery worker). | Clothes washing |
| Giarelli *et al.* 1992 [36] | 170 Cases of mesothelioma in Trieste, Italy (1968–1987) | Shipyard workers | 5/170 (2.9%) cases had domestic exposure and cleaned the clothes of their husbands who were shipyard workers. | 80% had no AB [a]; 20% had few AB; Clothes washing. |
| Schneider *et al.* 1996 [37] | 5 Pleural mesothelioma cases | Insulation mat manufacturing, turbine revision, roofer, asbestos cardboard manufacturing, and insulator. | "Causal relation established between the mesothelioma and inhalation of asbestos fibers while cleaning contaminated work-clothes and shoes". | 7–23 years of exposure; cleaning clothes and shoes |

**Table 1.** *Cont.*

| Author and Year | Population Studied | Occupation of Worker(s) | Results | Exposure Information |
|---|---|---|---|---|
| Seniori-Constantini & Chellini 1997 [34] | 335 Pleural mesothelioma cases from registry in Tuscany, Italy (1970–1996) | NR [b] | 30%–35% of 59 female cases were housewives; Same data source as Chellini *et al.* 1992 [33]. | NR |
| Rees *et al.* 1999 [38] | 123 Cases in South Africa | Mining workers | 13/123 cases (11%) noted contaminated clothing as source of exposure, along with working with asbestos or living in mining district. "No subject exclusively exposed to contaminated work clothes brought home". Three cases were reported to have only exposure to asbestos from contaminated clothing. | Mostly crocidolite and amosite; Contaminated clothing |
| Ascoli *et al.* 2000 (Italian) [39] | One female mesothelioma case | NR | Domestic exposure, duration of 20 years in an industrial town with a large chemical plant | NR |
| Barbieri *et al.* 2001 (Italian) [40] | 190 Cases of mesothelioma in Brescia, Italy diagnosed 1980–1999 | Asbestos hauler | 1/190 (0.5%) had domestic exposure; wife of asbestos hauler who washed his clothes | Clothes washing |
| Bianchi *et al.* 2001 [41] | 557 Malignant mesotheliomas of the pleura diagnosed 1968–2000 in the Trieste-Monfalcone area, Italy | Mainly shipbuilding town | 21/65 (32%) females and 0/492 males had histories of domestic exposure, cleaning clothes of an asbestos exposed worker; includes Giarelli *et al.* 1992 [36] cases. | 35% of domestic cases analyzed (n = 20) had AB; Clothes washing. |
| Mangone *et al.* 2002 (Italian) [42] | 323 Pleural and peritoneal mesothelioma cases in Emilia-Romagna, Italy (1996–2001) | NR | 13/325 (4%) were domestically exposed | NR |
| Miller 2005 [43] | 32 Pleural and peritoneal mesothelioma cases gathered from law firms (since 1990) | Shipyard workers, insulators, others. | 15 wives, 11 daughters, 3 sons, 1 sister-in-law, 1 niece, 1 boarder; Occupations of workers included: 13 shipyard workers, 7 insulators, 12 others | NR |
| Bianchi *et al.* 2007 [44] | 99 Cases in Trieste, Italy (2001–2006) | NR | 5 cases (5%) identified as "home exposure", where patients had washed asbestos-exposed husbands' work clothes | Clothes washing |

[a] AB = Asbestos bodies;
[b] NR = Not reported.

**Table 2.** Cohort and case-control studies of mesothelioma in domestically exposed populations.

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Newhouse & Thompson 1965 a, b [2,3] | Case-control | 76 cases from London hospital (1956–1963) | 76 "in patient" series (patient in medical and surgical wards of the hospital during early summer 1964) matched by sex and date of birth | PL, PE | Crocidolite, chrysotile, amosite | Spinners, engine room worker, boiler coverer, asbestos factory foreman, docker, railway carriage builder, asbestos factory worker | 9 cases had relative who worked with asbestos (7 pleural, 2 peritoneal) *vs*. 36 cases with no occupational exposure. 1 of "in patient" series had relative who worked with asbestos *vs*. 67 with no occupational exposure. **Crude OR = 16.75 (95% CI = 2.13–744.78)** [b,c,d] |
| Ashcroft & Heppleston 1970 [45] | Case-control | 22 cases in Tyneside (British shipbuilding town) | 46 hospital controls matched for age and sex, free of malignant disease | PL, PE | NR | Asbestos worker | One case was the widow of an asbestos worker who, for a period of 3 years, had come home with asbestos dust on his hair and shoes. |
| McEwen *et al*. 1971 [46] | Case-control | 80 cases from Scotland (1950–1967) | 2 sets of hospital controls with coronary artery disease or lung or gastric cancer, matched for age and sex | PL, PE | For one case: "Blue and white" asbestos | For one case: dock worker | "...only a few [cases] had shared a household with relatives who were known to have worked with asbestos. There was no statistical difference with regard to either household or spare-time exposure to asbestos between the three groups [cases, cancer controls, cardiovascular controls]. One individual case, however, was interesting. The husband of one of the female cases had worked regularly with asbestos, both blue and white, as a dock labourer, and quite frequently had come home with asbestos on his overalls. His wife (the case) had washed them at home". |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Rubino *et al*. 1972 [47] | Case-control | 50 cases from Piedmont, Italy (1960–1970) | Patients with same sex, age, and at same institution | PL | NR | Asbestos industry | 3/50 cases had "family exposure" 0/50 controls had "family exposure"<br>Thoracotomy cases:<br>1 (wife was employed in asbestos industry, no occupational exposure)/18 cases (3 with occupational exposure)<br>0 (no domestic or occupational exposure)/18 controls<br>(no domestic or occupational exposure)<br>No thoracotomy cases [e]:<br>2 (domestic exposure, unclear if could have occupational exposure)/32 cases (3 with occupational exposure)<br>0/32 controls (1 with occupational exposure) |
| Vianna & Polan 1978 [4] | Case-control | 52 female NY state residents 20+ year old (1967–1977) | 52 controls matched for age sex, race, marital status, county, year of death, and from non-cancer death | PL, PE | NR | Shoemaker, brake lining worker, pipefitter, heat insulation worker, heat electric wire worker, elevator insulation worker | 10 patients had husbands/fathers working in asbestos industry (9 pleural, 1 peritoneal), whereas their matched controls did not.<br>1 control had husband working in asbestos industry, whereas their matched case did not: RR = 10 (95% CI = 1.42–37.40) [e].<br>8 patients after excluded own occupational exposure, whereas their matched controls did not. 1 control had husband working in asbestos industry, whereas their matched case did not ($p < 0.02$). **OR = 8/1 = 8 (95% CI = 1–63.9)** [b] |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| McDonald & McDonald 1980 [5] | Case-control | 490 fatalities in Canada (1960–1972) and USA (1972) ascertained through 7,400 pathologists | Matched controls with pulmonary metastases from non-pulmonary malignancy by sex, age, year of death, and hospital | PL, PE | Chrysotile (at least 3 cases) and some amosite | Chrysotile production, insulation, or factory work | 2 males, 6 females exposed to dusty clothing of asbestos worker;<br>none among matched controls; 2 controls were exposed, but the paired cases were not ($p = 0.08$).<br>**OR = 4.0 (95% CI = 0.43–9.42)** [b,e];<br>5/8 cases were exposed during childhood;<br>3/8 cases (1 control) were exposed by clothing of a Quebec chrysotile production worker;<br>5/8 cases (1 control) were exposed by clothing of a insulation/factory worker. |
| Muscat & Wynder 1991 [48] | Case-control | 124 cases entering NY City hospital between 1981–1990 | 267 controls with non-tobacco disease, matched for age, sex, hospital, race, month of interview | M | NR | Auto mechanic | 1/16 women without occupational exposure reported domestic contact with asbestos (one husband was auto mechanic);<br>No information on controls;<br>1/105 males reported domestic exposure during childhood. |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Spirtas *et al*. 1994 [49] | Case-control | 208 cases from Veterans Administration hospital files and Los Angeles county and New York state cancer registries (1975–1980) | 533 controls from death certificates or VA benefit files died of other causes excluding cancer, respiratory disease, suicide, or violence | PL, PE | NR | Asked if "cohabitant ever exposed to asbestos". Separately asked if cohabitant performed any of 9 activities: (1) brake lining work/repair; (2) furnace/boiler installation/repair; (3) building demolition; (4) plumbing/heating; (5) insulation; (6) shipbuilding yard/repair; (7) elevator installation/repair; (8) production of textiles; (9) production of paper products. | OR for cohabitant ever exposed to asbestos: **Men: 13.2 (95% CI = 3.4–54.7) (12 pleural)** **Women: 3.4 (95% CI = 0.3–61.3)** **Crude OR = 6 (95% CI = 2.55–13.8)** [b,e] OR for cohabitant performed any of 9 activities: Men: 12.1 (95% CI = 4.6–33.3) (34 pleural, 4 peritoneal) Women: 1.4 (95% CI = 0.3–5.6) |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Howel *et al.* 1997 [50] | Case-control | 185 cases of mesothelioma from mesothelioma and cancer registries and post-mortem records in Yorkshire, England (1979–1991) | 159 controls from necropsy records excluded if had mesothelioma, bronchial or ovarian cancer, or circumstances that made gathering information difficult, matched for age, sex, and year of death | PL, PE | Unknown although crocidolite and amosite identified at factory that provoked concern for the study | NR | ORs for para-occupational exposure: Excluding subjects with likely occupational exposure: Likely *vs*. possible and unlikely 5.6 (95% CI = 1.9–16.5); Likely and possible *vs*. unlikely 1.8 (95% CI = 0.87–3.6); Excluding those with likely or possible occupational exposure: Likely *vs*. possible and unlikely 61.7 (95% CI = 3.4–1104); **Likely and possible *vs*. unlikely 5.8 (95% CI = 1.7–19.2).** |
| Case *et al.* 2002 [51] | Case-control | 10 female residents aged ≥50 years of Quebec mining regions identified through hospital records (1970–1989) | 150 controls selected from previously interviewed sample matched on age and area | PL | Chrysotile with some tremolite contamination (Thetford mines) | Chrysotile miners | 10 cases identified: 9 (90%) had lived with one or more asbestos workers (*vs*. 65% of controls); Never lived with asbestos worker OR = 1 Lived with 1 or 2 workers OR = 3.4 (95% CI = 0.4–30.8); Lived with 3 or more workers OR = 9.0 (95% CI = 0.9–87.4) **Crude OR = 4.92 (95% CI = 0.65–219.54)** [b,e] |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Magnani *et al.* 2000 [52] | Case-control | 53 mesothelioma cases in Italy, Spain, Switzerland without occupational exposure (1993–1997) | 232 controls from general population and hospitals without occupational exposure | PL | NR | Asbestos industry | OR for those with domestic exposure [f] and without environmental exposure: 4.92 (95% CI = 1.78–13.6); Probability domestic exposure [f] (adjusted for environmental exposure): Never exposed OR = 1; Low probability OR = 2.05 (95% CI = 0.83–5.09); Middle or high probability OR = 4.81 (95% CI = 1.77–13.1); Unknown OR = 0.74 (95% CI = 0.22–2.53); Intensity domestic exposure [f] (adjusted for environmental exposure): Never exposed OR = 1; Low intensity OR = 2.01 (95% CI = 0.84–5.06); Middle intensity OR = 5.68 (95% CI = 1.39–23.3); **High intensity OR = 7.83 (95% CI = 1.69–36.2)**; Unknown OR = 0.75 (95% CI = 0.21–2.69) |
| Welch *et al.* 2005 [53] | Case-control | 24 male cases treated at Washington Cancer Institute, Washington, DC (1989–2001) | 24 patients with appendical cancer treated at Washington Cancer Institute 1990–2000, matched for age and sex | PE | NR | Same 9 activities specified in Spirtas *et al.* 1994 [49], except brake lining work is grouped with tire work. | 8/24 (33%) cases cohabitated with persons involved in 9 specified "high-risk-for-asbestos-exposure processes" 2/24 (8%) controls cohabitated with persons involved in 9 processes **Crude OR = 5.5 (95% CI = 0.89–57.95) for co-habitating with one of the nine activities.**[b,e] |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Maule *et al.* 2007 [54] | Case-control | 103 cases from Casale Monferrato, Italy (1987–1993) | 272 controls matched by birth date, sex, vital status, date of death | PL | Crocidolite and chrysotile | Asbestos cement (AC) workers | OR for relative with AC occupation, adjusted for age, sex, and AC occupation: 2.4 (95% CI = 1.2–4.8); **RR for relatives' AC occupation accounting for age, sex, and domestic (home materials) exposure: Including distance to plant = 1.4 (95% CI = 0.7–2.9)** Not including distance to plant = 2.1 (95% CI = 1.0–4.5) Update to Magnani *et al.* 2001 [55]. |
| Rake *et al.* 2009/ Peto *et al.* 2009 [56,57] | Case-control | 622 patients in England, Wales and Scotland born since 1925 identified through physician records, cancer research network, and hospital records | 1,420 population controls matched for age and sex | M | Suggests that higher death rate in UK is due to amosite use. | NR | **OR living with a potentially exposed worker before 30 years of age: 2.0 (95% CI = 1.3–3.2);** Logistic regression results: OR living with a potentially exposed worker before 30 years of age (women): 2.3 (95% CI = 1.5–3.8) OR living with a potentially exposed worker before 30 years of age (men): 1.1 (95% CI = 0.9–1.4) OR living with a high-risk parent or sibling: 1.3 (95% CI = 1.0–1.6) OR living with a high-risk spouse: 2.1 (95% CI = 1.3–3.5) See also tables of Peto *et al.* 2009. |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Anderson 1982 [58] | Cohort | 2,218 household contacts of Unarco amosite factory workers first employed between 1941–1945; 663 deaths | State of New Jersey, age and sex-specific | M | Amosite | Amosite insulation factory workers | After 30+ years from onset of exposure, mesothelioma deaths in 3/170 (1.8%) deceased household contacts (2 female and 1 male, all children of workers) [c] Observed/expected for respiratory cancer was 1.25 for females and 1.7 for males. Same cohort as Joubert *et al*. 1991 [59], Anderson 1979 [60]; Anderson 1976 [61], Selikoff 1981 [62]. |
| Ferrante *et al*. 2007 [63] | Cohort | Cohort of 1,780 wives of asbestos cement workers in Casale Monferrato, Italy (deaths from Registrar's office, incidence from mesothelioma registry) Deaths: 1965–2003 Incidence: 1990–2001 | Used age and sex specific rates in Piedmont, Italy for reference | PL, PE | Crocidolite and chrysotile | Asbestos cement workers | Peritoneal cancer SMR = 2.51 (95% CI = 0.52–7.35) Pleural cancer SMR = 18.00 (95% CI = 11.14–27.52) **Pleural malignant mesothelioma SIR = 25.19 (95% CI = 12.57–45.07)** [d,e] Update to Magnani *et al.* 1993 [64]. |

**Table 2.** *Cont.*

| Author and Year | Study Design | Population Studied (dates of death/incidence) | Controls/ Unexposed | Disease | Fiber Type | Occupation of Worker(s) | Results [a] |
|---|---|---|---|---|---|---|---|
| Reid *et al.* 2008 [65] | Cohort | Followed 2,552 women and girls who lived in Wittenoom (crocidolite mining town) from 1943 to 1992 and were not involved in mining/milling (1950–2004) | Western Australia female population from 1970–2004 | PL (0 PE) | Crocidolite | Crocidolite miners | The risk of death from mesothelioma was increased, but not significantly, in residents known to have lived with (HR = 2.67, 95% CI = 0.77–9.21) [e] or **washed the clothes of an Australian Blue Asbestos Company asbestos worker (HR = 2.61, 95% CI = 0.85–7.99)** [d]; Update to Hansen *et al*. 1993 [66]. |
| Bourdès *et al.* 2000 [10] | Meta-analysis | Five studies: Yazicioglu *et al*. 1980 [67]; Newhouse & Thompson 1965 [3]; McDonald & McDonald 1980 [5]; Magnani *et al*. 1993 [64]; Howel *et al*. 1997 [50] | -- | PL | Chrysotile (McDonald & McDonald 1980 [5]); the rest are amosite or mixed fibers | -- | Meta RR: 8.1 (95% CI = 5.3–12) [e] |

[a] = Results in bold indicate values used in the meta- analysis;
[b] = Relative risk estimate and/or 95% CI was calculated because it was not provided in the study;
[c] = Appears exposure classification done in a hierarchy, so domestic cases and controls may contain subjects with neighborhood exposure (which itself is statistically significant);
[d] = Potential confounding by neighborhood exposure;
[e] = Potential confounding by occupational exposure;
[f] = Domestic exposure included exposures to asbestos-containing materials at home. Several with "high" domestic exposure included "crushed asbestos material in the courtyard";
PL = Pleural mesothelioma, PE = Peritoneal mesothelioma, M = Mesothelioma, OR = Odds ratio, RR = Relative risk, CI = Confidence interval, SMR = Standardized mortality ratio;
SIR = Standardized incidence ratio, HR = Hazard ratio;
NR = Not reported.

**Table 3.** Epidemiologic studies of interstitial and pleural abnormalities in domestically exposed populations.

| Author and Year | Study Design | Population Studied | Comparison Group | Disease | Fiber Type | Occupation of Worker | Results |
|---|---|---|---|---|---|---|---|
| Navratil & Trippe 1972 [68] | Cohort | 114 Blood relatives of asbestos workmen in Czechoslovakia | "General population" of district N with no known exposure | Pleural calcifications | Chrysotile | Asbestos product plant | 4/114 (3.5%) of blood relatives had pleural calcifications. Observed/expected = 4/0.39 |
| Anderson 1982 [58] | Cohort | 679 Household contacts (no occupational asbestos exposure) of amosite factory workers in Paterson, NJ employed between 1941–1954 | 325 urban NJ residence controls matched by sex, age, and residential community without asbestos exposure | Small opacities (≥1/0) and pleural abnormalities (pleural thickening, pleural calcification, pleural plaques) (1971 ILO [a]) | Amosite | Amosite asbestos factory workers | Household resident during index worker employment period. Cases (N = 679): Small opacities = 114 (17%), Pleural abnormalities = 178 (26%), Both = 239 (35%) ($p < 0.001$ compared to Controls). Controls (N = 325): Small opacities = 10 (3%), Pleural abnormalities = 6 (2%), Both = 15 (5%) Found statistically significant relationship between duration of exposure and year of first exposure and pleural thickening, pleural calcification, and both together, but not small opacities alone. |

**Table 3.** *Cont.*

| Author and Year | Study Design | Population Studied | Comparison Group | Disease | Fiber Type | Occupation of Worker | Results |
|---|---|---|---|---|---|---|---|
| Ferrante *et al.* 2007 [63] | Cohort | Cohort of 1,780 wives of asbestos cement workers in Casale Monferrato, Italy (deaths from Registrar's office: 1965–2003) | Used age and sex specific rates in Piedmont, Italy for reference | Nonmalignant respiratory disease | Crocidolite and chrysotile | Asbestos cement workers | SMR [b] = 0.86 (0.47–1.45) |
| Kilburn *et al.* 1986 [69] | Cross-sectional | 274 Wives, 79 sons, and 140 daughters of shipyard workers from Long Beach, CA who started work before 1961. Subjects volunteered and had no occupational exposure. | 1,347 Members of Long Beach Census tract in 1975 and random sample of adult population of Michigan during 1978–1979 without occupational asbestos exposure | Asbestosis and pleural abnormalities (refer to all as asbestosis) (ILO 1980, ≥1/0, and/or presence of pleural thickening or plaques) | NR | Shipyard workers | Asbestosis prevalence:<br>Wives: 11%<br>Sons: 8%<br>Daughters: 2%<br>Comparison populations:<br>Long Beach men: 3.7%<br>Long Beach women: 0.6%<br>Michigan men: 0.5%<br>Michigan women: 0.0%<br>Wives with abnormalities:<br>Pleural only: 39%<br>Parenchymal only: 52%<br>Parenchymal and pleural: 10%<br>75% of wives with asbestosis had husbands with asbestosis. |

**Table 3.** *Cont.*

| Author and Year | Study Design | Population Studied | Comparison Group | Disease | Fiber Type | Occupation of Worker | Results |
|---|---|---|---|---|---|---|---|
| Sider *et al.* 1987 [70] | Cross-sectional | 93 wives > 40 years of age of current and retired insulators screened from January to March 1986 at Northwestern Memorial Hospital in Chicago with no occupational exposure | Wives without radiographic abnormalities | Parenchymal opacities and pleural changes according to ILO 1980 | NR | Pipe covers and asbestos removers (insulation workers) | 18/93 (19.4%) demonstrated pleural changes consistent with asbestos exposure: pleural plaques (N = 16, 88.9%), diaphragm plaques (N = 6, 27.8%), pleural calcification (N = 3, 16.6%), and diffuse pleural thickening (N = 1, 5.5%). No parenchymal opacities. Radiographs of the husbands were compared for 17 of the 18 wives with pleural abnormalities. 14 of the husbands (82%) demonstrated more severe pleural involvement than their wives and 6 had parenchymal abnormalities. The remaining 3 wives with more severe changes had at least one household contact in addition to her husband. Only year of initial exposure was statistically different from the comparison group. |

**Table 3.** *Cont.*

| Author and Year | Study Design | Population Studied | Comparison Group | Disease | Fiber Type | Occupation of Worker | Results |
|---|---|---|---|---|---|---|---|
| Peipins *et al.* 2003 [71] | Cross-sectional | 6,668 Participants ≥ 18 years of age who had lived, worked, or played in Libby, MT for at least 6 months before December 31, 1990 | None | Pleural abnormality (any unilateral or bilateral pleuralcalcification on the diaphragm, chest wall, or other site or any unilateral or bilateralpleural thickening or plaque on the chest wall, diaphragm, or costophrenic angle site, consistent with asbestos-related pleural disease, using the PA view, the oblique views, or a combination of those views) and interstitial abnormality (ILO 1980, ≥1/0). | Libby amphibole (tremolite, actinolite, winchite, richterite) | Vermiculite miners | Lived with W.R. Grace workers (n = 1,376): Pleural abnormality N = 358 (26.0%); Interstitial abnormality N = 17 (1.2%); Does not exclude occupational or non-occupational exposure; Using logistic regression found having been ahousehold contact of a vermiculite miner associated with pleural abnormalities. |

[a] ILO = International Labour Organization disease classification;

[b] SMR = Standardized mortality ratio.

The case reports and case series include pleural and peritoneal mesothelioma in wives, children, mothers, and siblings of asbestos workers such as miners, asbestos factory workers, pipefitters, laggers/insulators, and shipyard workers. Unfortunately, these published case reports rarely identified the type of asbestos to which the case was exposed [1,14,23,38], with a few exceptions, all of which reported exposure to amphibole asbestos (amosite or crocidolite) (Table 1). None of the case reports provided information on the level of asbestos exposure experienced in each case, although a limited number of studies reported results of lung-burden analyses [28,36,41]. Two of these studies reported finding asbestos bodies in 20% to 35% of persons examined [36,41]. Several of the case reports specifically noted clothes washing as the source of exposure via the inhalation pathway [15–17,21,24,29,31–37,38,40,41,44].

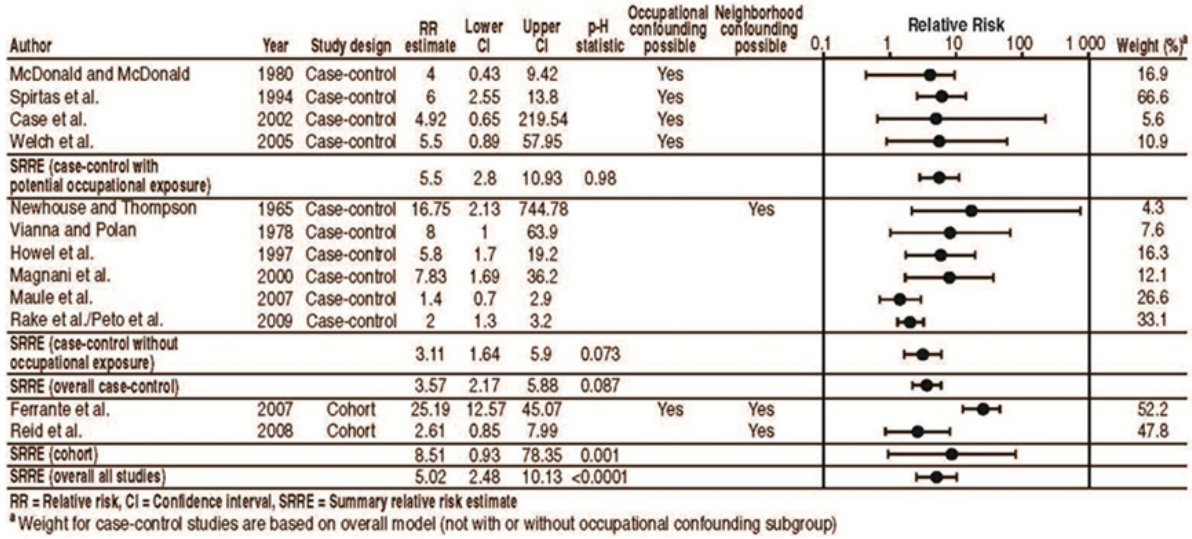
Among studies of the association between domestic exposure and asbestos-related disease, mesothelioma was the most common disease reported. Several cohort (n = 3) and case-control (n = 14) studies of mesothelioma evaluated domestically exposed populations or identified cases of mesothelioma in domestically exposed individuals (Table 2). One meta-analysis was also identified. The occupations of the workers included in the studies were primarily those associated with traditional high-risk trades: asbestos miners, asbestos factory workers, shipyard/dock workers, textile workers, furnace/engine/boiler room workers, railway carriage builders, pipefitters, and insulators. Our review included 14 case-control studies, of which 10 reported relative risk estimates or provided enough information to calculate a crude relative risk estimate [2–5,49–54,56], ranging from 1.4 [54] to 16.75 [2,3]. Two of the three cohort studies reported relative risk values [63,65]. In the first cohort study, a statistically significant standardized incidence ratio (SIR) of 25.19 (95% CI: 12.57–45.07) was reported for wives of Italian cement workers [63], although results were not adjusted for potential confounding by neighborhood or occupational exposure. In the second cohort study, a non-statistically significant hazard ratio (HR) of 2.61 (95% CI: 0.85–7.99) was reported in household members of workers of the Australian Blue Asbestos Company [65]. In this study, potential neighborhood exposures were also not evaluated in the estimation of relative risk.

The cohort and case-control studies evaluated both pleural and peritoneal mesotheliomas, with some studies not discerning between the two sites. In many studies, asbestos fiber type was also not reported. The fiber type to which the study participants were exposed is an important factor, as amphibole fibers (crocidolite, amosite or tremolite) are generally more potent then chrysotile fibers [72–75]. When reported, the workers via whom the individuals were domestically exposed were nearly always exposed to amphiboles. This fiber-type issue is further complicated by the fact that some chrysotile deposits have different degrees of co-occurrence of tremolite. One case-control study evaluated exposure to chrysotile in 10 female co-habitants of Quebec chrysotile miners, although the miners worked in the Thetford area, which the authors described has having the highest tremolite content of the Canadian mining sites. This study resulted in a non-significant increase in the risk of mesothelioma (odds ratio [OR] = 4.92, 95% CI: 0.65–219.54) among co-habitants [51].

Meta-analysis of all 12 cohort and case-control studies with reported relative risk estimates resulted in an SRRE of 5.02 (95% CI: 2.48–10.13; Figure 1). This SRRE indicates a statistically significant increase in the risk of mesothelioma for those domestically exposed, although heterogeneity was evident (p-H < 0.0001). The lower bound of the confidence interval in the Ferrante *et al.* study [63] is greater than the upper bound of the confidence interval from the overall summary effect. Removal of this study in a

sensitivity analysis resulted in an attenuation, albeit still statistically significant, of the overall effect (SRRE = 3.34, 95% CI: 2.15–5.19), and the model became more homogeneous (p-H = 0.126).

**Figure 1.** Meta-analysis of cohort and case-control studies of mesothelioma in domestically exposed populations.




RR = Relative risk, CI = Confidence interval, SRRE = Summary relative risk estimate

[a] Weight for case-control studies are based on overall model (not with or without occupational confounding subgroup)

A further sub-analysis by study type (cohort *vs.* case-control) was performed. The SRRE for the two cohort studies together [63,65] was elevated, but was not statistically significant (SRRE = 8.51, 95% CI: 0.93–78.35; p-H = 0.001). There is considerable heterogeneity between these two cohort studies; the disparity in risk estimates is likely due to potential confounding by occupational exposures (e.g., [63]) and neighborhood exposures (e.g., [63,65]). In contrast, the SRRE for the 10 case-control studies was elevated and statistically significant (SRRE = 3.57, 95% CI: 2.17–5.88; p-H = 0.087). Significant heterogeneity was present in both study design models. The case-control studies were further divided by whether the results could have been modified by the cases being occupationally exposed to asbestos themselves. The SRREs for the case-control studies, with and without potential modification by occupational exposure, were both statistically significantly increased, and the model of studies with potential occupational exposure was homogeneous (SRRE = 5.5, 95% CI: 2.8–10.93, p-H = 0.980 and SRRE = 3.11, 95% CI: 1.64–5.9, p-H = 0.073, respectively). Additionally, in the group of case-control studies without potential for occupational exposure, the highest relative risk value was from a study with increased likelihood of neighborhood exposure (16.75) [2,3]. As a sensitivity analysis, the cohort study by Reid *et al.* [65], which did not have potential occupational confounding, was analyzed with the six case-control studies that also did not have occupational confounding. The resulting SRRE is 2.87 (95% CI: 1.69–4.88). This value is not much different from the overall SRRE based on the six case-control studies alone, indicating that this study does not have a large effect on the analysis. As an additional sensitivity analysis, the case-control study by Newhouse and Thompson [2,3] was omitted from the six case-control studies that did not have potential occupational confounding. The relative risk estimate for this study is considerably greater than those for the other five. A point of deviation for Newhouse and Thompson [2,3] appears to be

study date, which may be a proxy for increased exposure or for less accurate categorization of exposure compared to the more recent studies. As noted earlier, the Newhouse and Thompson studies included persons exposed to various fiber types, including chrysotile, amosite, and crocidolite. The resulting SRRE based on five case-control studies is 2.83 (95% CI: 1.51–5.31). This value is also not much different from the overall SRRE based on the six case-control studies, including Newhouse and Thompson [2,3], indicating that this study does not have a large effect on the analysis.

#### 3.1.2. Lung Cancer

The epidemiologic studies of domestic exposure rarely evaluated the risk of lung cancer. Only two studies with results for lung cancer were identified [58,63]. In the first study, a cohort of 2,218 family contacts of amosite asbestos factory workers in New Jersey first employed between 1941 and 1945 was studied [58]. The authors reported a slight statistically significant increase in cancer of the respiratory system for male family contacts of the factory workers with more than 20 years latency (observed *vs.* expected = 1.97), but not for female contacts (observed *vs.* expected = 1.70). In the second study of 1,780 wives of asbestos cement workers in Casale Monferrato, Italy, no significant increase in lung cancer was reported (SMR = 1.17; 95% CI: 0.60–2.04) [63]. Although the fiber potency gradient is less pronounced for lung cancer than it is for mesothelioma, fiber type is an important factor in determining disease. The study by Ferrante *et al.* included persons exposed to chrysotile and crocidolite, while the Andersen study included amosite workers.

#### 3.1.3. Pleural and Interstitial Abnormalities

Case reports of pleural and interstitial abnormalities in domestically exposed individuals date back to the 1960s [7,76–80] and focus primarily on pleural plaques. Epidemiologic studies of pleural (*i.e.*, plaques and diffuse pleural thickening) and interstitial abnormalities were gathered and reviewed (Table 3). As with the studies of asbestos-related malignancy, information on fiber type was either not reported or indicated a mixed fiber exposure. Six cohort and cross-sectional studies were identified [58,63,68–71], half of which accounted for potential confounding by occupational exposure [58,69–71]. Sider *et al.* [70] collected chest radiographs of the male workers and their wives, reporting that the majority (82%) of the husbands, who worked in the insulation trades, demonstrated more severe radiographic changes than their wives. Likewise, Kilburn *et al.* [69] reported that 75% of the wives with pleural and/or parenchymal abnormalities had husbands who worked in shipyards and exhibited abnormalities. One of these studies [58] also reported a statistically significant relationship between the duration of domestic exposure and year of first exposure with pleural thickening, calcification, or both abnormalities combined, but not small opacities alone. Sider *et al.* [70] reported that only the year of initial domestic exposure was statistically different from the comparison group.

### *3.2. Review of Domestic Exposure Studies*

Unfortunately, none of the epidemiologic studies reported the level of asbestos exposures experienced by the domestic cases themselves. This was expected, given the findings of previous review articles, and the difficulty of characterizing exposures in a domestic setting in an epidemiologic study. At best, the epidemiologic studies characterized exposure by intensity (low, medium, high) or probability of exposure. In our review of household exposure studies, nineteen separate exposure studies were identified, although some reported on overlapping populations. These studies, in each of the four categories of interest, are shown in Table 4. As with the epidemiology studies, most exposures were to mixed fibers.

#### 3.2.1. Exposures in the Home Environment

Three of the studies reviewed provided results of sampling within the homes of asbestos workers [81–83]. Two of the three studies [81,83] reported airborne asbestos concentrations, while the third [82] summarized reports of fibers found in the settled dust. These three studies were primarily reviews or articles that reported exposure concentrations indirectly and did not provide sufficient information to attribute concentrations directly to worker clothing. For example, in their book, Selikoff and Lee [82] described a study performed by Mount Sinai regarding asbestos workers' homes, wherein workers were employed at asbestos factories during 1941 to 1954, and "small amounts" of amosite were identified in settled dust in the workers' homes and in neighboring homes of non-asbestos workers up to 400 yards downwind of factories. The authors attributed these amosite fibers found in workers' homes to the clothing workers brought home from the workplace. The amosite fibers identified in the homes of non-asbestos workers were attributed to atmospheric contamination and deposition; however, because samples were collected 20 to 25 years after the fact, it is difficult to attribute concentrations directly to a take-home source such as clothing. In addition, these samples involved settled dust from surfaces in the homes, rather than airborne asbestos concentrations. The observed dust concentrations are not representative of the air inhaled by household members.

In 1986, the World Health Organization (WHO) reported a mean concentration of 0.006 f/cc (range, 0.002–0.011 f/cc) in the homes of South African asbestos miners and estimated a range of 0.01–1 f/cc for "paraoccupational" exposures [83]. Although described as an environmental study, Nicholson *et al.* [81] found levels ranging from 100 to up to 5,000 ng/m$^3$ by weight (approximately 0.003–0.15 f/cc based on the conversion factor presented by the National Research Council [84]) in the homes of chrysotile miners in California and Newfoundland, where homes were described as having visible fibers and dust in living areas and laundry facilities.

#### 3.2.2. Exposures from Clothing

Our literature review identified only one study that provided airborne asbestos levels measured during laundering of workers' clothing [85]. This study evaluated concentrations associated with laundering clothes contaminated during an asbestos removal operation, reporting an average airborne concentration of 0.4 ± 0.1 f/cc (duration not specified) resulting from picking up contaminated clothing and loading it into the washer. No information was provided regarding specific sample duration;

however, earlier evaluations performed at the same building reported mean fiber counts that were typically associated with one-hour sampling duration. The exposure levels "dropped to zero" following a single wash cycle (Table 4). A maximum personal sample of 1.2 f/cc (corresponding mean = 0.4 f/cc, sample duration unknown) was measured during the complete laundry operation, and all asbestos fibers detected were chrysotile. This study was not conducted in a home laundry setting, but focused primarily on the sufficiency of the decontamination procedures used by 40 workers after the removal of an asbestos-containing ceiling. Although not reported specifically as 8-hour time-weighted averages, these exposure levels are clearly low.

Two studies regarding bulk samples of dust on workers' clothing performed by NIOSH at friction product manufacturing plants were also reviewed. Unfortunately, these studies did not discuss airborne exposures resulting from this dust [86,87]. One of these studies reported that asbestos was present in 85% of samples obtained from clothing and car seats of friction workers, but did not describe the fiber type.

**Table 4.** Domestic exposure studies.

| Author and Year | Population or Task Studied | Asbestos Fiber Type | Reported Exposure Information |
|---|---|---|---|
| *Studies reporting measurements of airborne or settled dust in homes of asbestos workers* | | | |
| Nicholson *et al.* 1980 [81] | Homes of chrysotile miners in Copperopolis, California and Baie Verte, Newfoundland | Chrysotile | Homes of miners: 100 to < 5,000 ng/m$^3$ (approx. 0.003–0.15 f/cc [a,b]) (n[c] = 13)<br>Homes of non-miners (Baie Verte): 32, 45, 65 ng/m$^3$ |
| Selikoff and Lee 1978 [82] | Settled dust in asbestos workers' homes | Amosite | "...small amounts of amosite were found 20–25 years later in the settled dust of asbestos workers' houses from factory operations over the period 1941–1954, and up to 400 yards downwind in the neighboring houses of nonasbestos workers". |
| WHO [d] 1986 [83] | Asbestos miners' homes | NR | Residences of asbestos miners in South Africa:<br>Mean = 0.006 f/cc (range, 0.002–0.011 f/cc);<br>Para-occupational range: 0.01–1.0 f/cc |
| *Study of clothing and laundering* | | | |
| Sawyer *et al.* 1977 [85] | Asbestos abatement workers | Chrysotile | Mean of personal samples (n = 12): 0.4 f/cc (max = 1.2 f/cc)<br>Mean of area samples:<br>During picking up clothing (n = 4): 0.4 f/cc<br>Loading washer (n = 5): 0.4 f/cc<br>Loading dryer (n = 6): 0.0 f/cc |
| *Exposure simulation studies* | | | |
| Jiang *et al.* 2008 [88] | Unpacking and repacking clutches | Chrysotile | 30 min PCM [e] -adjusted mean following clothing handling = 0.002 ± 0.002 f/cc (n = 4)<br>Estimated 8 h TWA [f] = 0.0001 f/cc. |
| Madl *et al.* 2008 [89] | Unpacking and repacking brakes | Chrysotile | 30 min PCM-adjusted mean (range) following clothing handling (n = 5):<br>0.011 f/cc (0.002–0.015 f/cc ) |

**Table 4.** *Cont.*

| Author and Year | Population or Task Studied | Asbestos Fiber Type | Reported Exposure Information |
|---|---|---|---|
| Madl *et al.* 2009 [90] | Mechanics performing brake repair on heavy equipment | Chrysotile | 30 min PCM-adjusted mean (range) following clothing handling:<br>For primary worker (n = 2): 0.036 f/cc (0.032–0.039 f/cc)<br>For bystander (n = 2): 0.010 f/cc (0.003–0.018 f/cc) |
| Mowat *et al.* 2007 [91] | Roofers removing dried material from laundered clothing | Chrysotile | 30 min PCM-E [g] mean (n = 12): 0.0017 f/cc (range = 0–0.011 f/cc)<br>Calculated TWAs = 0.003–0.002 f/cc |
| Weir *et al.* 2001 [92] | Brake mechanics | Chrysotile | Agitation of operator's coveralls (30 min) = 0.72 f/cc<br>Background concentration in laboratory ≤ 0.065 f/cc |
| *Lung burden studies* | | | |
| Dawson *et al.* 1993 [93] | Women with mesothelioma (n = 170) | Mixed | Women with domestic exposure (n = 14):<br>Total amphiboles = $4.9 \times 10^6$ f/g [h] (range = 0–251)<br>Chrysotile = $12.7 \times 10^6$ f/g (range = 0–2506)<br>Control group (n = 31):<br>Total amphiboles = $0.04 \times 10^6$ f/g (range = 0–1.0);<br>Unknown = $4.4 \times 10^6$ f/g (range = 0–20.1) |
| Dodson *et al.* 2003 [94] | Women with mesothelioma (n = 15) | Mixed | 4 women had potential domestic exposure through their father's/husband's work;<br>2/4 had ferruginous bodies (wife of crocidolite worker and wife of laborer/ship scaler/cement worker/*etc*.);<br>1 had uncoated amosite and tremolite (daughter of shipyard worker);<br>1 had uncoated tremolite and no commercial amphiboles (daughter of maintenance worker and wife of shipyard worker/painter/*etc*. |
| Giarelli *et al.* 1992 [36] | Family members of shipyard workers with mesothelioma in Trieste, Italy | --- | 5/170 (2.9%) cases had domestic exposure, cleaned clothes of spouse:<br>80% had no AB [i]<br>20% had few AB (1–5 AB/section) |

**Table 4.** *Cont*.

| Author and Year | Population or Task Studied | Asbestos Fiber Type | Reported Exposure Information |
|---|---|---|---|
| Gibbs *et al.* 1989 [95] | Mesothelioma cases with para-occupational exposure (n = 13) | Mixed | Mean (range) fiber counts of Group II para-occupational, e.g., wives of males working with asbestos (n = 13):<br>Total: 277.8 (5.6–2507)<br>Amosite: 1.5 (0–6.1)<br>Crocidolite: 31.8 (0–251.1)<br>Chrysotile: 218.9 (1.9–2507)<br>Mean (range) fiber counts of Group V, unexposed (n = 21):<br>Total: 42.5 (0–188.3)<br>Amosite: 0.7 (0–4.6)<br>Crocidolite: 5.5 (0–101.7)<br>Chrysotile: 19.6 (0–76.5)<br>Units in fiber × $10^6$/g dry lung |
| Gibbs *et al.* 1989 [95] | Mesothelioma cases without occupational exposure (n = 84). | Mixed | Para-occupational group averages (range) in dry lung:<br>Amosite: 1.5 × $10^6$ f/g (0–6.1)<br>Crocidolite: 31.8 × $10^6$ f/g (0–251)<br>Chrysotile: 218.9 × $10^6$ f/g (1.9–2507)<br>Unexposed group averages (range) in dry lung:<br>Amosite: 0.7 × $10^6$ f/g (0–4.6)<br>Crocidolite: 5.5 × $10^6$ f/g (0–102)<br>Chrysotile: 19.6 × $10^6$ f/g (0–77) |
| Gibbs *et al.* 1990 [96] | Mesothelioma cases with para-occupational exposure (n = 10) | Mixed | 9 exposed to their husbands' work clothes and 1 was the daughter of a man who had died of asbestosis. |
| Huncharek 1989 [97] | Wife of shipyard machinist | Mixed | Chrysotile: 2.5 × $10^6$ f/g<br>AC[j]: 0.8 × $10^6$ f/g<br>TAA[k]:<br>3.2 × $10^6$ f/g (in dry lung) |
| Roggli & Longo 1991 [98] | Women whose only known exposure was household contact with an asbestos worker with asbestos-related disease (n = 6) | NR | Household contacts: median = 1,700 AB/g (range, 2–8,200) Uncoated fibers (UF [l]): median = 24,300 UF/g<br>(range, 17,000–120,000) Normal range: median = 3,100 UF/g (range, 0–20) |

**Table 4.** *Cont.*

| Author and Year | Population or Task Studied | Asbestos Fiber Type | Reported Exposure Information |
|---|---|---|---|
| Roggli 1992 [99] | Household contacts with mesothelioma (n = 3) | NR | Wife of shipyard insulator: 8,200 AB/g (29 yr exposure)<br>Daughter of insulator: 2,330 AB/g, 17,000 UF/g (25 yr exposure)<br>Wife of shipyard worker: 2 AB/g, 24,300 UF/g (1–2 yr exposure)<br>Normal lungs: 0–22 AB/g, 1,600–5,600 UF/g |
| Roggli *et al.* 2002 [100] | Household contacts with mesothelioma (asbestosis confirmed in 8.3%) | Mixed | Mean (range) lung burden in wet lung of household: 130 AB/g (2–14,100)<br>AC: 3,400 f/g (450–116,000)<br>TAA: 5,200 f/g (980–22,400)<br>chrysotile: 1,800 f/g<br>Mean (range) lung burden in wet lung of reference cases:<br>AB: 3 f/g (2–22)<br>AC: <600 f/g (<100–<2,540)<br>TAA: 158,000 f/g (1700–455,000)<br>Chrysotile: <600 f/g (<100–<2,540) |

[a] based on conversion factor in NRC 1984;
[b] f/cc = fibers per cubic centimeter;
[c] n = number of samples or cases;
[d] WHO = World Health Organization;
[e] PCM = Phase contrast microscopy;
[f] TWA = time-weighted average;
[g] PCM-E = phase contrast microscopy equivalents;
[h] f/g = fibers per gram lung;
[i] AB = Asbestos bodies;
[j] AC = commercial amphiboles (amosite + crocidolite);
[k] TAA = noncommercial amphiboles (tremolite + actinolite + anthophyllite)
[l] UF = uncoated fiber;

### 3.2.3. Exposure Modeling and Simulation

Five exposure simulation studies were identified (Table 4). Four of these involved an evaluation of simulated domestic exposures resulting from those working with friction products, such as brakes and clutches [88–90,92], three of which were performed by the same group of investigators. The fifth study characterized exposures from roofers' clothing [91]. Phase contrast microscopy (PCM) was used in all simulations; transmission electron microscopy (TEM) was also used in all except the Weir *et al.* study [92] to analyze fiber type in clothing-related samples. In studies employing TEM, PCM-equivalent (PCM-E) concentrations were also reported.

The simulations of friction-product-related exposures involved laundering activities by agitating a brake mechanic's coveralls [92] and simulated clean-up of countertops and clothes-handling tasks,

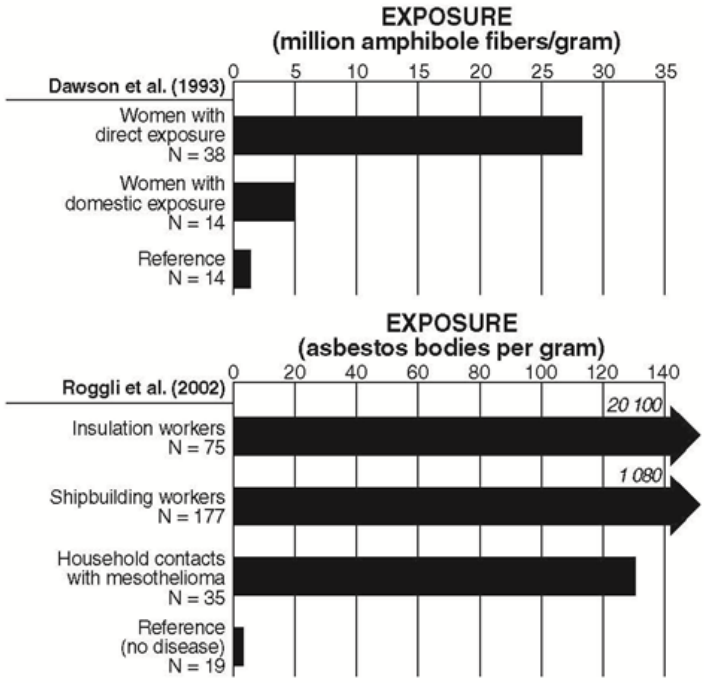
such as shaking and folding clothes worn by an operator, whose work activities involved packing and re-packing boxes of brakes and clutches [88,89] or performing repair work on heavy equipment [90]. All of these studies involved exposures only to chrysotile asbestos of unknown origin, because this was the fiber type used in the formulation of asbestos-containing friction materials [101]. Estimated 30 min PCM-E mean values were reported as 0.002 ± 0.002 f/cc (8 h time-weighted average [TWA] = 0.0001 f/cc) and 0.002–0.015 f/cc (mean = 0.011 f/cc) during clothes handling following unpacking and re-packing of clutches and brakes, respectively [88,89]. Similar asbestos levels were reported by Mowat *et al.* [91] in the simulation of potential exposures from asphalt-based roofing materials from scraping or picking dried material from laundered coveralls, with a 30 min exposure value of 0.0017 f/cc (range, non-detect [ND]–0.011 f/cc). For mechanics performing brake repair on heavy equipment, equivalent 30 min mean values following clothes handling were 0.036 f/cc and 0.010 f/cc for primary workers and bystanders, respectively [90]. During agitation of a brake mechanic's coveralls following brake work, the 30 min concentration was 0.72 f/cc [92].

#### 3.2.4. Lung-Burden Studies

Six unique lung-burden studies were identified that provide results related to domestic exposure, generally reporting fiber concentrations either as fibers × $10^6$/g dry lung (f/g) or asbestos bodies per gram of lung tissue analyzed (AB/g). Gibbs and colleagues [95,96] and Roggli and colleagues [102] reported multiple times on overlapping populations. Asbestos bodies are indicative of amphibole exposures, because asbestos bodies form primarily on amphibole fibers [102]. Of the studies identified, most reported that the domestically exposed persons were typically wives or daughters of insulators, boilermakers, or shipyard workers [36,93,94,96–100]. All six studies identified the fiber type detected in the lung tissue examined and found amphibole asbestos fibers, such as crocidolite and amosite, in the lungs of domestically exposed persons (Table 4). Only two studies [93,100] presented lung-burden data for domestic contacts compared to a reference group (Figure 2). Both studies indicated significantly higher concentrations of amphibole asbestos and/or AB/g of lung tissue in domestically exposed cases compared to the reference group, and even higher concentrations of amphibole asbestos in directly exposed insulation or shipyard workers, although the domestically exposed persons and directly exposed workers were not linked. No study compared the lung burdens of workers with those of their spouses.

In the series of studies by Roggli and colleagues, all of the asbestos workers were diagnosed with asbestosis, and three with lung cancer; all of the household contacts were diagnosed with either mesothelioma or lung cancer. In an update to their analyses involving 1,445 cases of mesothelioma, Roggli and colleagues reported that, in the household contacts identified, 57% were found to have pleural plaques, and 7.9% had asbestosis [100]. Of the four domestically exposed cases evaluated in their study, Dodson *et al.* [94] found ferruginous bodies in lung tissue of two of the four women, uncoated commercial amphibole asbestos fibers in another woman, uncoated non-commercial amphibole asbestos fibers in a third woman, and chrysotile fibers in another. Not surprisingly, high concentrations of crocidolite fibers were identified in lung tissue of the spouse of the crocidolite cement worker.

**Figure 2.** Lung-burden studies.




Some of the studies provided exposure estimates of those domestically exposed, but generally, the objectives for these studies were not related to an evaluation of domestic exposure, and no explanation of exposure-level estimation or quantitative analysis was performed. For example, Camus *et al.* [103] analyzed lung cancer risk among women living in asbestos mining areas wherein indoor household concentrations were estimated by extrapolation from fiber burden results in ten autopsied women who had lived with asbestos workers. Indoor asbestos concentrations associated with these observed fiber burdens were reported as being approximately 0.03 f/cc higher than existing outdoor levels, although the method by which this result was obtained was not described. These authors also reported an estimated cumulative exposure of 7.8 f/cc-years in household contacts using their approach.

## 4. Discussion and Conclusions

Overall, the results indicate a consistent elevated risk of mesothelioma in the domestically exposed populations, and summary results suggest that the association may be modified by the potential for additional occupational exposure. The SRRE for all cohort and case-control studies indicated a five-fold greater risk of mesothelioma for persons domestically exposed. For persons domestically exposed, the results of the meta-analysis indicated a three- to five-fold increased risk for case-control studies and 8.5-fold risk of mesothelioma for cohort studies, although the cohort studies suffered from heterogeneity (and there were only two studies). Comparatively, the Bourdès *et al.* [10] meta-analysis of pleural mesothelioma found an eight-fold greater risk. Our finding of increased risk applies to domestically exposed populations in which the associated workers were employed in traditionally high-risk occupations involving exposure to asbestos, where in many cases, possible confounding due

to direct asbestos exposures was not taken into account. For most of the included studies, exposures were to amphibole or mixed fiber exposures associated with traditionally high-risk occupations.

The domestic exposure studies of lung cancer were extremely limited and not supportive of an association between domestic exposure and lung cancer. In addition, both identified studies suffered from potential confounding by other occupational exposures and lack of consideration of smoking history. Fiber type was also not considered in these studies, though the two identified studies specifically included those exposed to amphibole asbestos. For the studies of pleural and interstitial abnormalities, results of pleural and interstitial abnormalities were often combined, despite them being two separate and distinct disease types, with reported exposures primarily being to mixed fibers. Even within pleural abnormalities themselves, the disease types differ (*i.e.*, pleural plaques *vs.* diffuse pleural thickening) in terms of their health impact and level of exposure required to cause the abnormality [104]. The studies supported an association between abnormalities and domestic exposure, but the association is largely due to pleural abnormalities. Similar to the mesothelioma studies, the workers themselves were likely highly exposed populations with exposure to amphiboles (e.g., asbestos product plant, amosite factory, and shipyard workers, insulators, and miners). These studies are unique, in that they provide linked data on husbands and wives (*i.e.*, data were collected on husbands and their wives, rather than workers in general and wives in general).

The findings of the lung-burden studies are consistent with the epidemiologic studies, in that they concluded that accumulated fiber burdens in persons exposed domestically might suggest a significant risk of mesothelioma, although the directly exposed workers in these studies were in traditional high-risk occupations, such as insulators, shipyard workers, and those in the building trades. All nine lung-burden studies (six unique studies total) detected amphibole fibers in the lungs of domestically exposed persons and, when compared to an appropriate reference group, were found to be present at significantly higher concentrations (Figure 2). In the Roggli series of studies, the lungs of household contacts were found to contain commercial amphiboles (defined as amosite and crocidolite) in 48% of cases, non-commercial amphiboles in 10.5% of cases, and chrysotile in 4.2% of the cases. Other studies also reported elevated amphibole fiber burdens [93,95,97]. These concentrations were reported as similar to those found in construction workers (190 AB/g), with higher lung fiber burdens reported in wives than children of these workers [100].

Ideally, airborne exposure estimates including asbestos fiber type information for the participants in the epidemiologic studies would exist in the peer-reviewed literature to allow for better evaluation of risk; however, this is not the case for epidemiologic studies of domestically exposed individuals. Instead, there are review articles with limited discussion of airborne measurements in asbestos miners' homes, one study of airborne monitoring during laundering the clothes of asbestos abatement workers exposed to chrysotile, and more recent controlled simulation studies of airborne concentrations during the handling of the clothes of workers who traditionally have low chrysotile exposures. Thus, the experiences of the domestically exposed populations in the epidemiologic studies (exposed via workers in high-risk occupations, with high levels of exposure to amphibole asbestos) do not correspond to the exposures characterized by the available airborne data (generally for low-level chrysotile exposures).

As noted above, the existing relevant airborne exposure data pertain to populations occupationally exposed to low-level chrysotile asbestos. Given the absence of epidemiologic studies of populations

exposed domestically by family members who were exposed occupationally to low-level chrysotile, alternative methods must be used to estimate the exposures and risk of mesothelioma for these populations.

First, it is logical that, if the worker is exposed to low levels of asbestos occupationally, then their co-habitants would experience even lower exposure concentrations. Automobile mechanics are a good population in which to test this hypothesis, because brake mechanics are exposed to low concentrations of solely chrysotile asbestos (e.g., [105,106]). For example, Paustenbach *et al.* [105] reported a typical 8-hour TWA exposure of 0.04 f/cc for automobile mechanics, based on review of numerous historical studies. When 8 h TWAs are calculated for the four simulation studies that involve clothing manipulation or potential take-home exposure from friction products [89,90,92], the exposure levels reported are approximately two orders of magnitude lower than the 8-hour TWA for automobile mechanics (0.0001 f/cc *vs.* 0.04 f/cc). In fact, the daily exposures resulting from clothing activities were indistinguishable from background concentrations of asbestos, reported as ranging between 0.00001 f/cc and 0.0001 f/cc [107].

The results of the simulation studies are based on a small sample size in some studies (n = 1 in Jiang *et al.* [88]) or involved a short period of time (45 seconds in Jiang *et al.* [88], to 2 min in Madl *et al.* [89]); however, in all four studies, the results were consistently low, well below current and historical occupational exposure limits and, in some cases, within ambient concentrations. The anomaly of higher concentrations reported in the Weir *et al.* [92] study can be explained, because the majority of the fibers present in the sample were non-asbestiform, such as cotton fibers. Although this comparison has limitations due to the small sample sizes and exposure durations attributed to clothing manipulation activities and differences in methods used to analyze for asbestos fibers, the comparison nonetheless indicates that, at a minimum, domestic asbestos exposures to persons derived from domestic relationships with automobile mechanics are likely to be lower than those observed in occupationally exposed career automobile mechanics. This is consistent with the lung-burden studies showing a gradation of fiber burden from occupationally exposed to domestically exposed persons [93,100].

In our review, only one study [48] identified a domestically exposed case of mesothelioma reportedly due to chrysotile exposure in a woman whose husband was an automobile mechanic. Although fiber type was not specifically reported, chrysotile was the only fiber type used in the manufacture of brake and clutch parts [101]. This study, and therefore this case, was not included in the meta-analysis, because it lacked the information to calculate an estimate of relative risk, namely a comparison group. Vianna and Polan [4], Spirtas *et al.* [49], and Welch *et al.* [53] combine the activity of brake lining work/repair with traditionally highly exposed asbestos activities (e.g., insulation, shipyard work); thus, any observed increase in risk cannot be attributed to automobile mechanic work or solely to chrysotile exposure, and instead is highly likely attributable to the other activities (e.g., [108]).

Second, if workers whose occupation involving low-level chrysotile exposure is not associated with an increased risk of mesothelioma, it follows that co-habitants of these workers also would not have an increased risk of mesothelioma. The existing epidemiologic studies of domestically exposed populations support this hypothesis, and demonstrate that the risk for the domestically exposed individual is remarkably less than that of the worker. While the exposure data is not complete in many of these studies with respect to both exposure level and fiber type, at least for one group—mechanics—the epidemiology shows that career workers exposed to low levels of chrysotile

asbestos are not at risk and, therefore, it follows that the families of these workers would also not be at increased risk for developing asbestos-related disease. This has also been demonstrated in other industries, where higher exposures have been reported. Maule *et al.* [54] provided risk estimates for those occupationally exposed during asbestos cement manufacturing, and their relatives, with the OR for the workers being remarkably greater than for those domestically exposed (27.5 *vs.* 1.4, non-significant). Likewise, the radiographic studies showed that the majority of the workers demonstrated more severe radiographic changes than their wives, and alternatively, if the wives showed radiographic abnormalities, so did their husbands [69,70]. Thus, if the existing studies of domestically exposed populations show trends of lower risk and disease than the worker population, it follows that if the worker population does not have increased risk, then the domestically exposed co-habitant would not either.

In conclusion, the epidemiologic and lung burden studies, as a surrogate of past exposure, support an increased risk of mesothelioma and interstitial, but more likely pleural, abnormalities in domestically exposed individuals whose associated worker was employed in traditionally high-risk occupations involving exposure to amphibole asbestos. Quantifiable exposure concentrations do not exist for these domestically exposed cohorts; however, some data exist for manipulation of worker clothing after low-level chrysotile exposure, mostly in the form of recent exposure simulations. These simulation data show that results for domestic exposures are lower than the workers' exposures and are commensurate with background concentrations.

**Acknowledgements**

The authors thank Meghan Mitchell of Exponent for her aid in reviewing the epidemiologic studies.

**Conflicts of Interest**

No funding was obtained to prepare this manuscript. Two of the authors (David L. Dahlstrom and Fionna Mowat) have been designated as experts in litigation matters related to asbestos, and all authors have been retained as consultants in asbestos-related litigation matters.

**References**

1. Wagner, J.C.; Sleggs, C.A.; Marchand, P. Diffuse pleural mesothelioma and asbestos exposure in the north western Cape Province. *Brit. J. Ind. Med.* **1960**, *17*, 260–271.
2. Newhouse, M.L.; Thompson, H. Epidemiology of mesothelial tumors in the London area. *Ann. N. Y. Acad. Sci.* **1965**, *132*, 579–588.
3. Newhouse, M.L.; Thompson, H. Mesothelioma of pleura and peritoneum following exposure to asbestos in the London area. *Brit. J. Ind. Med.* **1965**, *22*, 261–269.
4. Vianna, N.J.; Polan, A.K. Non-occupational exposure to asbestos and malignant mesothelioma in females. *Lancet* **1978**, *1*, 1061–1063.
5. McDonald, A.D.; McDonald, J.C. Malignant mesothelioma in North America. *Cancer* **1980**, *46*, 1650–1656.

6. Grandjean, P.; Bach, E. Indirect exposures: The significance of bystanders at work and at home. *Amer. Ind. Hyg. Assn. J.* **1986**, *47*, 819–824.
7. National Institute for Occupational Safety and Health (NIOSH). *Report* to *Congress* on *Workers' Home Contamination Study Conducted under the Workers' Family Protection Act*. United States; Department of Health and Human Services, Centers for Disease Control and Prevention: Cincinnati, OH, USA, 1995.
8. National Institute for Occupational Safety and Health (NIOSH). *Protecting Workers' Families: A Research Agenda*; United States Department of Health and Human Services, Centers for Disease Control and Prevention: Cincinnati, OH, USA, 2002.
9. Institut National de la Santé de la Recherche Médicale (INSERM). Effets sur la santé des principaux types d'exposition à l'amiante; INSERM: Paris, France, 1997.
10. Bourdès, V.; Boffetta, P.; Pisani, P. Environmental exposure to asbestos and risk of pleural mesothelioma: Review and meta-analysis. *Eur. J. Epidemiol.* **2000**, *16*, 411–417.
11. Donovan, E.P.; Donovan, B.L.; McKinley, M.A.; Cowan, D.M.; Paustenbach, D.P. Evaluation of take home (para-occupational) exposure to asbestos and disease: A review of the literature. *Crit. Rev. Toxicol.* **2012**, *42*, 703–731.
12. Sutton, A.J.; Jones, D.R.; Abrams, K.R.; Sheldon, T.A.; Song, F. Systematic reviews and meta-analysis: A structured review of the methodological literature. *J. Health. Serv. Res. Policy* **1999**, *4*, 49–55.
13. Andersson, T.; Ahlbom, A. Episheet software: Spreadsheets for the analysis of epidemiologic data. 2004. Available online: http://www.google.com/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=1&ved=0CC0QFjAA&url=http%3A%2F%2Fkrothman.hostbyet2.com%2FEpisheet.xls&ei=SeTyUZrgPOSqiALi2IDwDQ&usg=AFQjCNHeAwcg2RhXfkGM_b8RdX498JSs9A&sig2=U6MjH7LGvrV4FIowPto19g&bvm=bv.49784469,d.cGE (accessed on 23 October 2013).
14. Lieben, J.; Pistawka, H. Mesothelioma and asbestos exposure. *Arch. Environ. Health* **1967**, *14*, 559–563.
15. Rusby, N.L. Pleural manifestations following the inhalation of asbestos in relation to malignant change. *J. Roy. Nav. Med. Serv.* **1968**, *54*, 142–148.
16. Heller, R.M.; Janower, M.L.; Weber, A.L. The radiological manifestations of malignant pleural mesothelioma. *Amer. J. Roentgenol.* **1970**, *108*, 53–59.
17. Bittersohl, G.; Ose, H. The epidemiology of pleural mesotheliomas (in German). *Z. Gesante Hyg.* **1971,** *17*, 861–864.
18. Champion, P. Two cases of malignant mesothelioma after exposure to asbestos. *Amer. Rev. Resp. Dis.* **1971**, *103*, 821–826.
19. Knappmann, J. Observations made at post mortem examination of 251 cases of mesothelioma in Hamburg (1958–1968) (in German). *Pneumonology* **1972**, *148*, 60–65.
20. Greenberg, M.; Davies, T.A.L. Mesothelioma register 1967–1968. *Br. J. Ind. Med.* **1974**, *34*, 91–104.
21. Lillington, G.A.; Jamplis, R.W.; Differding, J.R. Conjugal malignant mesothelioma. *N. Engl. J. Med.* **1974**, *291*, 583–584.
22. Milne, J.E.H. Thirty-two cases of mesothelioma in Victoria, Australia: A retrospective survey related to occupational asbestos exposure. *Br. J. Ind. Med.* **1976**, *33*, 115–122.

23. Edge, J.R.; Choudhury, S.L. Malignant mesothelioma of the pleura in Barrow-in-Furness. *Thorax* **1978**, *33*, 26–30.
24. Li, F.P.; Lokich, J.; Lapey, J.; Neptune, W.B.; Wilkins, E.W. Familial mesothelioma after intense asbestos exposure at home. *J. Amer. Med. Assn.* **1978**, *240*, doi:10.1001/jama.1978.03290050057022.
25. Epler, G.R.; Gerald, M.X.; Gaensler, E.A.; Carrington, C.B. Asbestos-related disease from household exposure. *Respiration* **1980**, *39*, 229–240.
26. Vianna, N.J.; Maslowsky, J.; Roberts, S.; Spellman, G.; Patton, R.B. Malignant mesothelioma: Epidemiologic patterns in New York State. *N. Y. State J. Med.* **1981**, *81*, 735–738.
27. Martensson, G.; Larsson, S.; Zettergren, L. Malignant mesothelioma in two pairs of siblings: Is there a hereditary predisposing factor? *Eur. J. Resp. Dis.* **1984**, *65*, 179–184.
28. Krousel, T.; Garcas, N.; Rothschild, H. Familial clustering of mesothelioma: A report on three affected persons in one family. *Amer. J. Prev. Med.* **1986**, *2*, 186–188.
29. Li, F.P.; Drefus, M.G.; Antman, K.H. Asbestos-contaminated nappies and familial mesothelioma. *Lancet* **1989**, *1*: 909–910.
30. Kane, M.J.; Chahinian, A.P.; Holland, J.F. Malignant mesothelioma in young adults. *Cancer* **1990**, *65*, 1449–1455.
31. Konetzke, G.W.; Beck, B.; Mehnert, W.H. Uber berufliche und auberberufliche asbestwirkungen—Remarks on occupational and non-occupational effects of asbestos. *Pneumologie* **1990**, *44*, 858–861.
32. Oern, S.; Odden, S.; Osnes, M. Familial clustering of asbestos-related disease (in Norwegian). *Tidssker Nor Laegeforen* **1991,** *111*, 1099–1101.
33. Chellini, E.; Fornaciai, G.; Merler, E.; Paci, E.; Costantini, A.S.; Silvestri, S.; Zappa, M.; Buiatti, E. Pleural malignant mesothelioma in Tuscany, Italy (1970–1988): II. Identification of occupational exposure to asbestos. *Amer. J. Ind. Med.* **1992**, *21*, 577–585.
34. Seniori-Costantini, A.; Chellini, E. The experience of the mesothelioma registry of Tuscany in assessing health hazard associated with asbestos exposure. *Med. Lav.* **1997**, *88*, 310–315.
35. Dodoli, D.; del Nevo, M.; Fiumalbi, C.; Iaia, T.E.; Cristaudo, A.; Comba, P.; Viti, C.; Battista, G. Environmental household exposures to asbestos and occurrence of pleural mesothelioma. *Amer. J. Ind. Med.* **1992**, *21*, 681–687.
36. Giarelli, L.; Bianchi, C.; Grandi, G. Malignant mesothelioma of the pleura in Trieste, Italy. *Amer. J. Prev. Med.* **1992**, *22*, 521–530.
37. Schneider, J.; Straif, K.; Woitowitz, H.J. Pleural mesothelioma and household asbestos exposure. *Rev. Environ. Health* **1966**, *11*, 65–70.
38. Rees, D.; Goodman, K.; Fourie, E.; Chapman, R.; Blignaut, C.; Bachmann, M.O.; Myers, J. Asbestos exposure and mesothelioma in South Africa. *S. Afr. Med. J.* **1990**, *89*, 627–634.
39. Ascoli, V.; Fantini, F.; Carnovale, S.C.; Blasetti, F.; Bruno, C.; di Domenicantonio, R.; lo Presti, E.; Pasetto, R.; Nardi, F.; Comba, P. Malignant mesothelioma in the industrial area of Colleferro. *Med. Lav.* **2000**, *91*, 547–564.
40. Barbieri, P.G.; Lombardi, S.; Candela, A.; Pezzotti, C.; Binda, I. Incidence of malignant mesothelioma (1980–1999) and asbestos exposure in 190 cases diagnosed in the population of the Province of Brescia. *Med. Lav.* **2001**, *92*, 249–262.

41. Bianchi, C.; Brollo, A.; Ramani, L.; Bianchi, T.; Giarelli, L. Asbestos exposure in malignant mesothelioma of the pleura: A survey of 557 cases. *Ind. Health* **2001**, *39*, 161–167.
42. Mangone, L.; Romanelli, A.; Campari, C.; Candela, S. Malignant mesothelioma in Emilia-Romagna: Incidence and asbestos exposure. *Epidemiol. Prev.* **2002**, *26*, 124–129.
43. Miller, A. Mesothelioma in household members of asbestos-exposed workers: 32 United States cases since 1990. *Amer. J. Ind. Med.* **2005**, *47*, 458–462.
44. Bianchi, C.; Bianchi, T.; Tommasi, M. Mesothelioma of the pleura in the province of Trieste. *Med. Lav.* **2007**, *98*, 374–380.
45. Ashcroft, T.; Heppelston, A.G. Mesothelioma and asbestos on tyneside. In *Pneumoconiosis*: *Proceedings of the International Conference*, *Johannesburg 1969*; Shapiro, H.A., Ed.; Oxford University Press: Cape Town, South Africa, 1970; pp. 177–179.
46. McEwen, J.; Finlayson, A.; Mair, A.; Gibson, A.A. Asbestos and mesothelioma in Scotland: An epidemiological study. *Int. Arch. Arbeitsmed.* **1971**, *28*, 301–311.
47. Rubino, G.F.; Scansetti, G.; Donna, A.; Palestro, G. Epidmilogy of pleural mesotheioma in north-western Italy (Piedmont). *Br. J. Ind. Med.* **1972**, *29*, 436–442.
48. Muscat, J.E.; Wynder, E.L. Cigarette smoking, asbestos exposure, and malignant mesothelioma. *Cancer Res.* **1991**, *51*, 2263–2267.
49. Spirtas, R.; Heineman, E.F.; Bernstein, L.; Beebe, G.W.; Keehn, R.J.; Stark, A.; Harlow, B.L.; Benichou, J. Malignant mesothelioma: Attributable risk of asbestos exposure. *Occup. Environ. Med.* **1994**, *51*, 804–811.
50. Howel, D.; Arblaster, L.; Sinburne, L.; Schweiger, M.; Renvoize, E.; Hatton, P. Routes of asbestos exposure and the development of mesothelioma in an English region. *Occup. Environ. Med.* **1997**, *54*, 403–409.
51. Case, B.W.; Camus, M.; Richardson, L.; Parent, M.E.; Desy, M.; Siemiatycki, J. Preliminary findings for pleural mesothelioma among women in the Quebec chrysotile mining regions. *Ann. Occup. Hyg.* **2002**, *46*, S128–S131.
52. Magnani, C.; Agudo, A.; Gonzalez, C.A.; Andrion, A.; Calleja, A.; Chellini, E.; Dalmasso, P.; Escolar, A.; Hernandez, S.; Lvaldi, C.; *et al.* Multicentric study on malignant pleural mesothelioma and non-occupational exposure to asbestos. *Br. J. Cancer* **2000**, *83*, 104–111.
53. Welch, L.S.; Acherman, Y.I.Z.; Haile, E.; Sokas, R.K.; Sugarbaker, P.H. Asbestos and peritoneal mesothelioma among college-educated men. *Int. J. Occup. Environ. Health* **2005**, *11*, 254–258.
54. Maule, M.M.; Magnani, C.; Dalmasso, P.; Mirabelli, D.; Merletti, F.; Biggeri, A. Modeling mesothelioma risk associated with environmental asbestos exposure. *Environ. Health Perspect.* **2007**, *115*, 1066–1071.
55. Magnani, C.; Dalmasso, P.; Biggeri, A.; Ivaldi, C.; Mirabelli, D.; Terracini, B. Increased risk of malignant mesothelioma of the pleura after residential or domestic exposure to asbestos: A case-control study in Casale Monferrato, Italy. *Environ. Health Perspect.* **2001**, *109*, 915–919.
56. Rake, C.; Gilham, C.; Hatch, J.; Darnson, A.; Hodgson, J.; Peto, J. Occupational, domestic and environmental mesothelioma risks in the British population: A case-control study. *Br. J. Cancer* **2009**, *100*, 1175–1183.
57. Peto, J.; Rake, C.; Gilham, C.; Hatch, J. *Occupational, Domestic and Environmental Mesothelioma Risks in Britain: A Case-Control Study*; Health and Safety Executive: Norwich, UK, 2009.

58. Anderson, H.A. Family contact exposure. In *Proceedings of the World Symposium on Asbestos*; Canadian Asbestos Information Centre: Montreal, Canada, 1982; pp. 349–362.
59. Joubert, L.; Seidman, H.; Selikoff, I.J. Mortality experience of family contacts of asbestos factory workers. *Ann. N. Y. Acad. Sci.* **1991**, *643*, 416–418.
60. Anderson, H.A.; Lilis, R.; Daum, S.M.; Selikoff, I.J. Asbestosis among household contacts of asbestos factory workers. *Ann. N. Y. Acad. Sci*. **1979**, *330*, 387–399.
61. Anderson, H.A.; Lilis, R.; Daum, S.M.; Fischbein, A.S.; Selikoff, I.J. Household-contact asbestos neoplastic risk. *Ann. N. Y. Acad. Sci.* **1976**, *271*, 311–323.
62. Selikoff, I.J. Household risks with inorganic fibers. *Bull. N. Y. Acad. Med*. **1981**, *57*, 947–961.
63. Ferrante, D.; Bertolotti, M.; Todesco, A.; Mirabelli, D.; Terracini, B.; Magnani, C. Cancer mortality and incidence of mesothelioma in a cohort of wives of asbestos workers in Casale Monferrato, Italy. *Environ*. *Health Perspect*. **2007**, *115*, 1401–1405.
64. Magnani, C.; Terracini, B.; Ivaldi, C.; Botta, M.; Budel, P.; Mancini, A.; Zanetti, R. A cohort study on mortality among wives of workers in the asbestos cement industry in Casale Monferrato, Italy. *Br. J. Ind. Med*. **1993**, *50,* 779–784.
65. Reid, A.; Heyworth, J.; de Klerk, N.H.; Musk, B. Cancer incidence among women and girls environmentally and occupationally exposed to blue asbestos at Wittenoom, Western Australia. *Int*. *J*. *Cancer* **2008**, *122*, 2337–2344.
66. Hansen, J.; de Klerk, N.H.; Eccles, J.L.; Musk, A.W.; Hobbs, M.S. Malignant mesothelioma after environmental exposure to blue asbestos. *Int. J. Cancer* **1993**, 54, 578–581.
67. Yazicioglu, S.; Ilcayto, R.; Balci, K.; Sayli, B.S.; Yorulmaz, B. Pleural calcification, pleural mesotheliomas, and bronchial cancers caused by tremolite dust. *Thorax* **1980**, *35*, 564–569.
68. Navratil, M.; Trippe, F. Prevalence of pleural calcification in persons exposed to asbestos dust, and in the general population in the same district. *Environ*. *Res*. **1972**, *5*, 210–216.
69. Kilburn, K.H.; Warshaw, R.; Thornton, J.C. Asbestos diseases and pulmonary symptoms and signs in shipyard workers and their families in Los Angeles. *Arch*. *Int*. *Med*. **1986**, *146*, 2213–2220.
70. Sider, L.; Holland, E.A.; Davis, T.M.; Cugell, D.W. Changes on radiographs of wives of workers exposed to asbestos. *Radiology* **1987**, *164*, 723–726.
71. Peipins, L.A.; Lewin, M.; Campolucci, S.; Lybarger, J.A.; Miller, A.; Middleton, D.; Weis, C.; Spence, M.; Black, B.; Kapil, V. Radiographic abnormalities and exposure to asbestos-contaminated vermiculite in the community of Libby, Montana, USA. *Environ*. *Health Perspect*. **2003**, *111*, 1753–1759.
72. Hodgson, J.T.; Darnton, A. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann*. *Occup*. *Hyg*. **2000**, *44*, 565–601.
73. Yarborough, C.M. Chrysotile as a cause of mesothelioma: An assessment based on epidemiology. *Crit*. *Rev*. *Toxicol*. **2006**, *36*, 165–187.
74. Berman, W.D.; Crump, K.S. A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. *Crit*. *Rev*. *Toxicol*. **2008**, *38*, S49–S73.
75. Berman, W.D.; Crump, K.S. Update of potency factors for asbestos-related lung cancer and mesothelioma. *Crit*. *Rev*. *Toxicol*. **2008**, *38*, S1–S47.

76. Kiviluoto, T. Pleural plaques and asbestos: Further observations on endemic and other nonoccupational asbestosis. *Ann. N. Y. Acad. Sci.* **1965**, *132*, 235–239.
77. Dalquen, P.; Hinz, I.; Dabbert, A.F. Pleural plaques, asbestosis and exposure to asbestos: An epidemiological study from the Hamburg Area. *Pneumonologie* **1970**, *143*, 23–42.
78. Ebihara, I. Asbestos related pulmonary disorders in Japan: Occupational exposure, contact in the home and air pollutional exposure to asbestos in Japanese urban districts. *Rodo Kagaku (Journal of Science of Labour—Japanese Edition)* **1981**, *57*, 363–396.
79. Lander, F.; Viskum, B. The occurrence of benign pulmonary changes in the spouses of previously employed asbestos workers. *Ugeskr. Laeg.* **1985**, *147*, 1805–1806.
80. Bianchi, C.; Brollo, A.; Ramani, L.; Berte, R. Exposure to asbestos in Monfalcone, Italy. A necropsy-based study. In *Autopsy in Epidemiology and Medical Research*; Ribloi, E., Delendi, M., Eds.; International Agency for Research on Cancer (IARC): Lyon, France, 1991; pp. 127–140.
81. Nicholson, W.J.; Rohl, A.N.; Weisman, I.; Selikoff, I.J. Environmental asbestos concentrations in the United States. In *Biological Effects of Mineral Fibres*; Wagner, J.C., Ed.; International Agency for Research on Cancer (IARC): Lyon, France, 1980; Volume 2, pp. 823–827.
82. Selikoff, I.J.; Lee, D.H.K. *Asbestos and Disease*; Academic Press: New York, NY, USA, 1978.
83. World Health Organization (WHO). *Asbestos and Other Natural Mineral Fibres*; World Health Organisation: Geneva, Swizerland, 1986.
84. Committee on Nonoccupational Health Risks of Asbestiform Fibers; Board on Toxicology and Environmental Health Hazards; Commission on Life Sciences; and National Research Council (NRC). *Asbestiform. Fibers: Nonoccupational Health Risks*; National Academy Press: Washington, DC, USA, 1984.
85. Sawyer, R.N. Asbestos exposure in a Yale building. *Environ. Res.* **1977**, *13*, 146–169.
86. Seixas, N.; Ordin, D. *Health Hazard Evaluation Report*; National Institute for Occupational Safety and Health (NIOSH): Cincinnati, OH, USA, 1986.
87. Driscoll, R.J.; Elliott, L.J. *Health Hazard Evaluation Report*; National Institute for Occupational Safety and Health (NIOSH): Trenton, MI, USA, 1990.
88. Jiang, G.C.T.; Madl, A.K.; Ingmundson, K.J.; Murbach, D.M.; Fehling, K.A.; Paustenbach, D.J.; Finley, B.L. A study of airborne chrysotile concentrations associated with handling, unpacking, and repacking boxes of automobile clutch discs. *Regul. Toxicol. Pharmacol.* **2008**, *51*, 87–97.
89. Madl, A.K.; Scott, L.L.; Murbach, D.M.; Fehling, K.A.; Finley, B.L.; Paustenbach, D.J. Exposure to chrysotile asbestos associated with unpacking and repacking boxes of automobile brake pads and shoes. *Ann. Occup. Hyg.* **2008**, *52*, 463–479.
90. Madl, A.K.; Gaffney, S.H.; Balzer, J.L.; Paustenbach, D.J. Airborne asbestos concentrations associated with heavy equipment brake removal. *Ann. Occup. Hyg.* **2009**, *53*, 839–857.
91. Mowat, F.; Weidling, R.; Sheehan, P. Simulation tests to assess occupational exposure to airborne asbestos from asphalt-based roofing products. *Ann. Occup. Hyg.* **2007**, *51*, 451–462.
92. Weir, F.W.; Tolar, G.; Meraz, L.B. Characterization of vehicular brake service personnel exposure to airborne asbestos and particulate. *Appl. Occup. Environ. Hyg.* **2001**, *16*, 1139–1146.
93. Dawson, A.; Gibbs, A.R.; Pooley, F.D.; Griffiths, D.M.; Hoy, J. Malignant mesothelioma in women. *Thorax* **1993**, *48*, 269–274.

94. Dodson, R.F.; O'Sullivan, M.; Brooks, D.R.; Hammar, S.P. Quantitative analysis of asbestos burden in women with mesothelioma. *Amer. J. Ind. Med.* **2003**, *43*, 188–195.
95. Gibbs, A.R.; Jones, J.S.P.; Pooley, F.D.; Griffiths, D.M.; Wagner, J.C. Non-occupational malignant mesotheliomas. In *Non-Occupational Exposure to Mineral Fibres*; Bignon, J., Peto, J., Saracci, R., Eds.; International Agency for Research on Cancer: Lyon, France, 1989; pp. 219–228.
96. Gibbs, A.R.; Griffiths, D.M.; Pooley, F.D.; Jones, J.S.P. Comparisons of fibre types and size distributions in lung tissues of paraoccupational and occupational cases of malignant mesothelioma. *Br. J. Ind. Med.* **1990**, *47*, 621–626.
97. Huncharek, M.; Capotorto, J.V.; Muscat, J. Domestic asbestos exposure, lung fibre burden, and pleural mesothelioma in a housewife. *Br. J. Ind. Med.* **1989**, *46*, 354–355.
98. Roggli, V.L.; Longo, W.E. Mineral fiber content of lung tissue in patients with environmental exposures: Household contacts *vs.* building occupants. *Ann. N. Y. Acad. Sci.* **1991**, *643*, 511–518.
99. Roggli, V.L. Mineral fiber content of lung tissue in patients with malignant mesothelioma. In: *Malignant Mesothelioma*; Henderson, D.W., Shilkin, K.B., Langlois, S.L., Witaker, D, Eds.; Hemisphere Publishing Corp.: New York, NY, USA, 1992; pp. 201–222.
100. Roggli, V.L.; Sharma, A.; Butnor, K.J.; Sporn, T.; Vollmer, R.T. Malignant mesothelioma and occupational exposure to asbestos: A clinicopathological correlation of 1445 cases. *Ultrastruct. Pathol.* **2002**, *26*, 55–65.
101. Sheehy, J.W.; Cooper, T.C.; O'Brien, D.M.; McGlothlin, J.D.; Froehlich, P.A. *Control. of Asbestos Exposure During Brake Drum Service*; U.S. National Institute for Occupational Safety and Health: Cincinnati, OH, USA, 1989.
102. Roggli, V.L. Asbestos bodies and nonasbestos ferruginous bodies. In: *Pathology of Asbestos-associated Diseases*, 2nd ed.; Oury, T.D., Sporn, T.A., Eds.; Springer-Verlag: New York, NY, USA, 2004; pp. 34–70.
103. Camus, M.; Siemiatycki, J.; Meek, B. Nonoccupational exposure to chrysotile asbestos and the risk of lung cancer. *N. Engl. J. Med.* **1998**, *338*, 1565–1571.
104. American Thoracic Society (ATS). Diagnosis and initial management of nonmalignant diseases related to asbestos. *Amer. J. Respir. Crit. Care Med.* **2004**, *170*, 691–715.
105. Paustenbach, D.J.; Richter, R.O.; Finley, B.L.; Sheehan, P.J. An evaluation of the historical exposures of mechanics to asbestos in brake dust. *Appl. Occup. Environ. Hyg.* **2003**, *18*, 786–804.
106. Finley, B.L.; Richter, R.O.; Mowat, F.S.; Mlynarek, S.; Paustenbach, D.J.; Warmerdam, J.M.; Sheehan, P.J. Cumulative asbestos exposure for US automobile mechanics involved in brake repair (circa 1950s–2000). *J. Expo. Sci. Enviro. Epidemiol.* **2007**, *17*, 644–655.
107. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Asbestos*; Agency for Toxic Substances and Disease Registry: Atlanta, GA, USA, 2001.
108. Hessel, P.A.; Teta, M.J.; Goodman, M.; Lau, E. Mesothelioma among brake mechanics: An expanded analysis of a case-control study. *Risk Anal.* **2004**, *24*, 547–552.

STUDY No. 12

# FIBER ANALYSIS VIGNETTES – AN INCONVENIENT TRUTH

STUDY No. 12

# FIBER ANALYSIS VIGNETTES – AN INCONVENIENT TRUTH

**Victor L. Roggli, M.D.**

There has been considerable interest in the exposure doses that contribute to the various asbestos-associated diseases. Epidemiological studies have shown important differences in the contributions of the various fiber types to asbestos-related diseases, with the amphiboles showing a greater degree of potency as compared to chrysotile. However, epidemiological studies have occasionally provided misleading results. Over the past several decades, there have been several examples where fiber analysis using electron microscopy produced unexpected results which were important to our understanding of disease-exposure relationships. It is the purpose of this article to summarize these fiber analysis vignettes.

# Fiber Analysis Vignettes—An Inconvenient Truth

Victor L. Roggli, M.D.

*Duke University Medical Center*

PO Box 3712 DUMC
Durham, NC 27710
(919) 668-5440
rogg1002@mc.duke.edu

Victor L. Roggli, M.D., is a professor of pathology at Duke University Medical Center in Durham, North Carolina, and the director of the Electron Microscopy Laboratory at the Durham Veterans Administration Medical Center. His research interests include pneumoconiosis, asbestosrelated disease and analytical electron microscopy. Dr. Roggli has published more than 140 articles and 26 chapters in textbooks. He has also authored or coauthored four books, including *Pathology of Asbestos-Associated Diseases, 2nd Ed.*

# Fiber Analysis Vignettes—An Inconvenient Truth

## Table of Contents

# Fiber Analysis Vignettes—An Inconvenient Truth

## I. Introduction

Exposure to asbestos fibers has been associated with the development of a variety of neoplastic and non-neoplastic disease processes.[1-3] These diseases have been shown to follow a dose response relationship. It has also been determined that low levels of asbestos are present in lung tissue samples from individuals with no recognizable exposure to asbestos. There is no evidence that these background exposures cause or contribute to disease.[4] Consequently, there has been considerable interest in the exposure doses that do contribute to the various asbestos-associated diseases.

There are several types of asbestos, including the *serpentine* chrysotile and the amphiboles: amosite and crocidolite (*commercial amphiboles*) and tremolite, actinolite and anthophyllite (*non-commercial amphiboles*). It has long been known from animal studies that these fiber types do not accumulate in the lungs of experimental animals to the same degree.[5] The amphiboles accumulate progressively with increasing exposure doses, while chrysotile tends to reach a plateau after a certain level of exposure. In addition, epidemiological studies have shown important differences in the contributions of the various fiber types to asbestos-related diseases, with the amphiboles showing a greater degree of potency as compared to chrysotile.[6]

The analysis of lung tissue samples for concentrations and types of fibers provides a useful measure of the cumulative levels found in an individual patient.[7] This information may then be correlated with presence or absence of disease and exposure history. Over the past several decades, there have been several examples where fiber analysis produced unexpected results which were important in our understanding of disease-exposure relationships. It is the purpose of this presentation to summarize these fiber analysis vignettes.

## II. Analytical Methodology

The determination of concentrations of fibers in lung tissue samples involves several steps.[8] First is the selection of an appropriate specimen. Second involves the removal of the organic matrix, typically by wet chemical digestion. Third, the residue is collected on the surface of a filter. Fourth, the filter is mounted and prepared for examination by some form of electron microscopy. Some authors prefer to use transmission electron microscopy (TEM) while others have used scanning electron microscopy (SEM). A discussion of the relative advantages and disadvantages of SEM vs. TEM is beyond the scope of this paper but has been dealt with elsewhere.[8,9] Fiber types can be determined by means of energy dispersive x-ray analysis (EDXA). With TEM, additional information regarding crystalline structure may be readily obtained by selected area electron diffraction (SAED).

## III. Canadian Chrysotile Miners and Millers

When extensive epidemiological studies of Quebec chrysotile miners and millers were begun in the 1960s, it was believed that exposures in this cohort were only to chrysotile.[10] TEM studies of lung tissue samples from these miners and millers were conducted by Fred Pooley and the results reported in 1976.[11] To the surprise of these investigators, tremolite was found in concentrations similar to or even higher than chrysotile concentrations. Subsequent investigations showed that veins of tremolite occurred in association with chrysotile deposits, and tremolite exposures were greater in the Thetford area mines than in Asbestos. McDon-

ald and colleagues subsequently showed that tremolite levels were higher in the central as compared to the peripheral mines in Thetford, and the risk of mesothelioma was also greater in these central Thetford mines.[12]

Begin and colleagues challenged these conclusions, noting that mesothelioma rates were similar in miners and millers in Thetford as compared to Asbestos. Since tremolite levels were greater in Thetford mines, tremolite could not be the answer and it must be the chrysotile itself that was the main culprit.[13] However, subsequent studies showed that miners and millers from Asbestos had increased levels of crocidolite and/or amosite in their lung samples, whereas Thetford miners and millers had only tremolite and chrysotile.[14] Some crocidolite and amosite imported from South Africa had been used in the mills in Asbestos, accounting for the commercial amphibole exposures and the risk of mesothelioma in this cohort. Studies of other mesothelioma cases in Canada compared to controls similarly showed that mesothelioma risk is related to long (> 8 μm) amphibole fibers, with no apparent additional risk conferred by lung chrysotile content.[15]

In summary, these early studies set the groundwork for subsequent investigations showing a marked difference in potency between chrysotile and amphiboles in terms of mesothelioma risk.[6] In South African chrysotile mines where tremolite contamination is not observed, no increased mesothelioma risk has been identified.[16] In circumstances where an increased risk of mesothelioma from pure chrysotile exposure was claimed, SEM studies demonstrated that tremolite was indeed present and that tremolite to chrysotile ratios in lung samples were similar to those of Canadian chrysotile miners and millers.[17, 18]

## IV. Asbestos Textile Workers

In 1996 Allan Smith published a literature review claiming that chrysotile asbestos is the main cause of pleural mesothelioma.[19] Among the studies quoted in support of this proposition was that of Peto *et al.* regarding the Rochdale textile workers in the United Kingdom.[20] The type of asbestos used in the factory was predominantly chrysotile although small quantities of crocidolite had been historically used. Twelve deaths were due to pleural mesothelioma, and analysis of lung tissue samples for asbestos fiber content was undertaken using TEM.[21] Mineral fiber analysis was consistent with the known exposure to chrysotile, but the crocidolite content was about 300 times that of the general UK population. The finding of substantial crocidolite exposure implied that mesotheliomas that occurred in this textile factory could not be attributed with any certainty to chrysotile alone.

A similar situation exists with South Carolina asbestos textile workers, for which it has been claimed that 100 percent of the exposure was to chrysotile asbestos.[19, 22] Green and colleagues performed lung tissue analyses on 39 former asbestos textile workers and 31 controls.[23] Chrysotile was the predominant mineral fiber type identified along with substantial tremolite. However, crocidolite and amosite were also increased in the asbestos factory textile workers compared with controls, and in 28 percent of the workers exceeded one million fibers per gram of dry lung tissue.

More recently, Loomis and Dement published a survey of North Carolina asbestos textile workers, including four cases with mesothelioma from the Marshville plant, which according to the authors utilized only chrysotile.[24] The authors suggested that their findings would alter the potency ratios that had been published by Hodgson and Darnton.[6] However, one of the cases from this plant had been previously analyzed in our laboratory by means of SEM.[25] This 58 year old woman was a spinner, winder and weaver of chrysotile asbestos cloth, and as expected, elevated concentrations of chrysotile and tremolite were detected in her lung tissue samples. However, elevated concentrations of amosite were also detected. This patient's husband worked as an insulator and she regularly laundered his clothes. Domestic exposures are well-recognized sources of significant exposure to asbestos.[4, 25]

## V. Insulators

In 1965 Selikoff and colleagues reported that insulators had light and intermittent exposures to asbestos.[26] These investigators had previously reported high rates of lung cancer and mesothelioma in this cohort, and Becklake in 1976 reported that insulators were exposed almost exclusively to chrysotile.[27, 28] McDonald found a relative risk of mesothelioma of 46 for insulators, which was the highest of any of the categories studied.[10]

These disparate and contradictory observations are clarified by fiber analysis of lung tissue samples from individuals working with insulation products. Churg and Vedal reported fiber analysis results in 144 individuals from the Pacific Northwest, most of whom were insulators or shipyard workers (exposed to insulation products) or allied trades (*e.g.*, pipe fitters).[29] These authors used TEM and observed that amosite was the most common fiber type, often present at orders of magnitude higher levels than chrysotile or tremolite. For example, among 23 cases with asbestosis, the geometric mean amosite fiber count was 10 million fibers per gram of dry lung, whereas the corresponding values for chrysotile and tremolite were 0.005 and 0.034 million fibers per gram. The presence of specific diseases correlated with amosite concentrations but not with chrysotile or tremolite.

Most asbestos insulation products used in the United States prior to 1972 contained amosite and often chrysotile, so insulators were not exposed to chrysotile alone. The finding of high concentrations of amosite in these workers' lungs is at odds with the assessment of 'light and intermittent exposures to asbestos'. The author has analyzed lung tissue samples from 92 insulators, including 34 with mesothelioma and 43 with lung cancer[7, 9] (and unpublished observations). Forty-eight of the cases had asbestosis and 60 had pleural plaques. The median asbestos body count in 89 cases was 26,600 per gram of wet lung tissue (normal range 0-20 AB/gm). The median asbestos fiber count as determined by SEM was 300,000 fibers 5 μm or greater in length per gram of wet lung (median value for 20 controls was <600 fibers/gm). Similar to the findings of Churg and Vedal, the vast majority of fibers analyzed were amosite. Furthermore, insulators have the highest asbestos content of any occupational group that we have analyzed.[7, 30]

## VI. Railroad Workers

Railroad workers had ample opportunity for exposure to asbestos, especially during the steam era (up until approximately 1958) when large amounts of asbestos lagging were applied to and removed from the boilers of the steam engines. Mancuso reported on a cohort of railroad machinists with mesothelioma.[31, 32] These individuals often worked in the roundhouse, where repairs on locomotives were conducted. According to Mancuso, these workers were exposed almost exclusively to chrysotile.[31]

The author has had the opportunity to examine lung tissue samples from 33 individuals whose primary occupational exposure to asbestos was as a railroad worker [7-9, 30] (and unpublished observations). These included 12 patients with mesothelioma and 13 with lung cancer. Three patients had asbestosis and 13 had pleural plaques. The median asbestos body count was 68 AB/gm (normal range 0-20 AB/gm). The median asbestos fiber count as determined by SEM was 7670 fibers 5 μm or greater in length per gram of wet lung (median value for 20 controls was <600 fibers/gm). Amosite was identified in increased concentrations in many of these workers lungs, and in two cases with mesothelioma, crocidolite was the only commercial amphibole fiber type identified.[33] Tremolite and chrysotile fibers were also elevated in these latter two cases.

## VII. Jewelry Industry

In 1992, Kern et al reported a case of mesothelioma in an individual working in the jewelry industry.[34] The patient had worked for 35 years making asbestos soldering forms at a costume jewelry production

facility. Non-neoplastic asbestos-related disease had been described in similar workers, and it was believed that the exposure was to chrysotile asbestos.[35] The patient underwent an extrapleural pneumonectomy for his mesothelioma, and a fiber analysis was performed. There were 13,300 AB/gm of wet lung tissue by light microscopy, and there were 20,900 amosite fibers per gram of wet lung tissue by SEM. No chrysotile or tremolite was detected. On further investigation, it was determined that a distributor had supplied both chrysotile and amosite during the first 25 years that the patient fabricated soldering forms. The patient had also worked for nine months in the 1940s in a local shipyard, cleaning up after welders fabricating new hulls from steel plates.

## VIII. Auto Mechanics

Several investigators have expressed concern that auto mechanics might be at increased risk of asbestos related disease as a result of working with asbestos-containing friction products (brakes and clutches).[36-38] However, a number of epidemiological studies have failed to demonstrate an increased risk of mesothelioma among auto mechanics.[39-42] Furthermore, epidemiological analyses have concluded that working with friction products does not increase the risk of asbestos-related disease incurred from working with other asbestos products.[43] Some authors have thus concluded that whether or not asbestos exposure from brake and clutch repair work increases one's risk of mesothelioma is controversial.[44] It is therefore useful to examine the results of lung fiber analyses to see if they are informative in this regard.

Butnor *et al.* reported fiber analysis results on 10 cases of mesothelioma among individuals whose only known exposure to asbestos was from auto repair work.[45] In five of these individuals, the tissue asbestos content as determined by SEM was indistinguishable from that of our reference or control population. In five additional cases, there were elevated levels of commercial amphiboles (amosite in four cases, crocidolite in one). In three of these latter cases, either tremolite or chrysotile concentrations were also elevated. Since commercial amphiboles were not used in friction products in the United States, the authors concluded that these latter five individuals had some other unidentified exposure to asbestos. Marsh *et al.* reanalyzed these ten cases and added five additional cases, four of which had background levels of asbestos and one of which had elevated concentrations of crocidolite.[46] These authors concluded that there was a correlation between commercial amphibole levels and tremolite levels in these 15 cases, and further noted that there was no correlation between tremolite levels and duration of exposure as an auto mechanic. Together these findings added further support to the conclusion that the 6 individuals with elevated asbestos content had exposures in occupational settings other than brake repair work.

The author has had the opportunity to examine the tissue asbestos content in 33 individuals whose only known exposure to asbestos was working in the automotive industry, including 30 cases in the service industry, two in manufacturing, and one 'shade tree' mechanic[7, 9, 30, 46] (and unpublished observations). These included 17 with mesothelioma, 5 with lung cancer and 5 with pleural plaques. Ten cases had interstitial lung disease but none met criteria for asbestosis. The median asbestos body count was 12.5 AB/gm (normal range 0-20 AB/gm). The median asbestos fiber count by SEM was 1,040 fibers 5µm or greater in length per gram of wet lung. In addition, we have analyzed lung tissue from five household contacts of auto mechanics, and all five had tissue asbestos contents within the range of our reference population. A number of other investigators using TEM also reported either background levels of asbestos or elevated levels of commercial amphiboles among brake mechanics with mesothelioma.[47-50]

## IX. Summary and Conclusions

This report summarizes some of the more prominent and well-documented examples where electron microscopy has corrected misconceptions regarding asbestos and disease. The fiber analysis vignettes presented here are summarized in Table 1. There are numerous additional examples where electron microscopy has provided useful information regarding the causation of asbestos-associated diseases and their relationship to specific industrial or environmental exposures.

# Endnotes


1 Roggli VL, Oury TD, Sporn TA, eds. Pathology of Asbestos-Associated Diseases, 2nd Ed. Springer: New York, 2004.

2 Churg A. Nonneoplastic Disease Caused by Asbestos, CH 9, In: Pathology of Occupational Lung Disease, 2nd Ed. (Churg A, Green FHY, eds.), Williams & Wilkins: Baltimore, 1998, pg. 277.

3 Churg A. Neoplastic Asbestos-Induced Disease, CH 10, In: Pathology of Occupational Lung Disease, 2nd Ed. (Churg A, Green FHY, eds.), Williams & Wilkins: Baltimore, 1998, pg. 339.

4 Henderson DW, Rantanen J, Barnhart S, Dement JM, De Vuyst P, Hillerdal G, Huuskonen MS, Kivisaari L, Kusaka Y, Lahdensuo A, Langard S, Mowe G, Okubo T, Parker JE, Roggli VL, Rödelsperger K, Rösler J, Tossavainen A, Woitowitz HJ. Asbestos, asbestosis, and cancer: The Helsinki criteria for diagnosis and attribution. A consensus report of an international expert group. Scand J Work Environ Health 23: 311, 1997.

5 Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of the inhalation of asbestos in rats. Br J Cancer 29: 252, 1974.

6 Hodgson JT, Darnton A. The quantitative risk of mesothelioma and lung cancer in relation to asbestos exposure. Ann Occup Hyg 44: 565, 2000.

7 Roggli VL, Sharma A. Analysis of Tissue Mineral Fiber Content, CH 11, In: Pathology of Asbestos-Associated Diseases, 2nd Ed. (Roggli VL, Oury TD, Sporn TA, eds.), Springer: New York, 2004, pg. 309.

8 Roggli VL. Tissue Digestion Techniques, Appendix, In: Pathology of Asbestos-Associated Diseases, 2nd Ed. (Roggli VL, Oury TD, Sporn TA, eds.), Springer: New York, 2004, pg. 402.

9 Roggli VL, Vollmer RT. Twenty-five years of fiber analysis: What have we learned? Hum Pathol 39: 307, 2008.

10 McDonald JC. Epidemiology of malignant mesothelioma – An outline. Ann Occup Hyg 54: 851, 2010.

11 Pooley FD. An examination of the fibrous mineral content of asbestos in lung tissue from the Canadian chrysotile mining industry. Environ Res 12: 281, 1976.

12 McDonald AD, Case BW, Churg A, Dufresne A, Gibbs GW, Sebastien P, McDonald JC. Mesothelioma in Quebec chrysotile miners and millers: Epidemiology and aetiology. Ann Occup Hyg 41: 707, 1997.

13 Begin R, Gauthier J-J, Desmeules M, Ostiguy G. Work-related mesothelioma in Quebec, 1967-1990. Am J Ind Med 22: 531, 1992.

14 Dufresne A, Begin R, Churg A, Masse S. Mineral fiber content of lungs in patients with mesothelioma seeking compensation in Quebec. Am J Respir Crit Care Med 153: 711, 1996.

15 McDonald JC, Armstrong B, Case B, Doell B, McCaughey WTE, McDonald AD, Sebastien P. Mesothelioma and asbestos fiber type: Evidence from lung tissue analyses. Cancer 63: 1544, 1989.

16 White N, Nelson G, Murray J. South African experience with asbestos related environmental mesothelioma: Is asbestos fiber type important? Reg Toxicol Pharmacol 52 [Suppl 1]: S92, 2008.

17 Yano E, Wang Z-M, Wang X-R, Wang M-Z, Lan Y-J. Cancer mortality among workers exposed to amphibole-free chrysotile asbestos. Am J Epidemiol 154: 538, 2001.

18 Tossavainen A, Kotilainen M, Takahashi K, Pan G, Vanhala E. Amphibole fibres in Chinese chrysotile asbestos. Ann Occup Hyg 45: 145, 2001.

19 Smith AH, Wright CC. Chrysotile asbestos is the main cause of pleural mesothelioma. Am J Ind Med 30: 252, 1996.

[20] Peto J, Doll R, Hermon C, Binns W, Clayton R, Goffe T. Relationship of mortality to measures of environmental asbestos pollution in an asbestos textile factory. Ann Occup Hyg 29: 305, 1985.

[21] Wagner JC, Berry G, Pooley FD. Mesotheliomas and asbestos type in asbestos textile workers: A study of lung contents. Br Med J 285: 603, 1982.

[22] Dement JM, Brown DP, Okun A. Follow-up study of chrysotile asbestos textile workers: Cohort mortality and case-control analyses. Am J Ind Med 26: 431, 1994.

[23] Green FH, Harley R, Vallyathan V, Althouse R, Fick G, Dement J, Mitha R, Pooley F. Exposure and mineralogical correlates of pulmonary fibrosis in chrysotile asbestos workers. Occup Environ Med 54: 549, 1997.

[24] Loomis D, Dement JM, Wolf SH, Richardson DB. Lung cancer mortality and fiber exposures among North Carolina asbestos textile workers. Occup Environ Med 66: 535, 2009.

[25] Roggli VL, Oury TD, Moffatt EJ. Malignant mesothelioma in women. In: Anatomic Pathology, 1997, Vol. 2 (Rosen PP, Fechner RE, eds.), ASCP Press: Chicago, 1998, pg. 147.

[26] Selikoff IJ, Churg J, Hammond EC. Relation between exposure to asbestos and mesothelioma. N Engl J Med 272: 560, 1965.

[27] Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. JAMA 188: 22, 1964.

[28] Becklake MD. Asbestos-related disease of the lungs and other organs: Their epidemiology and implications for clinical practice. Am Rev Respir Dis 114: 187, 1976.

[29] Churg A, Vedal S. Fiber burden and patterns of asbestos-related disease in workers with heavy mixed amosite and chrysotile exposure. Am J Respir Crit Care Med 150: 663, 1994.

[30] Roggli VL, Sharma A, Butnor KJ, Sporn T, Vollmer RT. Malignant mesothelioma and occupational exposure to asbestos: A clinicopathological correlation of 1445 cases. Ultrastruct Pathol 26: 55, 2002.

[31] Mancuso TF. Relative risk of mesothelioma among railroad machinists exposed to chrysotile. Am J Ind Med 13: 639, 1988.

[32] Mancuso TF, Mesothelioma among machinists in railroad and other industries. Am J Ind Med 4: 501, 1983.

[33] Schneider F, Sporn TA, Roggli VL. Crocidolite and mesothelioma. Ultrastruct Pathol 32: 171, 2008.

[34] Kern DG, Hanley KT, Roggli VL. Malignant mesothelioma in the jewelry industry. Am J Ind Med 21: 409, 1992.

[35] Kern DG, Frumkin H. Asbestos-related disease in the jewelry industry: Report of two cases. Am J Ind Med 13: 407, 1988.

[36] Rohl AN, Langer AN, Wolff MS, Weisman I. Asbestos exposure during brake lining maintenance and repair. Environ Res 12: 110, 1976.

[37] Lemen RA. Asbestos in Brakes: Exposure and Risk of Disease. Am J Ind Med 45: 229, 2004.

[38] Welch LS. Asbestos Exposure Causes Mesothelioma, But Not *This* Asbestos Exposure: An Amicus Brief to the Michigan Supreme Court. Int J Occup Environ Health 13: 318, 2007.

[39] Goodman M, Teta MJ, Hessel PA, Garabrant DH, Craven VA, Scrafford CG, Kelsh MA. Mesothelioma and Lung Cancer among Motor Vehicle Mechanics: A Meta-analysis. Ann Occup Hyg 48: 309, 2004.

[40] Kelsh MA, Craven VA, Teta MJ, Mowat FS, Goodman M. Mesothelioma in vehicle mechanics: is the risk different for Australians? Occup Med 57: 581, 2007.

[41] Laden F, Stampfer MJ, Walker AM. Lung Cancer and Mesothelioma Among Male Automobile Mechanics: A Review. Rev Environ Health 19: 39, 2004.

[42] Wong O. Malignant Mesothelioma and Asbestos Exposure among Auto Mechanics: Appraisal of Scientific Evidence. Toxicol Pharmacol 84: 170, 2001.

[43] Hessel PA, Teta MJ, Goodman M, Lau E. Mesothelioma among Brake Mechanics: An Expanded Analysis of a Case-Control Study. Risk Analysis 24: 547, 2004.

[44] Hammar SP, Henderson DW, Klebe S, Dodson RF. Neoplasms of the Pleura, CH 43, In: Dail & Hammar's Pulmonary Pathology, 3rd Ed. (Tomashefski JF, Cagle PT, Farver CF, Fraire AE, eds.), Springer-Verlag: New York, 2008, pg. 558.

[45] Butnor KJ, Sporn TA, Roggli VL. Exposure to brake dust and malignant mesothelioma: A study of 10 cases with mineral fiber analyses. Ann Occup Hyg 47: 325, 2003.

[46] Marsh GM, Youk AO, Roggli VL. Asbestos fiber concentrations in the lungs of brake repair workers: Commercial amphibole levels are predictive of chrysotile levels. Inhal Toxicol 12: 681, 2011.

[47] Gordon RE, Dikman S. Asbestos fiber burden analysis of lung and lymph nodes in 100 cases of mesothelioma (abstr). Am J Respir Crit Care Med 179: A5892, 2009.

[48] Case BW. Exposure to brake dust and malignant mesothelioma: Lung-retained fibre analyses using transmission electron microscopy confirm previous findings at lower magnification by scanning electron microscopy (abstr.), presented at the British Occupational Hygiene Society, Stratford upon Avon, UK, April 5-7, 2011.

[49] Dodson RF, Hammar SP, Poye LW. A technical comparison of evaluating asbestos concentration by phase-contrast microscopy (PCM), scanning electron microscopy (SEM), and analytical transmission electron microscopy (ATEM) as illustrated from data generated from a case report. Inhal Toxicol 20: 723, 2008.

[50] Dodson RF, Graef R, Shepherd S, O'Sullivan M, Levin J. Asbestos burden in cases of mesothelioma from individuals from various regions of the United States. Ultrastruct Pathol 29: 415, 2005.

**Table 1. Examples Where Electron Microscopy Has Improved Understanding or Corrected Misconceptions Regarding Asbestos and Disease**

| Exposure Category | Misconception or Belief | Electron Microscopy Findings | References |
|---|---|---|---|
| Canadian chrysotile miners and millers | Exposure only to chrysotile | High levels of tremolite found in lungs of miners and millers | 10-12 |
| Thetford vs Asbestos miners and millers | Similar mesothelioma rates although tremolite exposure much higher in Thetford miners and millers | Miners and millers from Asbestos with mesothelioma have elevated levels of commercial amphiboles (amosite and/or crocidolite) | 13, 14 |
| Asbestos textile workers | These workers exposed exclusively or almost exclusively to chrysotile | Many of these workers have elevated levels of commercial amphiboles (amosite and/or crocidolite) in their lung tissue samples | 19-25 |
| Insulators | Insulators had light and intermittent exposures to chrysotile asbestos | Insulators have high concentrations of asbestos in their lung tissue samples, primarily amosite | 7, 9, 26, 28-30 |
| Railroad workers | Railroad machinists are exposed almost exclusively to chrysotile | Railroad workers with mesothelioma typically have elevated levels of amosite or crocidolite | 7, 9, 30-33 |
| Jewelry industry | Jewelry soldering board fabricators are exposed to chrysotile asbestos | Jewelry maker with mesothelioma had elevated levels of amosite Soldering boards were fabricated from chrysotile and amosite | 34, 35 |
| Auto mechanics | Exposure to chrysotile from brake and clutch repair work causes mesothelioma | Auto mechanics either have background asbestos contents (60%) or elevated levels of commercial amphiboles with or without elevated chrysotile or tremolite | 36-38, 45-50 |

STUDY No. 13

# HISTORICAL AMBIENT AIRBORNE ASBESTOS CONCENTRATIONS IN THE UNITED STATES – AN ANALYSIS OF PUBLISHED AND UNPUBLISHED LITERATURE (1960s – 2000s)

**STUDY No. 13**

# HISTORICAL AMBIENT AIRBORNE ASBESTOS CONCENTRATIONS IN THE UNITED STATES – AN ANALYSIS OF PUBLISHED AND UNPUBLISHED LITERATURE (1960s – 2000s)

**Inhalation Toxicology, 2015; 27(4); 754-766**

**Anders Abelmann, Meghan E. Glynn, Jennifer S. Pierce, Paul K. Scott, Samantha Serrano, and Dennis J. Paustenbach**

Outdoor concentrations of airborne asbestos have been measured throughout the US over time. However, a thorough review and analysis of these data has not been conducted. The purpose of this study is to characterize asbestos concentrations in ambient air by environment type (urban, rural) and by decade, using measurements collected in the absence of known asbestos emission sources. A total of 17 published and unpublished studies and datasets were identified that reported the results of 2058 samples collected from the 1960s through the 2000s across the US. Most studies did not report asbestos fiber type, and data based on different analytical methods (e.g. Phase Contrast Microscopy, Transmission Electron Microscopy, etc.) were combined in the dataset; however, only fibers ≥5 $\mu$m in length were considered. For a small subset of the measurements (n = 186, 9.0%), a conversion factor was used to convert mass-based data (e.g. ng/m$^3$) to count-based values (i.e. f/cc ≥5 $\mu$m). The estimated overall mean and median ambient asbestos concentrations for the 1960s through 2000s were 0.00093 f/cc and 0.00022 f/cc, respectively. Concentrations generally increased from the 1960s through the early 1980s, after which they declined considerably. While asbestos use decreased throughout the 1970s, these results indicate that ambient concentrations peaked during the early 1980s, which suggests the possible contribution of abatement or demolition activities. Lastly, ambient asbestos concentrations were higher in urban than rural settings, which is consistent with the greater use of asbestos-containing materials in more densely populated areas.

Inhalation Toxicology

http://informahealthcare.com/iht
ISSN: 0895-8378 (print), 1091-7691 (electronic)

Inhal Toxicol, 2015; 27(14): 754–766
 DOI: 10.3109/08958378.2015.1118172

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

# Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature (1960s–2000s)

Anders Abelmann[1], Meghan E. Glynn[1], Jennifer S. Pierce[1], Paul K. Scott[2], Samantha Serrano[3], and Dennis J. Paustenbach[3]

[1]Cardno ChemRisk, Chicago, IL, USA, [2]Cardno ChemRisk, Pittsburgh, PA, USA, and [3]Cardno ChemRisk, San Francisco, CA, USA

**Abstract**

Outdoor concentrations of airborne asbestos have been measured throughout the US over time. However, a thorough review and analysis of these data has not been conducted. The purpose of this study is to characterize asbestos concentrations in ambient air by environment type (urban, rural) and by decade, using measurements collected in the absence of known asbestos emission sources. A total of 17 published and unpublished studies and datasets were identified that reported the results of 2058 samples collected from the 1960s through the 2000s across the US. Most studies did not report asbestos fiber type, and data based on different analytical methods (e.g. Phase Contrast Microscopy, Transmission Electron Microscopy, etc.) were combined in the dataset; however, only fibers $\geq$5 μm in length were considered. For a small subset of the measurements ($n = 186$, 9.0%), a conversion factor was used to convert mass-based data (e.g. ng/m$^3$) to count-based values (i.e. f/cc $\geq$5 μm). The estimated overall mean and median ambient asbestos concentrations for the 1960s through 2000s were 0.00093 f/cc and 0.00022 f/cc, respectively. Concentrations generally increased from the 1960s through the early 1980s, after which they declined considerably. While asbestos use decreased throughout the 1970s, these results indicate that ambient concentrations peaked during the early 1980s, which suggests the possible contribution of abatement or demolition activities. Lastly, ambient asbestos concentrations were higher in urban than rural settings, which is consistent with the greater use of asbestos-containing materials in more densely populated areas.

**Keywords**

Ambient levels, asbestos, fibers, outdoor concentrations

**History**

Received 15 June 2015
Revised 29 September 2015
Accepted 5 November 2015
Published online 15 December 2015

## Introduction

Between the 1960s and the late 2000s, outdoor airborne asbestos fiber concentrations have been measured in both rural and urban environments in the US. Although the published literature contains a reasonably large number of datasets containing information on ambient asbestos, a thorough review and analysis of these data has not been conducted. The purpose of this study was to characterize historical to present day measurements of asbestos in outdoor air throughout the US, using measurements that were collected in the absence of known or potential asbestos emission sources. The data were also evaluated for temporal and spatial trends.

## Background

Asbestos is ubiquitous in the environment in the US (ATSDR, 2001). Sources of this asbestos are primarily related to anthropogenic activities, such as mining, milling, manufacturing and use of asbestos-containing materials (ACM), and transport and disposal of associated waste. Furthermore, in some areas of the US, concentrations of airborne asbestos are due to weathering of naturally occurring asbestos (NOA) in seams of exposed rock (ATSDR, 2001; IARC, 2012; NTP, 2014; U.S. EPA, 2010).

In the US, the use of asbestos has decreased dramatically from its peak in the mid-1970s (Figure 1) (Virta, 2002, 2006, 2010, 2014), mainly as a result of regulations promulgated as a response to concerns regarding health hazards associated with occupational exposures. As a consequence, emissions of asbestos to the environment have decreased; however, the presence of and activities involving ACM likely continue to affect the ambient air concentrations of asbestos to the present day (ATSDR, 2001; NTP, 2014; Wylie & Candela, 2015). For example, as noted by Wylie & Candela (2015), between 2003 and 2013, an average of 6500 tons of ''friable'' asbestos was disposed of or otherwise released to the environment annually in the US (U.S. EPA, 2015). Currently, there are no federal standards for outdoor air concentrations of asbestos to which the general public is exposed each day.

### Naturally occurring asbestos

Weathering of NOA and anthropogenic activities, such as excavation, agriculture, mining and road construction can

Address for correspondence: Dr. Anders Abelmann, PhD, Cardno ChemRisk, LLC, 30 N. LaSalle Street, Suite 3910, Chicago, IL 60602, USA. Tel: (312) 726-2154. Fax: (312) 726-2334. E-mail: anders.abelmann@cardno.com

DOI: 10.3109/08958378.2015.1118172



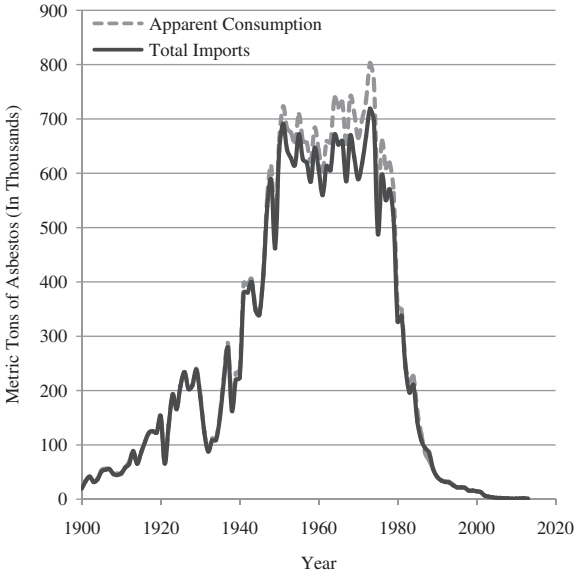



Figure 1. US historical asbestos imports and consumption 1900–2013 [based on data reported by Virta (2006, 2010, 2014)].

result in the release of measurable concentrations of asbestos fibers in the environment (Gunter et al., 2007; Hendrickx, 2009; Van Gosen, 2007; Walton, 1982). At least 35 states in the US have reported findings of NOA, with the major areas of concern located along the Appalachian Mountains and in the Western Cordillera (Harper, 2008; U.S. EPA, 2008). Additionally, large areas of exposed ultramafic bedrock in northern California, some now densely populated by housing and infrastructure, have become the focus of attention after they were found to contain chrysotile and tremolite-actinolite asbestos (Churchill & Hill, 2000; Clinkenbeard et al., 2002; Lee et al., 2008; Ross & Nolan, 2003). Furthermore, Wylie & Candela (2015) recently estimated that in the US, 400 tons of amphibole asbestos is released annually through weathering of naturally occurring minerals.

## Mining and milling

In the US, asbestos was mined commercially beginning in 1900, and mining occurred over time in 15 different states, with the largest production occurring in Arizona, California, North Carolina and Vermont (Virta, 2006). Asbestos production reached its maximum in 1973, after which it declined rapidly until the last mine closed in 2002 (Van Gosen, 2007; Virta, 2006). The vast majority of the total cumulative asbestos production in the US, as well as most imported materials, was chrysotile, whereas amphibole asbestos was imported to the US mainly from South Africa and Australia (Ross & Virta, 2001; Virta, 2002, 2006). However, some of the early production in the US was amphibole asbestos, and while such mines were most often short-lived, anthophyllite asbestos was mined in North Carolina from the early 1930s until 1979 (Virta, 2006). The control of emissions from mining and milling operations was reportedly often poor; however, very little quantitative data characterizing airborne asbestos concentrations in the ambient air near these operations exist (Gibbs & Du Toit, 1973; Harwood & Blaszak, 1974).

## Manufacturing operations

According to the US Environmental Protection Agency (EPA), manufacture of ACM occurred in at least 22 states (U.S. EPA, 1974a,b). Predominant asbestos-containing products historically manufactured in the US include cement pipe and sheets, and flooring, friction and roofing products, which together made up about 60 to 70% of the country's total annual demand for asbestos (USBM, 1976, 1985). Amid rising health concerns and associated regulations, asbestos use in the manufacture of commercial products in the US declined from a peak of about 800 000 metric tons in 1973 to 217 000 in 1983, and subsequently to 33 000 in 1992 (U.S. EPA, 1993b; USBM, 1976, 1985). Estimates of US asbestos consumption for recent years indicate that the decreasing trend has continued, with industry demand falling from 2230 metric tons in 2006 to 772 in 2013 (Virta, 2008, 2010, 2014).

Emissions of asbestos fibers from manufacturing operations occurred due to inadequate controls, through accidental releases, and from handling, transporting, and storing waste materials (Bruckman & Rubino, 1975; Harwood & Blaszak, 1974; U.S. EPA, 1981). Specifically, prior to regulations instituted in the early 1970s waste piles were often located in or near densely populated areas and were frequently kept uncovered, resulting in uncontrolled emissions of asbestos to the ambient environment (Harwood & Blaszak, 1974).

## Use of asbestos-containing products

Asbestos has been incorporated in various products because of its low cost and desirable qualities, such as heat and fire resistance, wear and friction characteristics, tensile strength, heat, electrical and sound insulation, adsorption capacity, and resistance to chemical and biological attack (ATSDR, 2001). Asbestos-containing products utilized in construction, industry, commerce and in the military can be found in encapsulated and friable forms. Encapsulated products, such as wallboard, ceiling tile, molded phenolic materials, roofing, piping and floor tile, contain asbestos fibers that are bound in a matrix such that they do not spontaneously release fibers unless the materials are disturbed (Lee & Van Orden, 2008; Michaels & Chissick, 1979; U.S. EPA, 1985). However, damage to some encapsulated ACM from severe weather, chemicals and mechanical forces (i.e. cutting, drilling, sanding or breaking) may result in release of fibers (U.S. EPA, 1985, 1990). Conversely, by definition, friable materials contain more than 1% asbestos and can be crumbled, pulverized or reduced to powder by hand pressures (U.S. EPA, 1985). Examples of friable products include sprayed or troweled-on materials on ceilings, walls and other surfaces (e.g. for decoration, fireproofing, or heat and sound insulation), as well as insulation on pipes, tanks, ducts and other equipment (Mangold, 1983). The application or use of such products was widespread from the 1950s to the mid-1970s (Mangold, 1983; U.S. EPA, 1987).

*Friction materials*

Beginning in the early 1900s, asbestos was used in the manufacture of automotive friction products (i.e. brakes and clutches); from 1965 to 2001, an estimated 1.4 million tons of chrysotile asbestos were used in friction products in the US (Paustenbach et al., 2003, 2004; Virta, 2006). Although wear debris from these products could be released into the environment during the high temperature and mechanical forces of the braking and clutching processes, asbestos fibers in friction materials degraded into forsterite, leaving only a very small percentage (<1%) of the chrysotile fibers intact. Therefore, the contribution to ambient concentrations of asbestos fiber from chrysotile-containing friction materials was limited. Forsterite has a chemical composition similar to chrysotile, but the material is amorphous and non-fibrous and does not pose the same threat to human health as asbestos (Anderson et al., 1973; Cha et al., 1983; Hickish & Knight, 1970; Jacko et al., 1973; Luxon, 1970; Lynch, 1968; Rowson, 1978; Sheehy et al., 1989; Williams & Muhlbaier, 1982). Furthermore, as noted by Langer (2003) and others, any remaining chrysotile fibers in the brake wear debris (i.e. those not subjected to mechanical destruction or thermal transformation to forsterite) do not retain their natural properties or biological activity.

*Shipyards*

Until the late 1970s, asbestos-containing insulation was used extensively within naval ships, and was composed mainly of amosite (up to 86%), and to a much lesser extent, chrysotile (Franke & Paustenbach, 2011; Murbach et al., 2008). Amosite was principally used due to its thermal conductivity, resistance to spreading fires, physical and chemical stability, light weight, strength and refractoriness. Additionally, sections of molded, fragile, amosite-containing insulation were typically covered with a protective layer of chrysotile asbestos (Fleischer & Viles, 1946; Harries, 1971; Rushworth, 2005). Between the 1930s and the 1970s, 30 to 500 tons of asbestos insulation could be used aboard a single warship (Murbach et al., 2008; Rushworth, 2005). Because of increasing concerns related to asbestos health risks, by the 1970s the amount had been reduced to between 3 and 50 tons per ship (Murbach et al., 2008; Rushworth, 2005). Subsequently, most ACM (e.g. gaskets, packing and insulation) were replaced by asbestos-free alternatives in the mid- or late 1970s, and, by 1979, the US Navy ceased using asbestos aboard warships completely (Hollins et al., 2009; Murbach et al., 2008; Rushworth, 2005). During the time that ACM were used in shipbuilding, fiber releases to the ambient air could have occurred due to inadequate exposure control policies, such as the use of dilution ventilation or ''open doors'' instead of local exhaust ventilation (Marr, 1964). In addition, asbestos-containing insulation materials were also fabricated at the shipyards, which could have resulted in further emissions into the environment (Hollins et al., 2009).

Substantial releases of asbestos fibers can also occur during the overhaul, repair and disposal of ships (Andersen, 2001; Harries, 1971; U.S. EPA, 2000). The disposal of a ship (also known as dismantling) entails removal of equipment, components and consumables for reuse or resale, whereupon the ship is broken up and the material is recycled or disposed of (Andersen, 2001). Inadequate procedures for removing ACM, such as pipe insulation or blankets could result in incidental asbestos releases. Exposure and emissions data related to handling of ACM during ship dismantling operations are not readily available. However, it is likely that although the use of ACM in shipbuilding was discontinued over 30 years ago, the sustained use of historical vessels, as well as maintenance activities will continue to be a source of asbestos emissions into the ambient environment. In addition, given that the service life of a warship can be up to 35 years, decommissioning of ships containing ACM will likely continue far into the twenty-first century (Koenig et al., 2008).

## Regulation of asbestos in ambient air

There are no current federal standards that limit the concentration of asbestos in ambient air in the US. Rather, federal regulations set forth restrictions on (1) emission levels from known point sources, (2) the manufacture, importation, processing and distribution of certain asbestos-containing products and ''new uses'' of asbestos, and (3) the use and handling of ACM during construction, demolition and renovation (U.S. EPA, 1988, 1993a, 1999a).

Starting in the early 1970s and driven by increasing concerns regarding health effects associated with exposure to asbestos, various restrictions and regulations related to ACM were instituted. However, it is interesting to note that demand for asbestos in the US did not peak until the late 1970s. US EPA's first regulations related to airborne asbestos were proposed in 1971 and promulgated in 1973 under the Clean Air Act; National Emissions Standards for Hazardous Air Pollutants (NESHAP) were intended to protect the public by minimizing release of fibers into the atmosphere during activities that involved processing, handling and disposal of ACM (i.e. materials containing more than 1% asbestos) during building demolition activities (U.S. EPA, 1971, 1973). NESHAP prohibited ''visible emissions'' from asbestos milling and nine major manufacturing operations through the use of process controls, such as air cleaning equipment and during demolition of structures that contained asbestos (U.S. EPA, 1973).

Through NESHAP, the US EPA also prohibited sprayed-on application of friable ACM for fireproofing and insulation and established routine maintenance procedures (including adequate wetting of any friable fireproofing, insulation or asbestos-insulated pipe prior to removal from a building) for handling ACM on boilers, pipe or load-bearing structural members (U.S. EPA, 1973). Over the following years, the regulations were amended to include additional activities (e.g. fabrication and renovation) and materials (U.S. EPA, 1974c, 1977, 1999b). Notably, in 1978, the US EPA extended its original ban on spray-on asbestos insulation to include banning all uses of spray-on ACM for decorative purposes (U.S. EPA, 1977). In addition, in the late 1970s, the US EPA began to focus on friable ACM in schools by initiating technical assistance programs and publishing guidance documents, as well as enacting and expanding the Asbestos Hazard Emergency Response Act (AHERA) in

DOI: 10.3109/08958378.2015.1118172 

1982 and 1986, respectively (U.S. EPA, 1979a,b, 1987). In addition, several states have established their own emission levels or ambient air standards (e.g. State of Connecticut, 2006; State of Vermont, 2011).

## Methods

### Identification of data sources

A thorough search of the peer-reviewed literature and other publicly available documents was performed to identify asbestos air sampling data for ambient settings in the US using several database search engines (e.g. PubMed, NTIS, Medline, TOXNET, ScienceDirect, ProQuest). To locate additional studies, a systematic review was performed of the reference lists of all studies identified by the initial search, as well as of key review papers. Furthermore, relevant entities were contacted to acquire any supplementary data. If multiple studies presented results pertaining to the same dataset, the study with the original data and/or that contained the final results (as opposed to preliminary analyses) was selected for analysis.

During the literature search, an unpublished dataset was identified, consisting of data collected by the RJ Lee Group, Inc., in urban settings across the US between 1986 and 1998 (Personal Communication, Van Orden, RJ Lee Group, 2013); a summary was previously reported by Lee & Van Orden (2008). These data were reportedly collected as reference samples in relation to demolition and asbestos abatement work at various worksites. For purposes of the analysis presented in this manuscript, only samples collected prior to any work commencing were included. Similarly, all samples collected in locations with known sources of NOA were excluded.

Data for ambient settings were defined as samples collected outdoors in the absence of a known or potential emission source. Data collected near operations likely to be associated with emissions of asbestos fibers, such as a shipyard or asbestos mine, were excluded. In some studies, such as those related to asbestos remediation work, both source-related and ambient data were reported; for such instances, only reference samples collected prior to this type of work were included.

Whenever possible, the following elements were identified and abstracted for each measurement; detailed descriptions of several of these elements are provided below:

- Sample collection date (year)
- Sampling location (name of city or town, state)
- Population category (urban, rural, unknown)
- Sample identifier
- Sampling duration
- Analytical method
- Analytical limit of detection
- Fiber definition or counting protocol (length and/or aspect ratio)
- Concentration (including units of measurement)

#### *Population category*

Sampling locations were classified as urban or rural, as designated by the study authors. If such designations were not explicitly assigned, data from the US census conducted on or immediately prior to the sampling year were used to classify the location based on population size. Locations with populations exceeding 50 000 persons were considered urban and populations less than or equal to 50 000 persons were considered rural (U.S. Census Bureau, 2013). If sampling location could not be determined, the population category was classified as ''unknown''.

#### *Analytical methods*

Several analytical methods exist for analyzing airborne asbestos samples. In addition, fiber counting protocols (i.e. the definition of what was counted as an asbestos fiber) varied between studies regardless of the analytical method used. Below is a brief description of analytical methods that were identified in the studies that were included in this analysis. Asbestos fibers are typically characterized by their length and aspect ratio (i.e. ratio of fiber length to width). All count-based samples which counted only those fibers equal to or longer than 5 μm, regardless of the analytical method employed or the aspect ratio considered, were combined into one category (i.e. ''$\geq$5 μm''). Thus, any count-based measurements for which fiber length was reported as $<5$ μm, or where fibers of ''all lengths'' were counted, were excluded from this analysis.

- *Phase contrast microscopy (PCM)*: PCM samples are analyzed using a standard optical microscope. In brief, any structure $\geq$5 μm and with a length to width ratio $\geq$3:1 is counted as a fiber; however, only fibers $\geq$0.2 μm are visible, and hence the presence of thinner fibers would not be detected. This method is often favored because it is relatively inexpensive and simple, and sample preparation is straightforward and does not require analysts to use specialized equipment (e.g. a complex electron microscope) (Perry, 2004). This method, however, cannot distinguish between asbestos and non-asbestos structures (e.g. asbestos versus fiberglass), or between asbestos fiber types (e.g. chrysotile versus amphiboles); therefore, in the presence of other fibers, the PCM method may overestimate the actual asbestos fiber concentration. Although in the past, researchers may incorrectly have referred to fiber concentrations based on PCM as ''asbestos fiber concentrations'', for the current analysis, fibers were assumed to be asbestos if the original study reported them as such.
- *Transmission electron microscopy (TEM) and electron microscopy (EM)*: The TEM technique relies on electron microscopy, rather than optical microscopy; thus, unlike PCM, it can be used to help distinguish between asbestiform and non-asbestiform structures, and also between different types of asbestos fibers based on their crystal structures (U.S. EPA, 1987). TEM also has much greater resolution than PCM, and can better detect fibers $<$5 μm in length and $<$0.2 μm in width (Kauffer et al., 1996; Mossman et al., 1990). Disadvantages of TEM include higher equipment costs and increased level of training required for operators (Stewart, 1988). Results from TEM analysis can be used to determine the percentage of asbestos fibers of all fibers in a sample.

For three additional studies included in this analysis, the analytical method was reported simply as ''electron microscopy'' (EM); for the purpose of this analysis, it was assumed that TEM was utilized for these samples. Additionally, the International Organization for Standardization (ISO) has developed a TEM method (i.e. ISO 10312) for determining concentration of asbestos fibers in ambient air (ISO, 1995). The main difference between the ISO TEM method and the TEM method most often employed in the US (i.e. NIOSH 7402) is that individual fibers are counted even when they are located inside higher-order structures when employing the ISO TEM protocol (ISO, 1995; NIOSH, 1994b). Data collected using the ISO TEM method were kept separate from other TEM and EM data in the analysis. Regardless of the protocol, only fibers reported as $\geq 5\,\mu m$ in length were included in the analysis.

- *Scanning electron microscopy (SEM)*: Like TEM, the SEM technique relies on electron microscopy rather than optical microscopy. SEM offers similar advantages as TEM, but has less sensitivity for smaller fibers (Burdett & Jaffrey, 1986). For purposes of the current analysis, only fibers with a reported length of $\geq 5\,\mu m$ were included in the dataset.

*Analytical limits of detection*

In a majority of studies included in this analysis, an analytical method-specific limit of detection (LOD) was provided. In three studies (Chesson et al., 1985; Nicholson et al., 1975; Sawyer, 1977), no LOD was provided, but one or more values of 0 f/cc (or equivalent) were reported. For the purposes of this analysis, it was assumed that these values were below the LOD, and a default value of 0.0010 f/cc was assigned based on the current LOD for PCM as described by OSHA and NIOSH; this is also the typical working minimum LOD for TEM (NIOSH, 1994a,b; OSHA, 1997).

As a result of the range of LODs reported across the studies included in the dataset, concentrations reported as detects in some data subsets were lower than the LODs for other samples. To assess the effect of this overlap, ranges of both detected data and data below the LOD are presented separately.

*Concentrations of asbestos in air and units of measurement.* Because the data considered were generated using a variety of analytical techniques, the dataset contained asbestos concentrations that were reported in both count-based [e.g. f/cc, structures per cc (s/cc), etc.] and mass-based (e.g. $ng/m^3$) units. To enable comparisons between these various units of measurement, a literature search was conducted to identify conversion factors to be used to convert mass-based concentrations to count-based (i.e. f/cc) concentrations. In 1986, the US EPA published a report, which included an evaluation of available mass-to-count concentration conversion factors based on samples collected in five studies conducted in occupational settings (principally manufacturing), as well as during one laboratory study (U.S. EPA, 1986). Based on these six studies, the agency suggested that $30\,\mu g/m^3$ per f/cc be used as a general conversion factor when count-based data were not available. This value was calculated as the geometric mean of the reported conversion factors, which ranged from 5 to $150\,\mu g/m^3$ per f/cc for fibers $\geq 5\,\mu m$. However, from a review of the studies included in US EPA's analysis, as well as the scientific literature as a whole, it was found that the conversion factor derived from Davis et al. (1978) of $5\,\mu g/m^3$ per f/cc may be more appropriate for use in the current analysis. This factor was based on the results of laboratory experiments that were conducted to determine the mass-to-fiber relationship using pure chrysotile asbestos (Davis et al., 1978). Thus, unlike conversion factors derived based on PCM measurements collected in occupational settings [such as those cited by the U.S. EPA (1986)], the mass-fiber ratios reported by Davis et al. (1978) would not have been influenced by the presence of non-asbestos fibers. This outcome was desirable since all mass-based concentrations included in this study were the result of TEM analysis, and were thus a measure of asbestos only (and not other agents or dusts). Moreover, given that chrysotile asbestos was the most widely used fiber type in the US, it would be expected that it would be the primary asbestiform fiber type found in the ambient air.

## Data analysis

Statistical analyses were performed using R software (V3.2.0, The R Foundation for Statistical Computing, Vienna, Austria) and the NADA package for R (Lee, 2013). Data were not normally or log-normally distributed, and as noted above a large fraction were below various LODs; thus, non-parametric methods were used for all statistical analyses. For the same reason, the median concentrations were estimated and were presented alongside the mean concentrations.

The reverse Kaplan-Meier (KM) estimator was used to estimate the mean and median asbestos concentrations, because this method tends to be insensitive to outliers and a good choice for analysis of relatively small datasets (Antweiler & Taylor, 2008; Gillespie et al., 2010). Mean asbestos concentrations were estimated by calculating the area between the reverse KM estimator's cumulative density function (CDF) and 1 for data $>0$; which is equivalent to summing the products of each detected value and its corresponding probability (Gillespie et al., 2010). Similarly, the median asbestos concentration was determined to be the smallest detected value in any given data subset for which the respective CDF was $\geq 0.5$. When the CDF exceeded 0.5 at the minimum detected concentration and the smallest value was $<$ LOD, the software did not estimate a median. For these subsets, the minimum detected concentration was reported as the median, and this was denoted in the table.

A high percentage of data was below the LOD in some subsets of the data used in the study. Traditional statistical methods for testing differences between subsets of data would not be informative or even appropriate. Therefore, comparisons by environment type (urban versus rural) or decade were instead evaluated by comparing differences in the proportion of samples for which asbestos concentrations exceeded a pre-selected cut-off value. The cut-off value was representative of an upper bound for the LODs reported across all the data sets. Because of the wide range of LODs for data below the detection limits across the studies evaluated (a result of the

DOI: 10.3109/08958378.2015.1118172 



variety of sampling and analytical methods), it was not feasible to select a cut-off point above the highest reported LOD. Therefore, the cut-off point was chosen as the 95th percentile of all reported LOD values and was estimated to be 0.0046 f/cc. The concept of using the 95th percentile as the cut-off point is commonly used in statistical analyses. The proportions of measurements above the cut-off for different subsets were compared through statistical analysis: a two-sample test of proportions was used to compare two subsets, and the Holm-Bonferroni corrected pair-wise two-sample test of proportions was used to compare more than two subsets simultaneously (Holm, 1979).

## Results

A total of 16 studies, either published in the scientific literature or as reports, were identified that described the results of 381 samples collected from the 1960s through the 1980s, and the 2000s in at least 30 states across the US (i.e. some studies only reported ''US'') (ATSDR, 2007; Baxter et al., 1983; Bruckman, 1978; Chesson et al., 1985; Heffelfinger et al., 1972; LeMoine, 1981; Mangold, 1982, 1983; Nicholson, 1971; Sawyer, 1977; U.S. EPA, 1974d, 1975, 2007, 2009; Wendlick, 1983, 1984). As noted above, a previously unpublished dataset was also identified, consisting of data ($n = 1677$) collected by the RJ Lee Group, Inc., between 1986 and 1998 in urban settings across at least 34 states in the US (Personal Communication, Van Orden, 2013); a summary was previously reported by Lee & Van Orden (2008). Thus, the total dataset contained 2058 data points collected in at least 40 states across the US between the 1960s and 2000s. A description of the studies included in this analysis is presented in Table 1.

The descriptive statistics for the overall dataset are presented in Table 2. The overall mean and median ambient asbestos concentrations based on all data were 0.00093 f/cc and 0.00022 f/cc, respectively. Histograms depicting the overall data distribution, as well as for subsets of data based on environment type and decade are included in Supplementary Appendix A.

### Environment type

The mean ambient asbestos concentrations in urban ($n = 1954$) and rural ($n = 102$) settings were 0.0011 f/cc and 0.00039 f/cc, respectively (Table 2). The median ambient asbestos concentration was 0.00050 f/cc in urban settings and 0.000020 f/cc in rural environments. The percentages of urban and rural data that exceeded the 95th percentile of the reported LOD values (i.e. 0.0046 f/cc) were 8.1 and 3.9%, respectively. There was no statistically significant difference between these two proportions ($p = 0.13$).

Data for both urban and rural settings were only available for the 1970s. While the mean rural concentration (0.0018 f/cc) exceeded the mean urban concentration (0.0010 f/cc) in the 1970s, the median urban concentration (0.00060 f/cc) was considerably higher than the median rural concentration (0.000021 f/cc). Surprisingly, while 19% of samples collected in rural settings in the 1970s exceeded the 95th percentile of the reported LOD values, only 4.6% of urban samples collected during the 1970s exceeded this cut-off; these two proportions were found to be statistically significantly different ($p = 0.017$).

### Temporal trends

As seen in Table 2, ambient air samples collected in the 1960s ($n = 64$), 1970s ($n = 132$), 1980s ($n = 659$), 1990s ($n = 1122$) and 2000s ($n = 81$) were identified. The mean ambient asbestos concentrations for these decades were 0.0012, 0.0011, 0.0022, 0.0016 and 0.000017 f/cc, respectively. The median asbestos concentrations were 0.00028, 0.00044, 0.00090, 0.0016 and 0.000014 f/cc. As shown in Table 2, the percentages of data that exceeded the 95th percentile of the reported LOD values varied, and increased from 4.7% in the 1960s to 6.8% in the 1970s and 20% in the 1980s. Then it decreased to 1.7% in the 1990s and 0% in the 2000s. The proportion of measurements exceeding the cut-off for the 1980s was statistically significantly different than for all other decades ($p$ values ranging from $<0.0001$ to 0.029), and the proportion from the 1970s was statistically significantly different than the proportion from the 1990s ($p = 0.0043$).

### Converted values

Approximately 9.0% ($n = 186$) of the samples included in this dataset were reported as mass-based concentrations (Table 3). These data were based on five studies (Bruckman, 1978; Heffelfinger et al., 1972; Nicholson, 1971; U.S. EPA, 1974d, 1975) and included samples collected in the 1960s and 1970s. The overall mean concentration for the converted data was 0.00096 f/cc, which is within a factor of 1.2 of the overall mean concentration for the non-converted data (0.00080 f/cc). However, the median concentration for the converted data (0.00032 f/cc) exceeded that for the non-converted data (0.000014 f/cc) by over 20-fold.

### Methods of analysis

Most ambient air samples were analyzed using TEM or EM [$n = 1943$; range: $<0.000031$–0.019 f/cc (note: the highest LOD was 0.025 f/cc)], with the others analyzed by PCM ($n = 105$; range: $<0.0010$–0.050 f/cc), SEM ($n = 9$; range: $<0.0000024$–0.016 f/cc), and ISO ($n = 1$; $< 0.00010$ f/cc). The mean and median PCM concentrations were higher than those reported for samples analyzed by TEM and SEM, which may be a result of date of sampling (1970s and early 1980s for all PCM, while a majority of TEM samples were collected in the 1990s), or the fact that PCM does not distinguish between asbestos and non-asbestos fibers.

## Discussion

The task of identifying data relevant to describe ambient concentrations of asbestos in the US over time, as described above, was rather straightforward. Anderson et al. (2015) recently published a review of ''ambient air asbestos concentrations''. There was some overlap between the data evaluated by Anderson et al. (2015) and those included in the current analysis; however, their dataset was smaller, the inclusion criteria appear to have been less rigorous (e.g. some data which could be attributed to point sources were

Table 1. Overview of studies reporting ambient asbestos concentrations.

| Reference | Objective for sample collection | Ambient data selected[a] | Sampling year | Analysis method | Sample size | Sampling location[b] | Environmental type |
|---|---|---|---|---|---|---|---|
| ATSDR (2007) | Evaluation of potential asbestos contamination of public beach | Reference samples | 1998 | TEM | 7 | Zion, IL | Rural |
| Baxter et al. (1983) | Evaluation of ambient concentrations throughout California | | 1983 | SEM | 9 | Various locations: CA | Urban, rural |
| Bruckman & Rubino (1978) | Study of airborne asbestos fiber concentrations in Connecticut | | 1977 | TEM | 2 | CT | Urban, rural |
| Chesson et al. (1985) | Project related to ACM removal in schools | Reference samples collected five months after completion project | 1985 | PCM | 6 | US, no further details provided | Urban |
| Heffelfinger et al. (1960s, 1970s) | Study to develop new sampling methods for ambient asbestos | Reference samples | 1972 | TEM | 33 | Various locations: CA, DC, KY, OH, PA, TX | Urban, rural |
| LeMoine (1981) | A survey of the asbestos levels in the ambient air of Seattle | | 1980 | PCM | 36 | Seattle, WA | Urban |
| Mangold (1982) | Evaluation of contribution to ambient concentrations from certain products | Reference samples | 1982 | PCM | 18 | Various locations: WA, OR | Urban |
| Mangold (1983) | Evaluation of ambient concentrations in the Greater San Francisco area | | 1983 | PCM | 25 | Various locations: CA | Urban |
| Nicholson (1960s, 1970s) | Evaluation of ambient concentrations in the U.S. | | 1971 | NR | 117 | Various locations: CO, GA, IL, MA, MD, MI, OH, TX | Urban |
| Personal Communication, Van Orden, RJ Lee Group (2013)[c] | Unpublished data collected as reference samples prior to demolition activities | | 1986–1998 | TEM | 1677 | Various locations and states ($n_{states} = 34$) | Urban |
| Sawyer (1977) | Evaluation of asbestos concentrations in buildings | Reference samples | 1977 | PCM | 1 | New Haven, CT | Urban |
| U.S. EPA (1974d) | Evaluation of ambient concentrations in Minnesota | | 1974 | EM | 33 | Various locations and states ($n_{states} = 17$) | Urban, rural |
| U.S. EPA (1975) | Evaluation of asbestos concentrations in buildings | Reference samples | 1975 | EM | 1 | Berkeley, CA | Urban |
| U.S. EPA (2007) | Evaluation of naturally occurring asbestos | Reference samples | 2007 | TEM | 1 | Laramie County, WY | Rural |
| U.S. EPA (2009) | Evaluation of methods for reducing exposures to naturally occurring asbestos | Reference samples | 2006–2008 | TEM | 73 | Eureka, MT, Helena, MT | Rural |
| Wendlick (1983) | Evaluation of contribution to ambient concentrations from certain products | Reference samples | 1983 | PCM | 10 | Portsmouth, VA, Newport News, VA | Urban |
| Wendlick (1984) | Study of airborne asbestos fiber concentrations in Pennsylvania | | 1984 | PCM | 9 | Philadelphia, PA | Urban |

[a]Unless otherwise noted, any data reported which met the inclusion criteria of the current study were selected for analysis.
[b]NR = Not Reported.
[c]A summary of these data was previously reported by Lee & Van Orden (2008).

DOI: 10.3109/08958378.2015.1118172 

Table 2. Descriptive statistics of ambient asbestos concentrations, overall and by environment type and decade.

| Data subset | | *n* | Fraction <LOD (%) | Fraction of converted samples[a] (%) | Range of values < Limit of detection (LOD) (f/cc) | Range of detected values (f/cc) | Median[b] (f/cc) | Mean[b] (f/cc) | Fraction > Cut-off[c] (%) |
|---|---|---|---|---|---|---|---|---|---|
| Overall | | 2058 | 84.9 | 9.0 | <0.0000024 – <0.025 | 0.0000048–0.050 | 0.00022 | 0.00093 | 7.9 |
| Environment | Urban | 1954 | 85.3 | 8.8 | <0.00071–<0.025 | 0.000040–0.050 | 0.00050 | 0.0011 | 8.1 |
| | Rural | 102 | 76.5 | 14.7 | <0.0000024–<0.0027 | 0.0000048–0.013 | 0.000020 | 0.00039 | 3.9 |
| | Unknown | 2 | 100 | 0 | <0.0000024[d] | – | – | – | – |
| Decade | 1960s | 64 | 0 | 100 | – | 0.000040–0.019 | 0.00028 | 0.0012 | 4.7 |
| | 1970s | 132 | 6.1 | 92.4 | <0.0000024–<0.0020 | 0.0000048–0.016 | 0.00044 | 0.0011 | 6.8 |
| | 1980s | 659 | 83.6 | 0 | <0.00071–<0.025 | 0.00010–0.050 | 0.00090 | 0.0022 | 20 |
| | 1990s | 1122 | 99.2 | 0 | <0.0017–<0.0069 | 0.0016–0.0037 | 0.0016 | 0.0016 | 1.7 |
| | 2000s | 81 | 92.6 | 0 | <0.000031– <0.0027 | 0.000014_0.000092 | 0.000014 | 0.000017 | 0 |
| Urban | 1960s | 64 | 0 | 100 | – | 0.000040–0.019 | 0.00028 | 0.0012 | 4.7 |
| | 1970s | 109 | 2.8 | 98.2 | <0.0010–<0.0020 | 0.000040–0.016 | 0.00060 | 0.0010 | 4.6 |
| | 1980s | 659 | 83.6 | 0 | <0.00071–<0.025 | 0.00010–0.050 | 0.00090 | 0.0022 | 20 |
| | 1990s | 1122 | 99.2 | 0 | <0.0017–<0.0069 | 0.0016–0.0037 | 0.0016 | 0.0016 | 1.7 |
| | 2000s | 0 | – | – | – | – | – | – | – |
| Rural | 1960s | 0 | – | – | – | – | – | – | – |
| | 1970s | 21 | 14.3 | 71.4 | <0.0000024–<0.0020 | 0.0000048–0.013 | 0.000021 | 0.0018 | 19 |
| | 1980s | 0 | – | – | – | – | – | – | – |
| | 1990s | 0 | – | – | – | – | – | – | – |
| | 2000s | 81 | 92.6 | 0 | <0.000031–<0.0027 | 0.000014–0.000092 | 0.000014 | 0.000017 | 0 |

[a]Conversion factor derived from Davis et al. (1978) (i.e. 5 μg/m$^3$ per f/cc).
[b]Calculated using the reverse Kaplan-Meier estimator.
[c]Reported as percentage of samples with a concentration above 0.0046 f/cc (i.e. the 95th percentile of all LOD values in the dataset).
[d]All samples below the LOD.
–: Data not available/not applicable.

included), and no summary statistics were presented (i.e. only ranges were reported). Therefore, to the best of the authors' knowledge, this study represents the most comprehensive and exhaustive analysis of the body of literature on this topic to date.

During the past decade, a commonly cited reference for ambient asbestos concentrations in the US has been a report from the Agency for Toxic Substances and Disease Registry (ATSDR) on the toxicology of asbestos (2001). In the current analysis, all of the references cited by the ATSDR were evaluated in detail and incorporated into the dataset if they met the inclusion criteria. Notably, the ATSDR did not conduct a statistical analysis per se, but rather reported several ranges of ambient asbestos concentrations identified in the literature. As such, the ATSDR report is similar to the recent Anderson et al. (2015) publication, while the current analysis provides a more thorough evaluation and analysis of the available data.

The results suggest that ambient asbestos concentrations in urban environments were generally higher than in rural environments. Specifically, the median concentration for all samples collected in urban environments was 25-fold higher than the median concentration for all rural samples. This relationship held true for the 1970s, the only decade for which samples were collected in both urban and rural environments, whereby the median concentration for samples collected in urban environments was nearly 29-fold higher than the median concentration for rural samples. While the mean concentration for rural samples collected in the 1970s was higher than in urban areas, due to the small sample size for rural environments, the mean was greatly influenced by a limited number of samples collected in Minnesota and California. It is possible that these concentrations were impacted by nearby (but not identified) sources of asbestos, such as NOA or mining operations. Because these samples skewed the distribution of this subset of data, the median concentration is likely a better indicator of the central tendency. The observation of higher ambient air asbestos concentrations in urban versus rural environments may be explained by factors, such as the greater usage of friable ACM in more densely populated areas, especially in the construction industry where spray insulation was common.

Overall, when considering both the estimated concentrations and the percentage of data above the cut-off value of 0.0046 f/cc, ambient asbestos concentrations generally increased from the 1960s through the 1980s, after which they declined considerably. The continued increase in ambient air concentrations of asbestos throughout the 1980s was somewhat unexpected given that use of ACM in construction decreased dramatically throughout the 1970s. However, this observation may be the result of new federal regulations promulgated by the US EPA that led to increased abatement and demolition activity, which possibly resulted in uncontrolled emissions of asbestos. This conclusion is further supported by a granular analysis of the 1980s data, which revealed that a majority of the higher concentrations were from samples collected in the early 1980s. The higher concentrations in the early 1980s may also be an artifact of the inclusion of PCM data, which, as previously noted, may overestimate the true asbestos fiber concentration compared

Inhal Toxicol, 2015; 27(14): 754–766

Table 3. Descriptive statistics of ambient asbestos concentrations by conversion factor and analytical method.

| Data subset | Environmental Type | *n* | Fraction < LOD (%) | Fraction of converted samples (%) | Range of values < LOD (f/cc) | Range of detected values (f/cc) | Median[a] (f/cc) | Mean[a] (f/cc) |
|---|---|---|---|---|---|---|---|---|
| *Mass- to count-based conversions* | No conversion | 1872 | 93.2 | 0 | <0.0000024–<0.025 | 0.000014–0.050 | 0.000014[c] | 0.00080 |
| | Urban | 1783 | 93.4 | 0 | <0.00071–<0.025 | 0.00010–0.050 | 0.00060 | 0.0013 |
| | Rural | 87 | 88.5 | 0 | <0.0000024–<0.0027 | 0.000014–0.013 | 0.000014[c] | 0.00032 |
| Converted data[b] | Overall | 186 | 1.6 | 100 | <0.0010–<0.0020 | 0.0000048–0.019 | 0.00032 | 0.00096 |
| | Urban | 171 | 1.2 | 100 | <0.0010–<0.0020 | 0.000040–0.019 | 0.00040 | 0.00097 |
| | Rural | 15 | 6.7 | 100 | <0.0020 | 0.0000048–0.0066 | 0.000020 | 0.00077 |
| *Analytical method* | TEM/EM | 1943 | 89.3 | 9.6 | <0.000031–<0.025 | 0.0000048–0.019 | 0.00014 | 0.00044 |
| | ISO | 1 | 100 | 0 | <0.00010[d] | – | – | – |
| | SEM | 9 | 44.4 | 0 | <0.0000024 | 0.0019– 0.016 | 0.0019 | 0.0056 |
| | PCM | 105 | 5.7 | 0 | <0.0010–<0.0020 | 0.00010–0.050 | 0.0063 | 0.0086 |

[a]Calculated using the reverse Kaplan-Meier estimator.
[b]Conversion factor derived from Davis et al. (1978) (i.e. 5 μg/m$^3$ per f/cc).
[c]As described in the ''Methods'' section, the median was not estimated for these subsets because the cumulative density function (CDF) exceeded 0.5 at the minimum detected concentration, and the minimum concentration was < LOD. Therefore, the reported value is the minimum detected concentration.
[d]All samples below the LOD.

to TEM. To evaluate the impacts of the inclusion of PCM data, all analyses were rerun following the exclusion of the PCM dataset (Tables B1–B3 in Supplementary Appendix B). This exclusion had no considerable impact on the time-trend analysis.

The median ambient asbestos concentration for count-based data was considerably lower (i.e. by a factor of >20) than the estimated median for the converted mass-based data, this possibly because mass-based data were only available for the 1960s and 1970s, whereas the highest count-based concentrations were reported for the 1980s. It could also be a function of the variability in the LODs across studies, with the range of LODs for converted data being wider than for non-converted data (Table 3). These differences could also be a result of the conversion factor chosen to convert mass-based concentrations to count-based concentrations. To evaluate the potential impact of the choice of conversion factor on the results, the dataset was analyzed using the conversion factor suggested by the US EPA (30 μg/m$^3$ per f/cc; as described in the ''Methods'' section). These results are shown in Tables B4 and B5 in Supplementary Appendix B. The overall mean ambient concentration using this conversion factor instead of the Davis et al. (1978) conversion factor is 0.00069 f/cc, which is 1.3 times lower than the overall mean of 0.00093 f/cc from Table 2. Similarly, the overall median using this conversion factor is 0.000043 f/cc, which is 5.1 times lower than 0.00022 f/cc from Table 2. For converted data only, the overall mean concentrations using the Davis et al. (1978) and US EPA conversion factors were 0.00096 f/cc and 0.00016 f/cc, respectively; the corresponding median concentrations were 0.00032 f/cc and 0.000053 f/cc, respectively. The six-fold difference in concentrations determined using the two conversion factors corresponds exclusively to the relative magnitude of the two factors. Moreover, as a result of this six-fold difference, concentrations for subsets with high percentages of converted data (i.e. 1960s = 100%, 1970s = 92.4%) were found to be much lower when using the US EPA factor versus the Davis et al. (1978) factor, and when compared to other subsets of data with no converted measurements, such as the 1980s, these estimates appear to be lower than expected.

One limitation of the dataset was the combination of data collected using various analytical methods (i.e. PCM, TEM, SEM, ISO). Thus, data meeting either the simple criteria of ≥5 μm in length and a length-to-width ratio of >3:1 were combined, regardless of fiber type and diameter. Ideally, all data should have been converted to a single metric (e.g. PCME) prior to conducting any statistical analysis, but sufficient information for such conversions was not available for the vast majority of data points. Furthermore, as was noted by the ATSDR (2001, pp. 157–158) and the U.S. EPA (2001), the relationship between TEM and PCM fiber counts is too variable to allow for development of a conversion factor to be used across settings and fiber types. Nonetheless, the aggregation of data generated using different analytical methods may have had an impact on the results. For example, as noted in the ''Methods'' section, PCM does not distinguish between asbestos and other fibers, and the inclusion of PCM data may have inflated the concentrations for certain subsets of the data. Similarly, TEM analysis allows for detecting fibers with a diameter <0.2 μm, which could have inflated the

DOI: 10.3109/08958378.2015.1118172 

resulting concentrations compared to if PCME data had been available. However, because certain subsets of the data heavily relied upon one analytical method (e.g. all early 1980s data were based on PCM, whereas all 1960s and almost all 1970s data were based on TEM or EM), it was deemed appropriate to include all metrics, to enable further statistical analyses (e.g. evaluations of concentration differences over time).

Considerable efforts were taken to identify all published and unpublished air sampling data for ambient asbestos; however, additional studies and reports containing relevant data may still exist. No attempt was made to differentially weight the studies in this analysis; however, there is likely some degree of variability in the quality of data collection and interpretation methods, particularly with respect to air sampling techniques and fiber counting protocols. One likely reason for the scarcity of data in some subsets is that, in general, sampling strategies for outdoor concentrations of asbestos may have entailed characterizing the highest potential ''near source'' concentrations (i.e. data not considered in this analysis). This strategy is analogous to that commonly employed in occupational settings, in which sampling generally is conducted in areas with high potential for exposure (Damiano & Mulhausen, 1998). Indeed, in several of the datasets used in the current analysis, only background or reference samples were identified as ambient data. Some of the studies included in this analysis were conducted in order to investigate the potential impact on ambient concentrations from certain point sources. For example, Mangold (1982, 1983) noted the likely contribution to ambient fiber concentrations in Bremerton, WA from a nearby shipyard. This is an important finding, although for purposes of the current analysis, per the inclusion criteria, samples specific to such settings (i.e., ''near a known source'') were excluded from the dataset.

As shown in Tables 2 and 3, a limitation of this analysis was the high percentage of data below the LOD for certain subsets, especially for some of the later decades (e.g. 99.2% of samples collected in the 1990s and 92.6% of samples collected in the 2000s were $<$LOD). The statistical analysis attempted to account for these issues by comparing the proportions of samples below an expected upper-bound LOD for different data subsets, but lack of actual detected concentrations most likely has impacted the results nonetheless. It is also possible that any identified temporal trends are due to the decrease in the LODs over time instead of actual concentration decreases. For example, it is unknown whether a concentration reported as $<$LOD in the 1970s is higher or lower than a concentration reported as $<$LOD in the 2000s, yet due to the lower LOD in the 2000s, the resulting mean concentration may have been estimated to be lower than that in the 1970s solely due to the lower LOD. However, this is unlikely to account for the entire difference, given the drastic decline in asbestos use in the US between the 1970s and the present.

Moreover, for purposes of comparing the results from this analysis to those generated using other standard methods, an additional analysis was performed in which all measurements below the LOD were assumed to be equal to the LOD/2. This method is commonly used to deal with values below the LOD, but has limitations (e.g. there is a potential for bias, for data sets with multiple LODs, when some LODs are in the upper range of the actual data distribution) compared to the more appropriate methods employed in the current analysis (Gillespie et al., 2010). However, with few exceptions, the results of this additional analysis were consistent with those generated using the reverse KM method. There were a limited number of instances in which the reverse KM method generated a considerably lower estimate than the LOD/2 substitution method. For example, this was the case for the mean for the overall dataset and the 2000s data, and the median for the urban environment data. Nonetheless, this result was to be expected, as the reverse KM estimator considers the distribution of the data when calculating means (graphic representations of distributions are shown in Supplementary Appendix A), whereas the LOD/2 substitution method does not. In other words, for the overall dataset, the detected values tended to fall below the LOD/2 values; therefore, the mean concentration estimated using the reverse KM estimator was lower than the mean estimated using the LOD/2 method.

Lastly, the unpublished RJ Lee Group dataset represented a majority of the data included in this analysis, especially for urban environments in the 1990s. Given that these data were collected in a variety of sampling locations across the US (including many different cities and states), they were considered to be an important addition to the literature and this analysis. Nonetheless, in an effort to evaluate the relative impact of the RJ Lee Group data on the overall results, the statistical analysis was also performed without this dataset; the results are shown in Tables B6 and B7 in Supplementary Appendix B. Notably, the overall trends and conclusions did not change when evaluating the results of this alternative analysis.

## Conclusions

In conclusion, the objective of this research was to provide a review and analysis of concentrations of asbestos in the ambient air in the US over time, an analysis that has not been published in the scientific literature. Based on the results of this study, it was found that ambient air concentrations of asbestos were higher in urban versus rural environments. In addition, ambient asbestos concentrations likely peaked sometime in the 1980s, and appear to have declined since then. These results are consistent with the patterns of use of asbestos in the US, and suggest that federal regulations introduced in the 1970s (such as asbestos abatement) aimed at decreasing asbestos exposure in the general public may have resulted in an unintended and transient increase in the ambient air concentration of asbestos. These data may also be useful in retrospectively assessing human exposures to asbestos present in ambient air.

## Acknowledgements

The authors would like to thank Dr Peter Ruestow of Cardno ChemRisk and Dr Brent D. Kerger of Exponent for providing valuable technical feedback. In addition, we thank Dr Richard J. Lee and Drew Van Orden of the RJ Lee Group for giving us permission to use their data in this analysis.

## Declaration of interest

Cardno ChemRisk is a consulting firm which has been engaged by companies that have been, and are, involved in asbestos-related litigation, to provide general consulting and expert advice on scientific matters, as well as litigation support. This work was funded in its entirety by Cardno ChemRisk, and the preparation of the paper is the exclusive professional work of the authors. Drs Pierce and Paustenbach have served as experts in asbestos-related litigation, and they, along with others, may be called upon in the future to serve as expert witnesses in related litigation.

## References

Andersen AB. (2001). Worker safety in the ship-breaking industries. Sectoral Activites Programme. Geneva, Switzerland: International Labour Office (ILO).

Anderson AE, Gealer RL, McCune RC, Sprys JW. (1973). Asbestos emissions from brake dynamometer tests. SAE Paper No. 730549. New York, NY: Society of Automotive Engineers, Inc.

Anderson KE, Hoppe Parr KA, Boyd CA. (2015). A literature review of the ambient air asbestos concentrations. J Safety Health Environ Persp 11:211–21.

Antweiler AC, Taylor HE. (2008). Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. summary statistics. Environ Sci Technol 42:3732–8.

ATSDR. (2001). Toxicological profile for asbestos. Atlanta, GA: U.S. Department of Health and Human Services (DHHS), Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR).

ATSDR. (2007). Health consultation: asbestos contamination at Illinois Beach State Park. Zion, Lake County, Illinois. EPA Facility ID: ILD984840140. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR).

Baxter D, Ziskind R, Shokes R. (1983). Final report: ambient asbestos concentrations in California, Volume I. Sacramento, CA: California Air Resources Board.

Bruckman L. (1978). A study of airborne asbestos fibers in Connecticut. Proceedings of the Workshop on Asbestos: Definitions and Measurement Methods held; July 18–20, 1977; NBS Gaithersburg, MI (Issued November 1978). National Bureau of Standards Special Publication 506.

Bruckman L, Rubino RA. (1975). Asbestos: rationale behind a proposed air quality standard. J Air Pollut Control Assoc 25:1207–15.

Burdett GJ, Jaffrey S. (1986). Airborne asbestos concentrations in buildings. Ann Occup Hyg 30:185–99.

Cha S, Carter P, Bradow RL. (1983). Simulation of automobile brake wear dynamics and estimation of emissions, SAE Paper no. 831036. Warrendale, PA: Society of Automotive Engineers, Inc.

Chesson J, Margeson DP, Ogden J, et al (1985). Evaluation of asbestos abatement techniques, phase 1: removal. Washington, DC: U.S. Environmental Protection Agency, Office of Toxic Substances.

Churchill RK, Hill RL. (2000). A general location guide for ultramafic rocks in California – areas more likely to contain naturally occurring asbestos. Sacramento, CA: California Department of Conservation, Division of Mines and Energy.

Clinkenbeard JP, Churchill RK, Lee K. (2002). Guidelines for geologic investigations of naturally occurring asbestos in California: Sacramento. California Geological Survey Special Publication 124.

Damiano J, Mulhausen J. (1998). A strategy for assessing and managing occupational exposures. Fairfax, VA: American Industrial Hygiene Association (AIHA) Press.

Davis JM, Beckett ST, Bolton RE, et al (1978). Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. Br J Cancer 37:673–88.

Fleischer WE, Viles Jr FJ, et al (1946). A health survey of pipe covering operations in constructing naval vessels. J Ind Hyg Toxicol 28:9–16.

Franke K, Paustenbach D. (2011). Government and Navy knowledge regarding health hazards of asbestos: a state of the science evaluation (1900 to 1970). Inhal Toxicol 23:1–20.

Gibbs GW, Du Toit RSJ. 1973. Environmental data in mining. In: Bogovski P, Gilson J, Timbrell V, Wagner J. (eds.) Biological effects of asbestos. Lyon, France: International Agency for Research on Cancer (IARC), 138–44.

Gillespie BW, Chen Q, Reichert H, et al (2010). Estimating population distributions when some data are below a limit of detection by using a reverse Kaplan-Meier estimator. Epidemiology 21:S64–70.

Gunter ME, Sanchez MS, Williams TJ. (2007). Characterization of chrysotile samples for the presence of amphiboles: The Carey Canadian Deposit, Southeastern Quebec, Canada. Can Mineral 45: 263–80.

Harper M. (2008). 10th Anniversary critical review: naturally occurring asbestos. J Environ Monit.10:1394–408.

Harries PG. (1971). Asbestos dust concentrations in ship repairing: a practical approach to improving asbestos hygiene in naval dockyards. Ann Occup Hyg 14:241.

Harwood CF, Blaszak TP. (1974). Characterization and control of asbestos emissions from open sources. Chicago, IL: IIT Research Institute.

Heffelfinger RE, Melton CW, Kiefer DI. (1972). Development of a rapid survey method of sampling and analysis for asbestos in ambient air. Battelle Laboratories, Prepared for: U.S. Environmental Protection Agency (U.S. EPA), Division of Atmospheric Surveillance, Air Quality Analytical Branch, Contract no. CPA 22-69-110.

Hendrickx M. (2009). Naturally occurring asbestos in eastern Australia: a review of geological occurrence, disturbance, and mesothelioma risk. Environ Geology 57:909–26.

Hickish DE, Knight KL. (1970). Exposure to asbestos during brake maintenance. Ann Occup Hyg 13:17–21.

Hollins DM, Paustenbach DJ, Clark K, Mangold CA. (2009). A visual historical review of exposure to asbestos at puget sound naval shipyard (1962-1972). J Toxicol Environ Health B Crit Rev 12:124–56.

Holm S. (1979). A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70.

IARC. (2012). Volume 100: a review of carcinogens. Part C: arsenic, metals, fibres, and dusts. Lyon, France: International Agency for Research on Cancer.

ISO. (1995). Ambient air – determination of asbestos fibres – direct transfer transmission electron microscopy method. ISO Standards, Air Quality, Ambient Atmospheres. ISO 10312:1995. Geneva, Switzerland: International Organization of Standards (ISO).

Jacko MG, DuCharme RT, Somers JH. (1973). Brake and clutch emissions generated during vehicle operation, SAE Paper No. 730548. New York, NY: Society of Automotive Engineers, Inc.

Kauffer E, Vigneron JC, Fabries, et al (1996). The use of a new static device based on the collection of the thoracic fraction for the assessment of the airborne concentration of asbestos fibres by transmission electron microscopy. Ann Occup Hyg 40:311–19.

Koenig P, Nalchajian D, Hootman J. (2008). Ship service life and naval force structure. Engineering the total ship. Tysons Corner, VA: American Society of Naval Engineers.

Langer AM. (2003). Reduction of the biological potential of chrysotile asbestos arising from conditions of service on brake pads. Regul Toxicol Pharmacol 38:71–7.

Lee L. (2013). NADA: Nondetects and data analysis for environmental data. R Package Version: 1-5. Available from: https://cran.r-project.org/web/packages/NADA/index.html. [Last accessed: 12 Aug 2015].

Lee RJ, Strohmeier BR, Bunker KL, Van Orden DR. (2008). Naturally occurring asbestos: a recurring public policy challenge. J Hazard Mater 153:1–21.

Lee RJ, Van Orden DR. (2008). Airborne asbestos in buildings. Regul Toxicol Pharmacol 50:218–25.

LeMoine, GS. (1981). A survey of the asbestos levels in the ambient air of Seattle [thesis]. Seattle. WA: Public Health and Community Medicine, University of Washington.

Luxon S. (1970). Technical implementation of the new asbestos regulations. Ann Occup Hyg 13:23–4.

Lynch JR. (1968). Brake lining decomposition products. J Air Pollut Control Assoc 18:824–6.

Mangold C. (1982). The actual contribution of Garlock asbestos gasket materials to the occupational exposure of asbestos workers. Bellevue, WA: Environmental Control Sciences, Inc.

Mangold C. (1983). Asbestos fibers in the ambient air in the greater San Francisco area. Bellevue, WA: Environmental Control Sciences, Inc.

DOI: 10.3109/08958378.2015.1118172 

Marr WT. (1964). Asbestos exposure during naval vessel overhaul. Am Ind Hyg Assoc J 25:264–8.

Michaels L, Chissick SS. (1979). Asbestos: properties, applications, and hazards. Vol. 1. Chichester: John Wiley and Sons.

Mossman BT, Bignon J, Corn M, et al (1990). Asbestos: scientific developments and implications for public policy. Science 247: 294–301.

Murbach DM, Madl AK, Unice KM, et al (2008). Airborne concentrations of asbestos onboard maritime shipping vessels (1978-1992). Ann Occup Hyg 52:267–79.

Nicholson WJ. (1971). Measurement of asbestos in ambient air, Final Report; National Air Pollution Administration (Contract CPA 70-92).

Nicholson WJ, Rohl AN, Weisman I. (1975). Asbestos contamination of the air in public buildings. Research Triangle Park, NC: U.S. Environmental Protection Agency.

NIOSH. (1994a). NIOSH Method 7400: asbestos and other fibers by phase contrast microscopy. NIOSH manual of analytical methods National Institute for Occupational Safety and Health. Cincinnati, OH: National Institute for Occupational Safety and Health.

NIOSH. (1994b). NIOSH Method 7402: asbestos by transmission electron microscopy (TEM). NIOSH manual of analytical methods. DHHS Publication No. 94-113. Washington, DC: National Institute for Occupational Safety and Health.

NTP. (2014). Report on carcinogens. 13th Edition. Research Triangle Park, NC: U.S. Department of Health and Human Services. Public Health Service. National Toxicology Program (NTP).

OSHA. (1997). Asbestos in air. ID-160. Sampling and analytical methods. Salt Lake City, UT: Branch of Physical Measurements and Analysis, Occupational Safety and Health Administration (OSHA) Salt Lake Technical Center.

Paustenbach DJ, Finley BL, Lu ET, et al. (2004). Environmental and occupational health hazards associated with the presence of asbestos in brake linings and pads (1900 to present): a ''state-of-the-art'' review. J Toxicol Environ Health B Crit Rev 7:25–80.

Paustenbach DJ, Richter RO, Finley BL, Sheehan PJ. (2003). An evaluation of the historical exposures of mechanics to asbestos in brake dust. Appl Occup Environ Hyg 18:786–804.

Perry A. (2004). A discussion of asbestos detection techniques for air and soil. Washington, DC: Prepared for USEPA, Office of Solid Waste and Emergency Response.

Ross M, Nolan RP. (2003). History of asbestos discovery and use and asbestos-related disease in context with the occurrence of asbestos within ophiolite complexes. In: Dilek Y, Newcomb S. (eds.) Ophiolite concept and the evolution of geological thought: Geological Society of America Special Papers. Boulder, CO: Geological Society of America, 447–70.

Ross M, Virta RL. (2001). Occurrence, production and uses of asbestos. Can Mineral Spec Publ 5:79–88.

Rowson DM. (1978). The chrysotile content of wear debris of brake linings. Wear 47:315–21.

Rushworth DH. (2005). The Navy and asbestos thermal insulation. Nav Eng J 117:35–48.

Sawyer RN. (1977). Asbestos exposure in a Yale building. Analysis and resolution. Environ Res 13:146–69.

Sheehy JW, Cooper TC, O'Brien DM, et al (1989). Control of asbestos exposure during brake drum service. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Services (DHHS), Center for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health (NIOSH).

State of Connecticut. (2006). Hazard limiting values and ambient air quality standards. Hazardous Air Pollutants. Connecticut Regulations for the Abatement of Air Pollution. Section 22a-174-29. Department of Environmental Protection (CDEP), State of Connecticut.

State of Vermont. (2011). Air pollution control regulations. Air Pollution Control Division, Department of Environmental Conservation, Agency of Natural Resources.

Stewart IM. (1988). Asbestos — analytical techniques. Appl Indust Hygiene 3:F24-F26, R-3.

U.S. Census Bureau. (2013). Urban and rural classification. U.S. Department of Commerce, U.S. Census Bureau. Available from: http://www.census.gov/geo/reference/urban-rural.html. [Last accessed: 10 March 2013].

U.S. EPA. (1971). National Emission Standards for Hazardous Air Pollutants (NESHAP): proposed standards for asbestos, beryllium, mercury. 36 FR 23239-23256. 7 Dec 1971.

U.S. EPA. (1973). Title 40 — protection of environment, Chapter 1, Subchapter C. Air programs, Part 61. National emission standards for hazardous air pollutants: asbestos, beryllium, and mercury. 38 FR 8820-8850. 6 April 1973.

U.S. EPA. (1974a). Development document for effluent limitations guidelines and new source performance standards for the textile, friction materials and sealing devices segment of the asbestos manufacturing. Point Source Category. Washington, DC: U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=10004JLM.txt. [Last accessed: 2 Sep 2015].

U.S. EPA. (1974b). Development document for effluent limitations guidelines. Building, construction, and paper segment of the asbestos manufacturing. Point Source Category. Washington, DC: U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=10004CFZ.txt. [Last accessed: 2 Sep 2015].

U.S. EPA. (1974c). Part 61. National emission standards for hazardous air pollutants: asbestos, beryllium, and mercury. 39 FR 15396-15398. 3 May 1974.

U.S. EPA. (1974d). A preliminary report on asbestos in the Duluth, Minnesota Area. United States Environmental Protection Agency (U.S. EPA), Office of Enforcement and General Counsel, Office of Technical Analysis. Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000UW9G.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1975). Asbestos contamination of the air in public buildings. Research Triangle Park, NC: U.S. Environmental Protection Agency (U.S. EPA), Office of Air and Waste Management, Office of Air Quality Planning and Standards. Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100MKAP.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1977). National emission standards for hazardous air pollutants: proposed amendments to asbestos standard, 40 CFR Part 61. 42 FR 12122-12123. 2 March 1977.

U.S. EPA. (1979a). Asbestos-containing materials in school buildings: a guidance document. Part 1. U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100F2AH.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1979b). Asbestos-containing materials in school buildings: a guidance document. Part 2. U.S. Environmental Protection Agency (U.S. EPA). Available from: http://eric.ed.gov/?id=ED170947. [Last accessed: 2 Sep 2015].

U.S. EPA. (1981). Characterizing baghouse performance to control asbestos manufacturing source emissions. Cincinnati, OH: U.S. Environmental Protection Agency (U.S. EPA), Industrial Environmental Research Laboratory. Available from: http:/nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000TKK2.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1985). Guidance for controlling asbestos-containing materials in buildings. Washington, D.C.: Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000I0LQ.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1986). Airborne asbestos health assessment update. Washington, DC: U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=20009EBT.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1987). Asbestos-containing materials in schools; Final Rule and Notice (AHERA), 40 CFR Part 61. 52 FR 41826-41905. 30 October 1987.

U.S. EPA. (1988). Asbestos-in-schools: a guide to new federal requirements for local education agencies. Washington, DC: Office of Toxic Substances, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000UJP8.TXT. [Last accessed: 2 Sep 2015].

U.S. EPA. (1990). Asbestos/NESHAP regulated asbestos containing materials guidance. U.S. Environmental Protection Agency (U.S. EPA). Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=50000LZF.txt. [Last accessed: 2 Sep 2015].

U.S. EPA. (1993a). Asbestos, manufacture, importation, processing, and distribution prohibitions. U.S. Environmental Protection Agency (EPA). 58 Fed. Reg. 58964 to be codified at 40 CFR Part 763.

U.S. EPA. (1993b). Asbestos: industry profile. Final Report. Research Triangle Park, NC: U.S. Environmental Protection Agency (U.S. EPA), Office of Air Quality Planning and Standards, Innovative Strategies and Economics Group (ISEG). Available from: http://www.epa.gov/ttnecas1/regdata/IPs/Asbestos%20Processing_IP.pdf. [Last accessed: 2 Sep 2015].

U.S. EPA. (1999a). National emission standards for asbestos, U.S. Environmental Protection Agency (EPA). 40 CFR Part 61 Subpart M.

U.S. EPA. (1999b). U.S. EPA asbestos materials bans: clarification. Washington, DC: U.S. Environmental Protection Agency (U.S. EPA). Available from: http://epa.sownar.com/asbestos/pubs/asbbans2.pdf. [Last accessed: 2 Sep 2015].

U.S. EPA. (2000). A guide for ship scrappers: tips for regulatory compliance. Washington, DC: U.S. Environmental Protection Agency (U.S. EPA), Office of Enforcement and Compliance Assurance, Federal Facilities Enforcement Office.

U.S. EPA. (2001). Asbestos (CASRN 1332-21-4), carcinogenicity assessment for lifetime exposure, Last Revised 22 Feb 2001. U.S. Environmental Protection Agency (US EPA). Available from: http://www.epa.gov/iris/subst/0371.htm#woe. [Last accessed: 15 Sep 2015].

U.S. EPA. (2007). Air and soil sampling report for the Lupe road site, Laramie County, Wyoming. U.S. Environmental Protection Agency Emergency Response Team: Las Vegas, NV.

U.S. EPA. (2008). Naturally occurring asbestos: approaches for reducing exposure. Fact Sheet. U.S. Environmental Protection Agency (U.S. EPA), Office of Superfund Remediation and Technology Innovation. Available from: http://www.epa.gov/superfund/health/contaminants/asbestos/pdfs/noa_factsheet.pdf. [Last accessed: 2 Sep 2015].

U.S. EPA. (2009). Summary of outdoor ambient air monitoring for asbestos at the Libby asbestos site, Libby, Montana. U.S. Environmental Protection Agency, Denver, CO. Available from: http://www2.epa.gov/sites/production/files/documents/AmbientAirReportFinal09Feb2009.pdf. [Last accessed: 2 Sep 2015].

U.S. EPA. (2010). Naturally occurring asbestos (NOA). U.S. Environmental Protection Agency (U.S. EPA). Available from: http://www2.epa.gov/asbestos/learn-about-asbestos#exposed. [Last accessed: 2 Sep 2015].

U.S. EPA. (2015). Toxics Release Inventory (TRI) Program. U.S. Environmental Protection Agency (U.S. EPA). Available from: http://iaspub.epa.gov/triexplorer/tri_release.chemical. [Last accessed: 2 Sep 2015].

USBM. (1976). Mineral facts and problems. 1975 edition. Bulletin 667. Washington, DC: U.S. Department of the Interior, Bureau of Mines (USBM).

USBM. (1985). Mineral facts and problems. 1985 edition. Bulletin 675. Washington, DC: U.S. Department of the Interior, Bureau of Mines (USBM).

Van Gosen BS. (2007). The geology of asbestos in the United States and its practical applications. Environ Eng Geosci 13:55–68.

Virta RL. (2002). Asbestos: geology, mineralogy, mining, and uses. Open-File Report 02-149. US Department of the Interior, US Geological Survey. Available from: http://pubs.usgs.gov/of/2002/of02-149/index.html. [Last accessed: 2 Sep 2015].

Virta, RL. (2006). Worldwide asbestos supply and consumption trends from 1900 through 2003. U.S. Geological Survey Circular 1298:2. Reston, VA: U.S. Geological Survey.

Virta RL. (2008). 2007 Minerals yearbook, asbestos. U.S. Department of the Interior, U.S. Geological Survey. Available from: http://minerals.usgs.gov/minerals/pubs/commodity/asbestos/index.html#myb. [Last accessed: 2 Sep 2015].

Virta RL. (2010). 2009 Minerals yearbook, asbestos. U.S. Department of the Interior, U.S. Geological Survey. Available from: http://minerals.usgs.gov/minerals/pubs/commodity/asbestos/index.html#myb. [Last accessed: 2 Sep 2015].

Virta RL. (2014). 2013 Minerals yearbook, asbestos. U.S. Department of the Interior, U.S. Geological Survey. Available from: http://minerals.usgs.gov/minerals/pubs/commodity/asbestos/index.html#myb. [Last accessed: 2 Sep 2015].

Walton W. (1982). Nature and occurrence of asbestos dust. Ann Occup Hyg 25:121–54.

Wendlick JD. (1983). Ambient asbestos fiber levels in the metropolitan areas of Norfolk, Portsmouth, and Newport News, Virginia. Federal Way, WA. December. (Unpublished).

Wendlick JD. (1984). Ambient asbestos fiber levels at selected sites in Philadelphia, Pennsylvania. Federal Way, WA. November. (Unpublished).

Williams RL, Muhlbaier JL. (1982). Asbestos brake emissions. Environ Res 29:70–82.

Wylie AG, Candela PA. (2015). Methodologies for determining the sources, characteristics, distribution, and abundance of asbestiform and non-asbestiform amphibole and serpentine in ambient air and water. J Toxicol Environ Health B 18:1–42.

**Supplementary material available online**

Supplementary Appendices A and B

Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix A

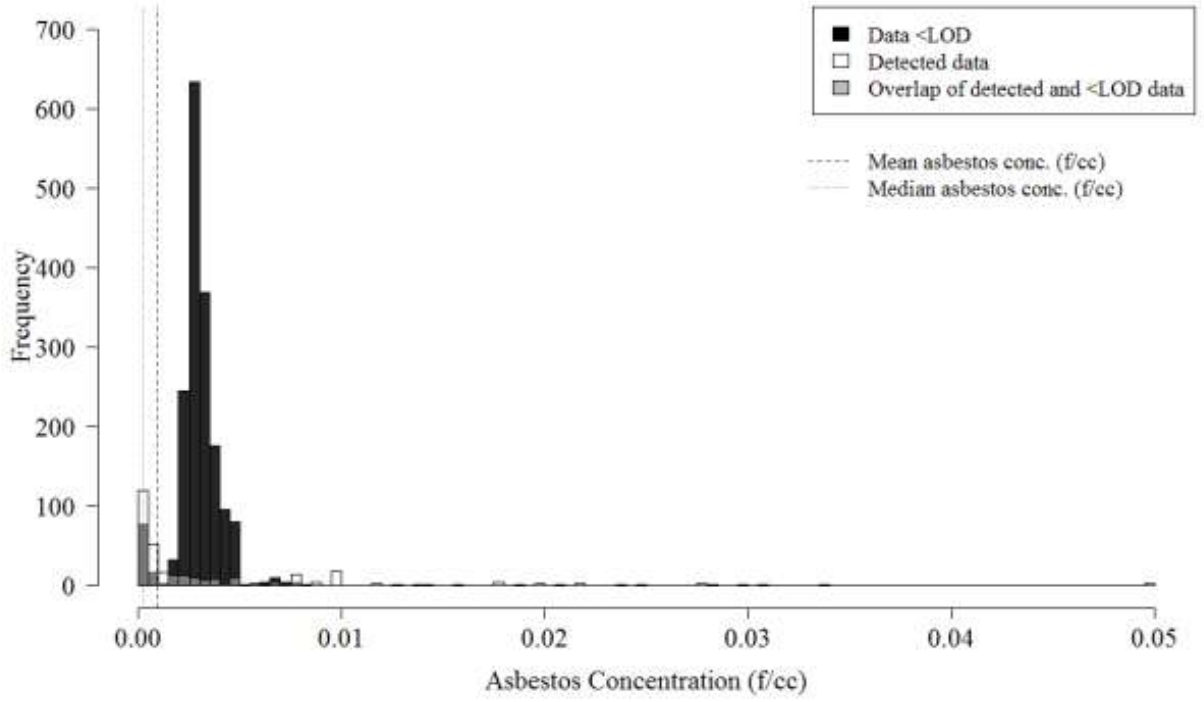
**Appendix A:**

The figures below show the distribution of the data. Data shown in black and white are data < LOD and detected data, respectively. The grey is where there were both detected and non-detected data.

The x-axes are intentionally kept the same in order to facilitate comparisons between figures.

The estimated mean value is shown by a darker dashed line, and the estimated median value is shown as a lighter dot-dashed line. These are provided in order to facilitate comparisons between figures but also to facilitate comparisons between estimated values and the distribution of the data for each subset.

**FIGURE A1: All Data**



Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix A

**FIGURE A2: Urban Data**

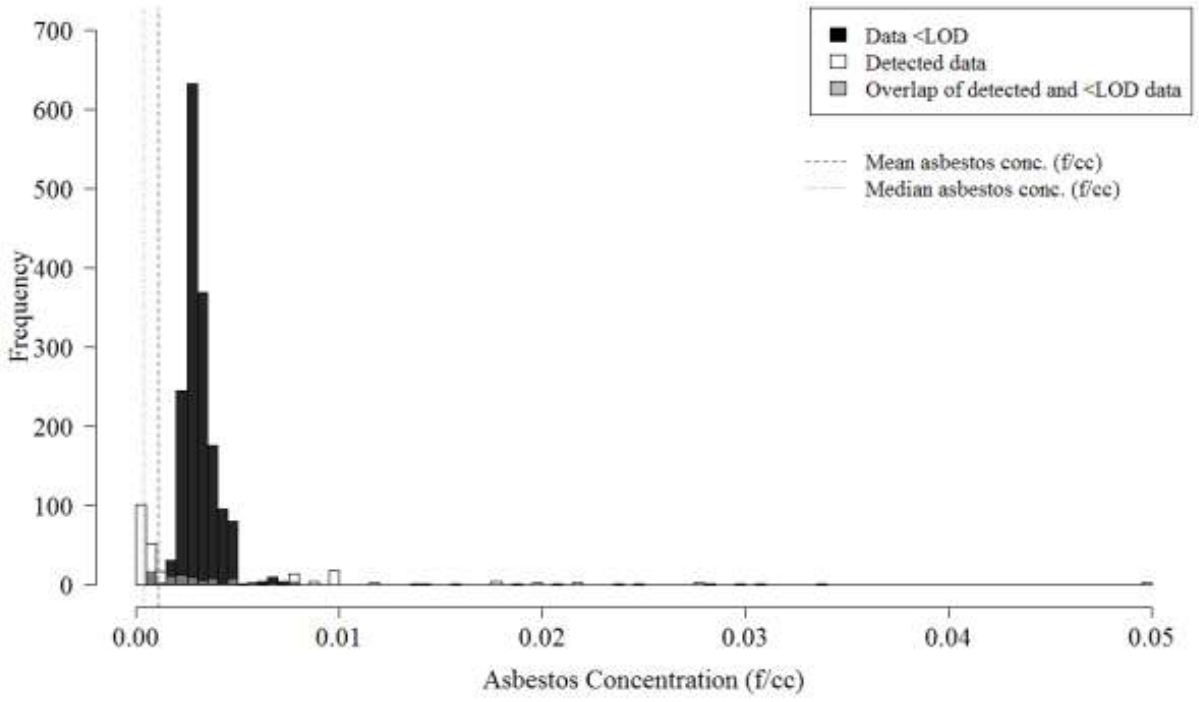
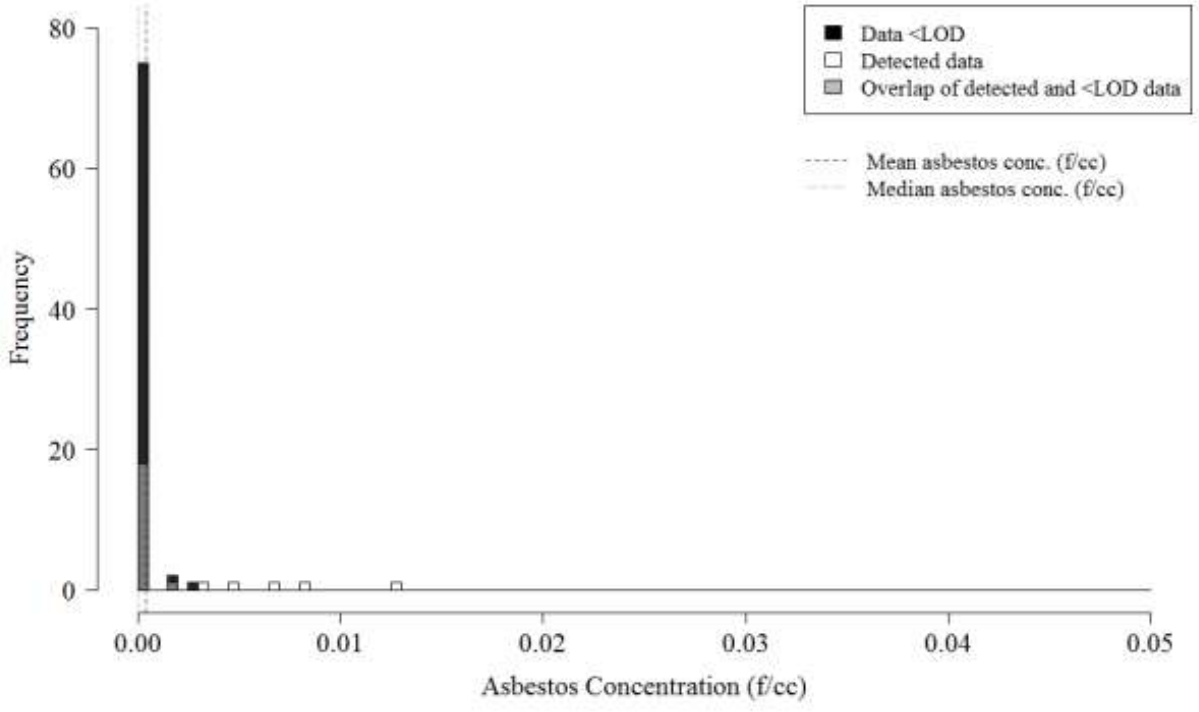



**FIGURE A3: Rural Data**



Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix A

**FIGURE A4: Data Collected During the 1960s**

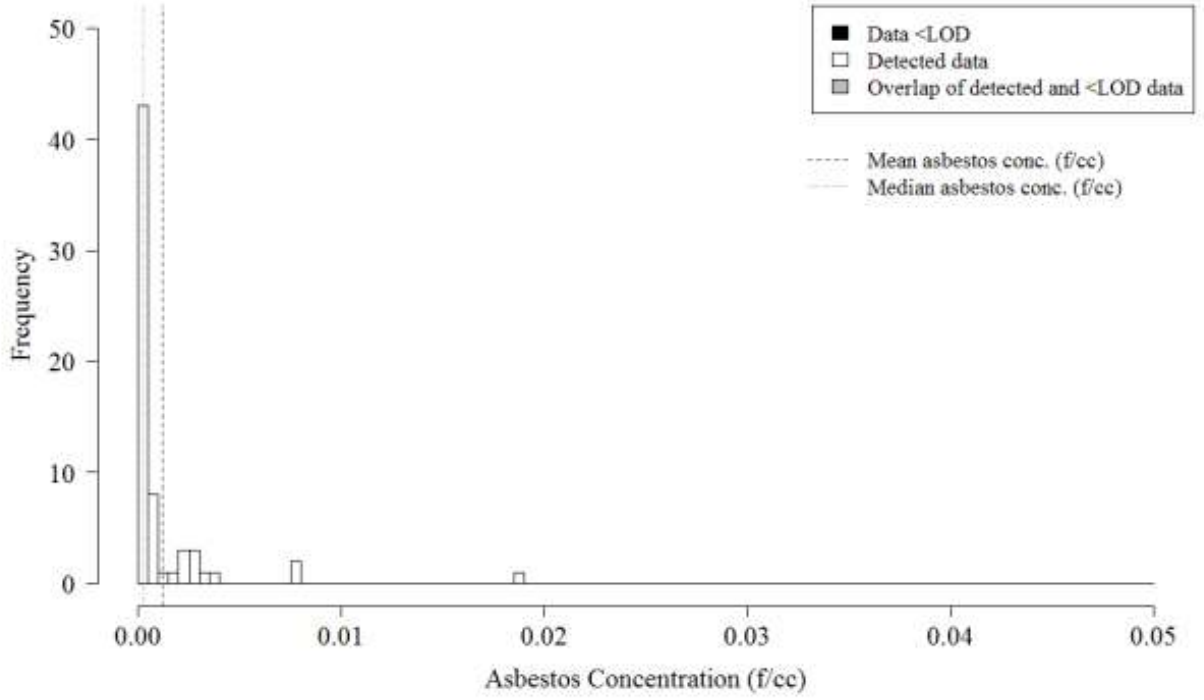



**FIGURE A5: Data Collected During the 1970s**

Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix A

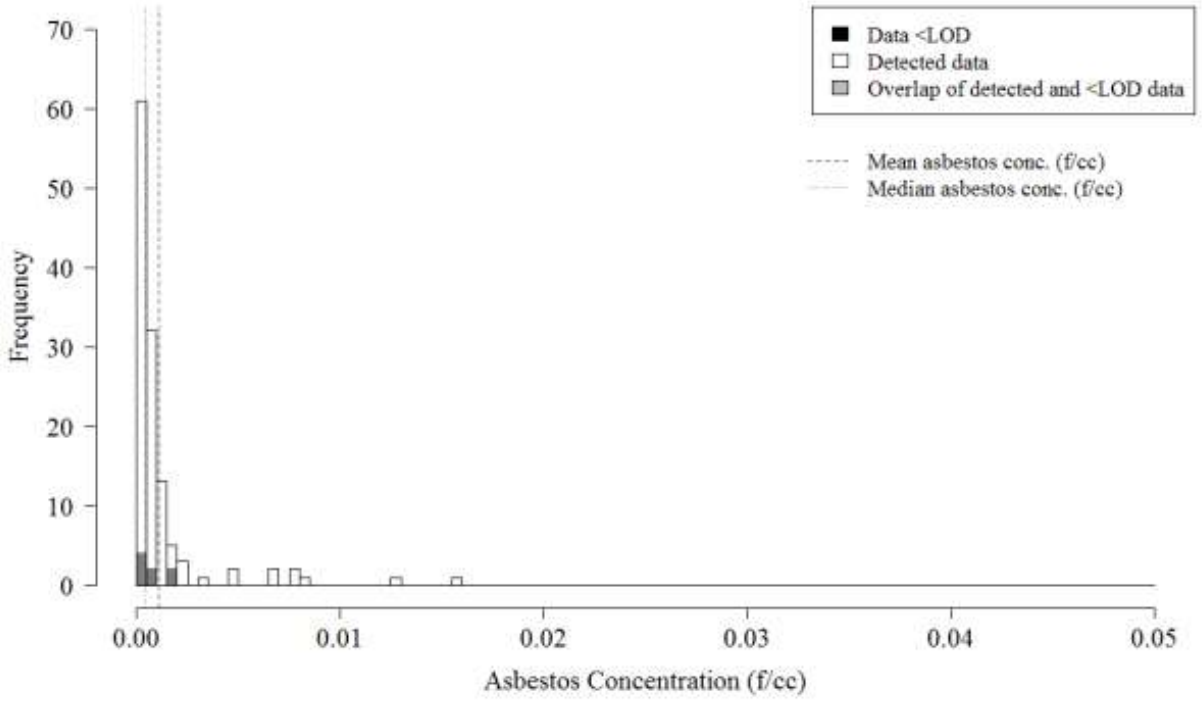



**FIGURE A6: Data Collected During the 1980s**

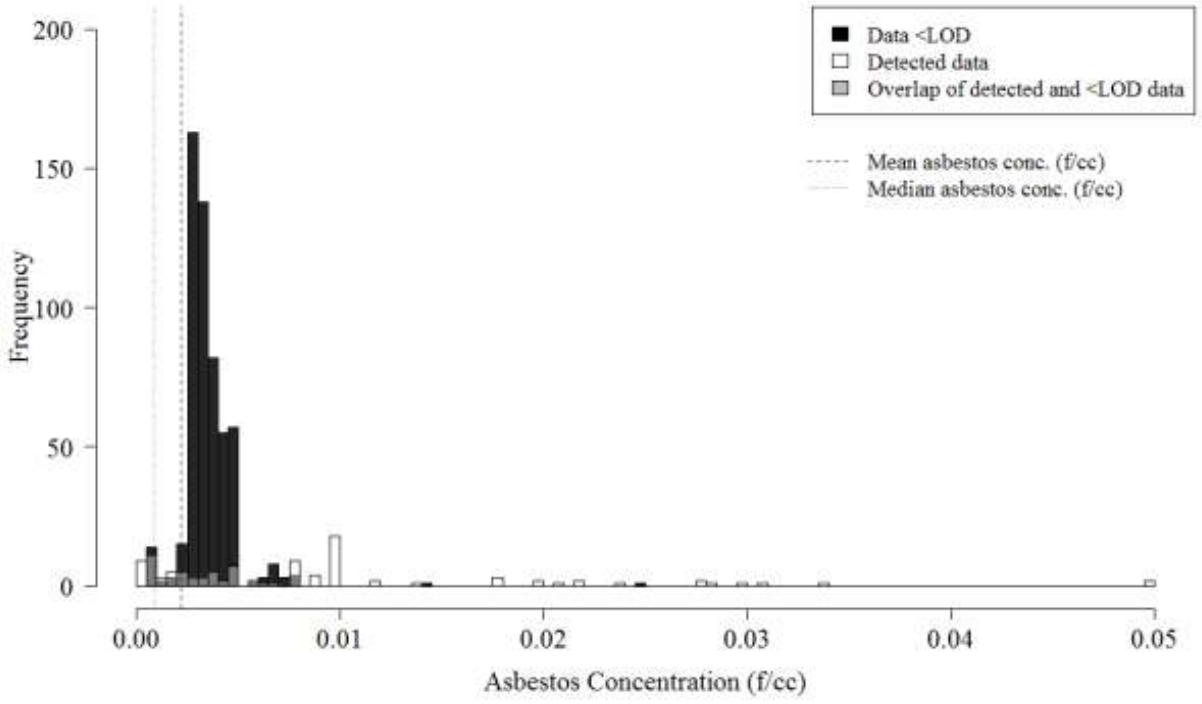



**FIGURE A7: Data Collected During the 1990s**

Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix A

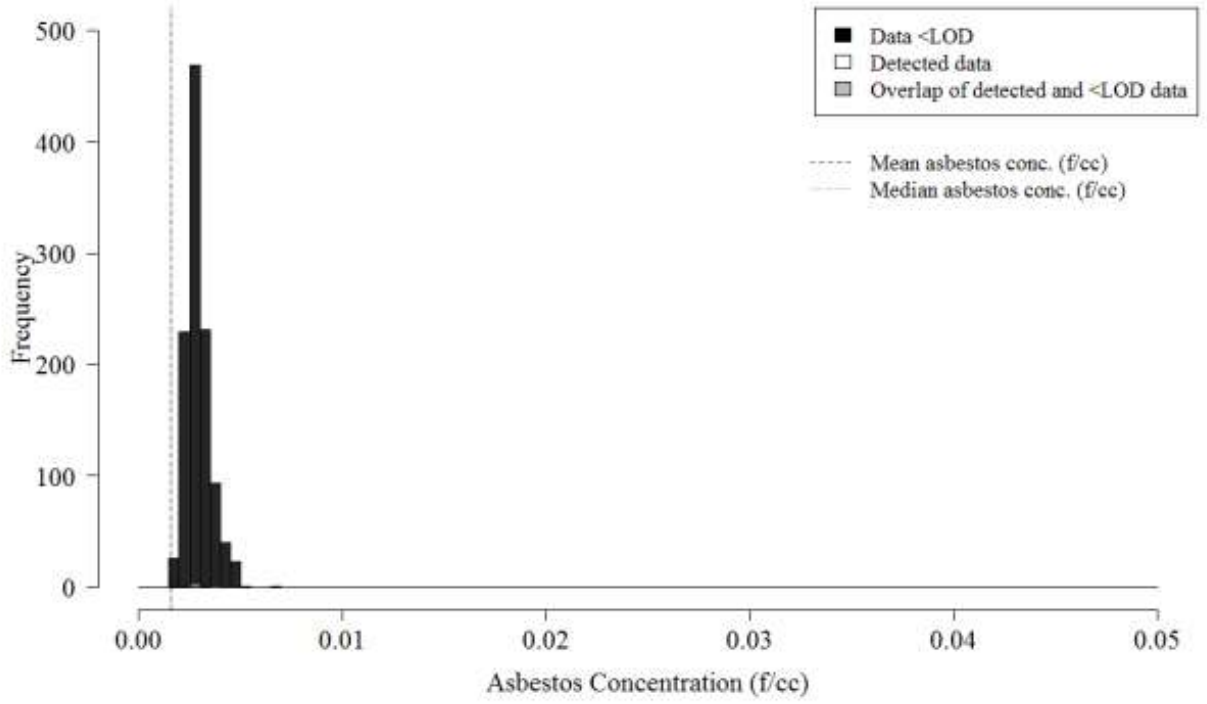
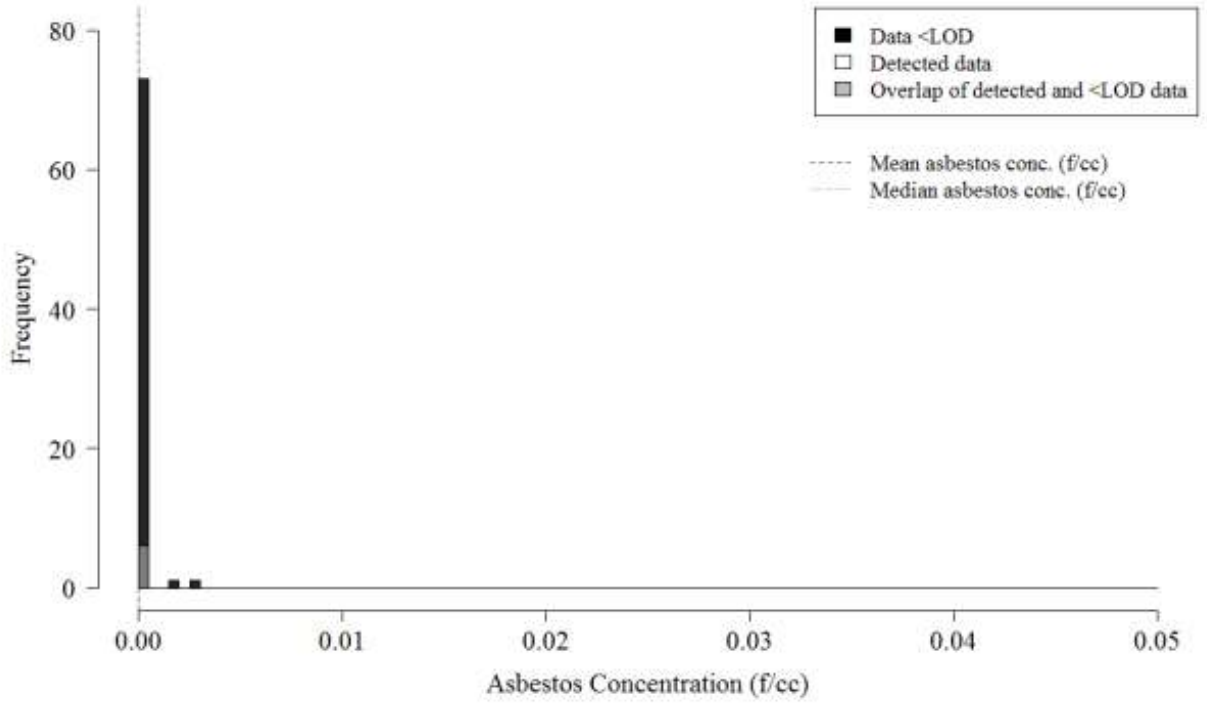



**FIGURE A8: Data Collected During the 2000s**



**APPENDIX B:**

**Table B1: Descriptive statistics of ambient asbestos concentrations, overall and by environment type and decade (excluding PCM data)**

| Data Subset | | n | Fraction < LOD (%) | Fraction of Converted Samples‡ (%) | Range of Values < Limit of Detection (LOD) (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|---|
| *Overall* | | 1953 | 89.1 | 9.5 | <0.00071 - <0.025 | 0.000040 - 0.019 | 0.00014 | 0.00046 |
| *Environment* | Urban | 1849 | 89.8 | 9.2 | <0.00071 - <0.025 | 0.000040 - 0.019 | 0.00030 | 0.00062 |
| | Rural | 102 | 76.5 | 14.7 | <0.0000024 - <0.0027 | 0.0000048 - 0.013 | 0.000020 | 0.00039 |
| | Unknown | 2 | 100 | 0 | < 0.0000024§ | -- | -- | -- |
| *Decade* | 1960s | 64 | 0 | 100 | -- | 0.000040 - 0.019 | 0.00028 | 0.0012 |
| | 1970s | 131 | 5.3 | 93.1 | <0.0000024 - <0.0020 | 0.0000048 - 0.016 | 0.00044 | 0.0011 |
| | 1980s | 555 | 98.4 | 0 | <0.00071 - <0.025 | 0.0025 - 0.0047 | 0.0025¥ | 0.0025 |
| | 1990s | 1122 | 99.2 | 0 | <0.0017 - <0.0069 | 0.0016 - 0.0037 | 0.0016 | 0.0016 |
| | 2000s | 81 | 92.6 | 0 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |
| *Urban* | 1960s | 64 | 0 | 100 | -- | 0.00004 – 0.019 | 0.00028 | 0.0012 |
| | 1970s | 108 | 1.9 | 99.1 | <0.0010 - <0.002 | 0.00004 - 0.016 | 0.0006 | 0.0010 |
| | 1980s | 555 | 98.4 | 0 | <0.00071 - <0.025 | 0.0025 - 0.0047 | 0.0025¥ | 0.0025 |
| | 1990s | 1122 | 99.2 | 0 | <0.0017 - <0.0069 | 0.0016 - 0.0037 | 0.0016 | 0.0016 |
| | 2000s | 0 | -- | -- | -- | -- | -- | -- |
| *Rural* | 1960s | 0 | -- | -- | -- | -- | -- | -- |
| | 1970s | 21 | 14.3 | 71.4 | <0.0000024 - <0.0010 | 0.0000048 - 0.013 | 0.000021 | 0.0018 |
| | 1980s | 0 | -- | -- | -- | -- | -- | -- |
| | 1990s | 0 | -- | -- | -- | -- | -- | -- |
| | 2000s | 81 | 92.6 | 0 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |

-- Data not available/not applicable
† Calculated using the reverse Kaplan-Meier estimator
‡ Conversion factor as recommended by the US EPA (1986) (i.e. 30 µg/m$^3$ per f/cc)
§ All samples below the LOD
¥ As described in the Methods section, the median was not estimated for these subsets because the cumulative density function (CDF) exceeded 0.5 at the minimum detected concentration, and the minimum concentration was < LOD. Therefore, the reported value is the minimum detected concentration.

Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix B

**Table B2: Descriptive statistics of ambient asbestos concentrations by conversion factor and analytical method (excluding PCM data)**

| Data Subset | | Environmental Type | n | Fraction < LOD (%) | Fraction of Converted Samples (%) | Range of Values < LOD (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|---|---|
| *Mass- to Count-Based Conversions* | No conversion | Overall | 1767 | 98.4 | 0 | <0.0000024 - <0.025 | 0.000014 - 0.050 | 0.000014¥ | 0.00013 |
| | | Urban | 1678 | 98.9 | 0 | <0.00071 - <0.025 | 0.0016 - 0.016 | 0.0016¥ | 0.0016 |
| | | Rural | 87 | 88.5 | 0 | <0.0000024 - <0.0027 | 0.000014 - 0.013 | 0.000014¥ | 0.00032 |
| | Converted data‡ | Overall | 186 | 1.6 | 100 | <0.0010 - <0.002 | 0.0000048 - 0.019 | 0.00032 | 0.00096 |
| | | Urban | 171 | 1.2 | 100 | <0.0010 - <0.002 | 0.00040 - 0.019 | 0.0004 | 0.00097 |
| | | Rural | 15 | 6.7 | 100 | <0.0020 | 0.0000048 - 0.0066 | 0.00002 | 0.00077 |
| *Analytical Method* | TEM/EM | Overall | 1943 | 89.3 | 9.6 | <0.000031 - <0.025 | 0.0000048 - 0.019 | 0.00014 | 0.00044 |
| | ISO | | 1 | 100 | 0 | <0.00010§ | -- | -- | -- |
| | SEM | | 9 | 44.4 | 0 | <0.0000024 | 0.0019 - 0.016 | 0.0019 | 0.0056 |

† Calculated using the reverse Kaplan-Meier estimator.
‡ Conversion factor as recommended by the US EPA (1986) (i.e. 30 µg/m$^3$ per f/cc)
¥ As described in the Methods section, the median was not estimated for these subsets because the cumulative density function (CDF) exceeded 0.5 at the minimum detected concentration, and the minimum concentration was < LOD. Therefore, the reported value is the minimum detected concentration.

**Table B3: Descriptive statistics of ambient asbestos concentrations, overall and by environment type and decade (TEM/EM data only, excluding mass-based data)**

| Data Subset | | n | Fraction < LOD (%) | Range of Values < Limit of Detection (LOD) (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|
| *Overall* | | 1757 | 98.6 | <0.000031 - <0.025 | 0.000014 - 0.0047 | 0.000014 | 0.000095 |
| *Environment* | Urban | 1677 | 98.9 | <0.00071 - <0.025 | 0.0016 - 0.0047 | 0.0016 | 0.0016 |
| | Rural | 80 | 92.5 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014¥ | 0.000017 |
| | Unknown | 0 | -- | -- | -- | -- | -- |
| *Decade* | 1960s | 0 | -- | -- | -- | -- | -- |
| | 1970s | 0 | -- | -- | -- | -- | -- |
| | 1980s | 555 | 98.4 | <0.00071 - <0.025 | 0.0025 - 0.0047 | 0.0025¥ | 0.0022 |
| | 1990s | 1122 | 99.2 | <0.0017 - <0.0069 | 0.0016 - 0.0037 | 0.0016 | 0.0016 |
| | 2000s | 80 | 92.5 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |
| *Urban* | 1960s | 0 | -- | -- | -- | -- | -- |
| | 1970s | 0 | -- | -- | -- | -- | -- |
| | 1980s | 555 | 98.4 | <0.00071 - <0.025 | 0.0025 - 0.0047 | 0.0025¥ | 0.0025 |
| | 1990s | 1122 | 99.2 | <0.0017 - <0.0069 | 0.0016 - 0.0037 | 0.0016 | 0.0016 |
| | 2000s | 0 | -- | -- | -- | -- | -- |
| *Rural* | 1960s | 0 | -- | -- | -- | -- | -- |
| | 1970s | 0 | -- | -- | -- | -- | -- |
| | 1980s | 0 | -- | -- | -- | -- | -- |
| | 1990s | 0 | -- | -- | -- | -- | -- |
| | 2000s | 80 | 92.5 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |

-- Data not available/not applicable
† Calculated using the reverse Kaplan-Meier estimator
¥ As described in the Methods section, the median was not estimated for these subsets because the cumulative density function (CDF) exceeded 0.5 at the minimum detected concentration, and the minimum concentration was < LOD. Therefore, the reported value is the minimum detected concentration.

Abelmann et al.
Historical ambient airborne asbestos concentrations in the United States – an analysis of published and unpublished literature
Appendix B

**Table B4: Descriptive statistics of ambient asbestos concentrations, overall and by environment type and decade [using US EPA (1986) conversion factor]**

| Data Subset | | n | Fraction < LOD (%) | Fraction of Converted Samples‡ (%) | Range of Values < Limit of Detection (LOD) (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|---|
| *Overall* | | 2058 | 84.9 | 9.0 | <0.0000024 - <0.025 | 0.00000080 - 0.050 | 0.000043 | 0.00069 |
| *Environment* | Urban | 1954 | 85.3 | 8.8 | <0.00071 - <0.025 | 0.0000067 - 0.050 | 0.00012 | 0.00077 |
| | Rural | 102 | 76.5 | 14.7 | <0.0000024 - <0.0027 | 0.00000080 - 0.013 | 0.0000034 | 0.00029 |
| | Unknown | 2 | 100 | 0 | < 0.0000024§ | -- | -- | -- |
| *Decade* | 1960s | 64 | 0 | 100 | -- | 0.0000067 - 0.0032 | 0.000047 | 0.00019 |
| | 1970s | 132 | 6.1 | 92.4 | <0.0000024 - <0.0010 | 0.00000080 - 0.016 | 0.000073 | 0.00046 |
| | 1980s | 659 | 83.6 | 0 | <0.00071 - <0.025 | 0.00010 - 0.050 | 0.00090 | 0.0022 |
| | 1990s | 1122 | 99.2 | 0 | <0.0017 - <0.0069 | 0.0016 - 0.0037 | 0.0016 | 0.0016 |
| | 2000s | 81 | 92.6 | 0 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |
| *Urban* | 1960s | 64 | 0 | 100 | -- | 0.0000067 - 0.0032 | 0.000047 | 0.00019 |
| | 1970s | 109 | 2.8 | 98.2 | <0.0010 | 0.0000067 - 0.016 | 0.00060 | 0.0010 |
| | 1980s | 659 | 83.6 | 0 | <0.00071 - <0.025 | 0.00010 - 0.050 | 0.00090 | 0.0022 |
| | 1990s | 1122 | 99.2 | 0 | <0.0017 - <0.0069 | 0.0016 - 0.0037 | 0.0016 | 0.0016 |
| | 2000s | 0 | -- | -- | -- | -- | -- | -- |
| *Rural* | 1960s | 0 | -- | -- | -- | -- | -- | -- |
| | 1970s | 21 | 14.3 | 71.4 | <0.0000024 - <0.0010 | 0.00000080 - 0.013 | 0.0000035 | 0.0014 |
| | 1980s | 0 | -- | -- | -- | -- | -- | -- |
| | 1990s | 0 | -- | -- | -- | -- | -- | -- |
| | 2000s | 81 | 92.6 | 0 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |

-- Data not available/not applicable
† Calculated using the reverse Kaplan-Meier estimator
‡ Conversion factor as recommended by the US EPA (1986) (i.e. 30 µg/m$^3$ per f/cc)
§ All samples below the LOD

**Table B5: Descriptive statistics of ambient asbestos concentrations by conversion factor and analytical method [using US EPA (1986) conversion factor]**

| Data Subset | | Environmental Type | n | Fraction < LOD (%) | Fraction of Converted Samples (%) | Range of Values < LOD (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|---|---|
| *Mass- to Count-Based Conversions* | No conversion | Overall | 1872 | 93.2 | 0 | <0.0000024 - <0.025 | 0.000014 - 0.050 | 0.000014¥ | 0.00080 |
| | | Urban | 1783 | 93.4 | 0 | <0.00071 - <0.025 | 0.00010 - 0.050 | 0.00060 | 0.0013 |
| | | Rural | 87 | 88.5 | 0 | <0.0000024 - <0.0027 | 0.000014 - 0.013 | 0.000014¥ | 0.00032 |
| | Converted data‡ | Overall | 186 | 1.6 | 100 | <0.0010 | 0.00000080 - 0.0032 | 0.000053 | 0.00016 |
| | | Urban | 171 | 1.2 | 100 | <0.0010 | 0.0000067 - 0.0032 | 0.000067 | 0.00016 |
| | | Rural | 15 | 6.7 | 100 | <0.0010 | 0.00000080 - 0.0011 | 0.0000034 | 0.00013 |
| *Analytical Method* | TEM/EM | Overall | 1943 | 89.3 | 9.6 | <0.000031 - <0.025 | 0.00000080 - 0.0047 | 0.000037 | 0.00016 |
| | ISO | | 1 | 100 | 0 | <0.00010§ | -- | -- | -- |
| | SEM | | 9 | 44.4 | 0 | <0.0000024 | 0.0019 - 0.016 | 0.0019 | 0.0056 |
| | PCM | | 105 | 5.7 | 0 | <0.0010 - <0.0020 | 0.00010 - 0.050 | 0.0063 | 0.0086 |

† Calculated using the reverse Kaplan-Meier estimator.
‡ Conversion factor as recommended by the US EPA (1986) (i.e. 30 µg/m$^3$ per f/cc)
§ All samples below the LOD
¥ As described in the Methods section, the median was not estimated for these subsets because the cumulative density function (CDF) exceeded 0.5 at the minimum detected concentration, and the minimum concentration was < LOD. Therefore, the reported value is the minimum detected concentration.

**Table B6: Descriptive statistics of ambient asbestos concentrations, overall and by environment type and decade (RJ Lee data excluded)**

| Data Subset | | n | Fraction < LOD (%) | Fraction of Converted Samples‡ (%) | Range of Values < Limit of Detection (LOD) (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|---|
| *Overall* | | 381 | 23.1 | 48.8 | <0.0000024 - <0.0027 | 0.0000048 - 0.050 | 0.00040 | 0.0030 |
| *Environment* | Urban | 277 | 2.9 | 61.7 | <0.0010 - <0.0020 | 0.000040 - 0.050 | 0.00086 | 0.0039 |
| | Rural | 102 | 76.5 | 14.7 | <0.0000024 - <0.0027 | 0.0000048 - 0.013 | 0.000020 | 0.00039 |
| | Unknown | 2 | 100 | 0 | < 0.0000024§ | -- | -- | -- |
| *Decade* | 1960s | 64 | 0 | 100 | -- | 0.000040 - 0.019 | 0.00028 | 0.0012 |
| | 1970s | 132 | 6.1 | 92.4 | <0.0000024 - <0.0020 | 0.0000048 - 0.016 | 0.00044 | 0.0011 |
| | 1980s | 104 | 4.8 | 0 | <0.0010 - <0.0020 | 0.00010 - 0.050 | 0.0063 | 0.0087 |
| | 1990s | 0 | -- | -- | -- | -- | -- | -- |
| | 2000s | 81 | 92.6 | 0 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |
| *Urban* | 1960s | 64 | 0 | 100 | -- | 0.000040 - 0.019 | 0.00028 | 0.0012 |
| | 1970s | 109 | 2.8 | 98.2 | <0.0010 - <0.0020 | 0.000040 - 0.016 | 0.00060 | 0.0010 |
| | 1980s | 104 | 4.8 | 0 | <0.0010 - <0.0020 | 0.0010 - 0.050 | 0.0063 | 0.0087 |
| | 1990s | 0 | -- | -- | -- | -- | -- | -- |
| | 2000s | 0 | -- | -- | -- | -- | -- | -- |
| *Rural* | 1960s | 0 | -- | -- | -- | -- | -- | -- |
| | 1970s | 21 | 14.3 | 71.4 | <0.0000024 - <0.0020 | 0.0000048 - 0.013 | 0.000021 | 0.0018 |
| | 1980s | 0 | -- | -- | -- | -- | -- | -- |
| | 1990s | 0 | -- | -- | -- | -- | -- | -- |
| | 2000s | 81 | 92.6 | 0 | <0.000031 - <0.0027 | 0.000014 - 0.000092 | 0.000014 | 0.000017 |

-- Data not available/not applicable
† Calculated using the reverse Kaplan-Meier estimator
‡ Conversion factor derived from Davis et al. (1978) (i.e. 5 µg/m$^3$ per f/cc)
§ All samples below the LOD

**Table B7: Descriptive statistics of ambient asbestos concentrations by conversion factor and analytical method (RJ Lee data excluded)**

| Data Subset | | Environmental Type | n | Fraction < LOD (%) | Fraction of Converted Samples (%) | Range of Values < LOD (f/cc) | Range of Detected Values (f/cc) | Median† (f/cc) | Mean† (f/cc) |
|---|---|---|---|---|---|---|---|---|---|
| *Mass- to Count-Based Conversions* | No conversion | Overall | 195 | 43.6 | 0 | <0.0000024 - <0.0027 | 0.000014 - 0.050 | 0.00050 | 0.0049 |
| | | Urban | 106 | 5.7 | 0 | <0.0010 - <0.0020 | 0.00010 - 0.050 | 0.0063 | 0.0087 |
| | | Rural | 87 | 88.5 | 0 | <0.0000024 - <0.0027 | 0.000014 - 0.013 | 0.000014¥ | 0.00032 |
| | Converted data‡ | Overall | 186 | 1.6 | 100 | <0.0010 - <0.0020 | 0.0000048 - 0.019 | 0.00032 | 0.00096 |
| | | Urban | 171 | 1.2 | 100 | <0.0010 - <0.0020 | 0.000040 - 0.019 | 0.00040 | 0.00097 |
| | | Rural | 15 | 6.7 | 100 | <0.0020 - <0.0020 | 0.0000048 - 0.0066 | 0.000020 | 0.00077 |
| *Analytical Method* | TEM/EM | Overall | 266 | 28.9 | 69.9 | <0.000031 - <0.0027 | 0.0000048 - 0.019 | 0.00016 | 0.00068 |
| | ISO | | 1 | 100 | 0 | <0.00010§ | -- | -- | -- |
| | SEM | | 9 | 44.4 | 0 | <0.0000024 | 0.0019 - 0.016 | 0.0019 | 0.0056 |
| | PCM | | 105 | 5.7 | 0 | <0.0010 - <0.0020 | 0.00010 - 0.050 | 0.0063 | 0.0086 |

† Calculated using the reverse Kaplan-Meier estimator.
‡ Conversion factor derived from Davis et al. (1978) (i.e. 5 µg/m$^3$ per f/cc)
§ All samples below the LOD
¥ As described in the Methods section, the median was not estimated for these subsets because the cumulative density function (CDF) exceeded 0.5 at the minimum detected concentration, and the minimum concentration was < LOD. Therefore, the reported value is the minimum detected concentration.

STUDY No. 14

# MESOTHELIOMA AND ANALYSIS OF TISSUE FIBER CONTENT

**STUDY No. 14**

# MESOTHELIOMA AND ANALYSIS OF TISSUE FIBER CONTENT

**Volker Neumann , Stefan Löseke, and Andrea Tannapfel**

The strong relationship between mesothelioma and asbestos exposure is well established. The analysis of lung asbestos burden by light and electron microscopy assisted to understand the increased incidence of mesothelioma in asbestos mining and consuming nations.

The data on the occupational exposure to asbestos are important information for the purpose of compensation of occupational disease No. 4105 (asbestos-associated mesothelioma) in Germany.

However, in many cases the patients have forgotten conditions of asbestos exposure or had no knowledge about the used materials with components of asbestos. Mineral fiber analysis can provide valuable information for the research of asbestos-associated diseases and for the assessment of exposure. Because of the variability of asbestos exposure and long latency periods, the analysis of asbestos lung content is a relevant method for identification of asbestos-associated diseases. Also, sources of secondary exposure, so called “bystander exposition” or environmental exposure can beexamined by mineral fiber analysis.

Household contacts to asbestos are known for ten patients (1987–2009) in the German mesothelioma register; these patients lived together with family members working in the asbestos manufacturing industry.

Analysis of lung tissue for asbestos burden offers information on the past exposure. The predominant fiber-type identified by electron microscopy in patients with mesothelioma is amphibole asbestos (crocidolite or amosite). Latency times (mean 42.5 years) and mean age at the time of diagnose in patients with mesothelioma are increasing (65.5 years). The decrease of median asbestos burden of the lung in mesothelioma patients results in disease manifestation at a higher age.

Lung dust analyses are a relevant method for the determination of causation in mesothelioma. Analysis of asbestos burden of the lung and of fiber type provides insights into the pathogenesis of malignant mesothelioma. The most important causal factor for the development of mesothelioma is still asbestos exposure.

# 6 Mesothelioma and Analysis of Tissue Fiber Content

Volker Neumann, Stefan Löseke, and Andrea Tannapfel

**Abstract** The strong relationship between mesothelioma and asbestos exposure is well established. The analysis of lung asbestos burden by light and electron microscopy assisted to understand the increased incidence of mesothelioma in asbestos mining and consuming nations.

The data on the occupational exposure to asbestos are important information for the purpose of compensation of occupational disease No. 4105 (asbestos-associated mesothelioma) in Germany.

However, in many cases the patients have forgotten conditions of asbestos exposure or had no knowledge about the used materials with components of asbestos. Mineral fiber analysis can provide valuable information for the research of asbestos-associated diseases and for the assessment of exposure. Because of the variability of asbestos exposure and long latency periods, the analysis of asbestos lung content is a relevant method for identification of asbestos-associated diseases. Also, sources of secondary exposure, so called "bystander exposition" or environmental exposure can be examined by mineral fiber analysis.

Household contacts to asbestos are known for ten patients (1987–2009) in the German mesothelioma register; these patients lived together with family members working in the asbestos manufacturing industry.

Analysis of lung tissue for asbestos burden offers information on the past exposure. The predominant fiber-type identified by electron microscopy in patients with mesothelioma is amphibole asbestos (crocidolite or amosite). Latency times (mean 42.5 years) and mean age at the time of diagnose in patients with mesothelioma are increasing (65.5 years). The decrease of median asbestos burden of the lung in mesothelioma patients results in disease manifestation at a higher age.

Lung dust analyses are a relevant method for the determination of causation in mesothelioma. Analysis of asbestos burden of the lung and of fiber type provides insights into the pathogenesis of malignant mesothelioma. The most important causal factor for the development of mesothelioma is still asbestos exposure.

[AU1] V. Neumann (✉) and S. Löseke
German Mesothelioma Register,
University Hospital Bergmannsheil,
Bochum, Germany
e-mail: volker.neumann@ruhr-uni-bochum.de

A. Tannapfel
German Mesothelioma Register,
University Hospital Bergmannsheil, Bochum,
Germany and
Institut für Pathologie, Ruhr-Universität Bochum,
BG Kliniken Bergmannsheil, Bürke-de-la-Camp
Platz 1, 44789 Bochum, Germany
e-mail: andrea.tannapfel@ruhr-uni-bochum.de

A. Tannapfel (ed.), *Malignant Mesothelioma*, Recent Results in Cancer Research 189,
DOI: 10.1007/978-3-642-10862-4_6, © Springer-Verlag Berlin Heidelberg 2011

6

## 6.1 Introduction

Occupational exposure to asbestos dust has been widespread in all industrial nations and exposure still exists in Canada, Russia, China, and Africa. Asbestos is a group of minerals with particular properties but only six asbestiform minerals are of commercial importance. There are two large groups of asbestos fibers, first amphibole asbestos including five asbestiform members (crocidolith, amosite, tremolite, actinolite, anthophyllite) and secondly serpentine asbestos of which chrysotile is the only asbestiform member. Crocidolith, amosite, and chrysotile are the most common commercially used asbestiform minerals. The other amphiboles have only limited commercial importance but are relevant as contaminants of other mineral species. Asbestos minerals have been used in over 3,000 commercial applications [2, 39].

The strong relationship between mesothelioma and asbestos exposure is well established [35, 36, 61, 63, 65, 122, 132]. There is a direct relationship between the national asbestos consumption (kg per head per year) in industrial nations and the number of deaths per million people per year by mesothelioma and asbestosis [75]. Historical asbestos consumption is a significant predictor for death by mesothelioma. Whereas in the so-called normal population mesothelioma have an incidence of 1–2 cases per 1 million inhabitants [83], the number of mesothelioma after asbestos exposure is much higher [32, 96]. The highest incidence rates – about 30 cases per 1 million- were estimated in Australia [72], Belgium [15], and Great Britain [87].

Although the usage of asbestos containing products was forbidden in most industrialized countries long time ago the number of mesothelioma is still growing due to long and variable latency periods (20 up to over 40 years) between exposure and diagnosis [20, 93, 104, 121]. Therefore, the incidence of mesothelioma is expected to peak between the years 2010 and 2020 [9, 64, 103, 106].

The commercial use of asbestos peaked in Germany at more than 200,000 t/year between 1968 and 1977 (Fig. 6.1). At present, as well as

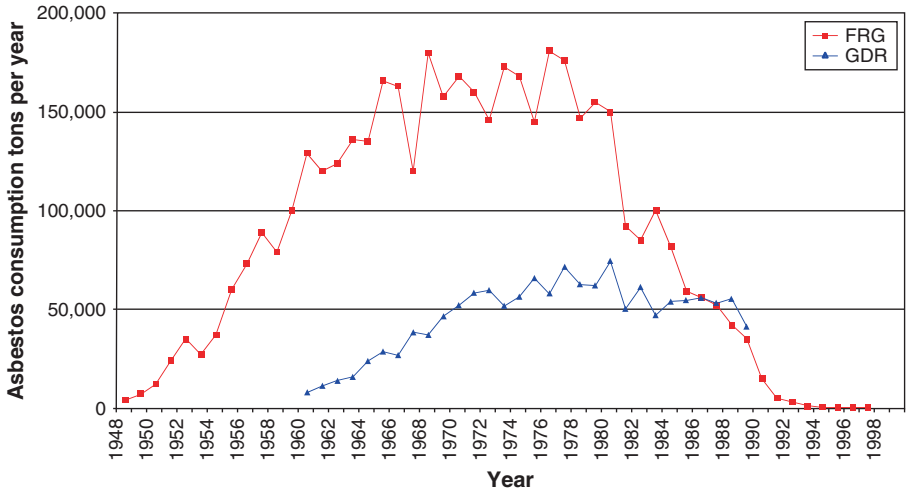



**Fig. 6.1** Consumption of asbestos in Germany (GDR/FRG) (German democratic republic/FRG Federal Republik of Germany)

in the near future asbestos-related diseases are considered to be a public health problem in Germany [99].

In 2008, 905 new cases (Fig. 6.2) were recognized as asbestos-related mesothelioma in Germany [38].

Mesothelioma often develop in patients with long-term occupational asbestos exposure, but can also occur in patients with low level or minor exposure to asbestos [59, 93]. Mesothelioma cases have been reported in wives and children of asbestos workers who were exposed to asbestos dust by cleaning and storing workers' clothes [44, 93, 129].

Such household contacts are known for ten patients (1989–2009) in the German mesothelioma register; these patients had lived together with family members working in the asbestos manufacturing industry.

The analysis of asbestos content of lung tissue provides important information concerning the understanding of the relationship between asbestos exposure and causation of asbestos-associated diseases [113].

So mineral fiber analysis is an essential tool to obtain valuable information for the research of asbestos-associated diseases and for the assessment of asbestos exposure [93]. The exact determination of asbestos exposure may often be problematic because of the variability of asbestos exposure in patient's histories, long latency times, and subsequent frequently forgotten episodes of asbestos exposure. So the analysis of asbestos lung content is a relevant method for identification of an asbestos-associated disease. The sole measurement of airborne asbestos fibers by using air samplers has some disadvantages and cannot solve the previously mentioned problems in the evaluation of a patient's individual history of asbestos exposure. The disadvantage of airborne measurements of asbestos fibers is caused by:

- Different sampling techniques over the time.
- Measurement of fibers ≥ 5 μm does not differentiate between respirable and nonrespirable.
- No fiber size distributions are given.
- Concentrations based on counts using the phase contrast microscopy.

Only measuring the asbestos content in lung tissue will give the relevant fiber burden retained in the lung at the time of analysis. Thus, this method is able to subsume the deposition and clearance of asbestos fibers in the

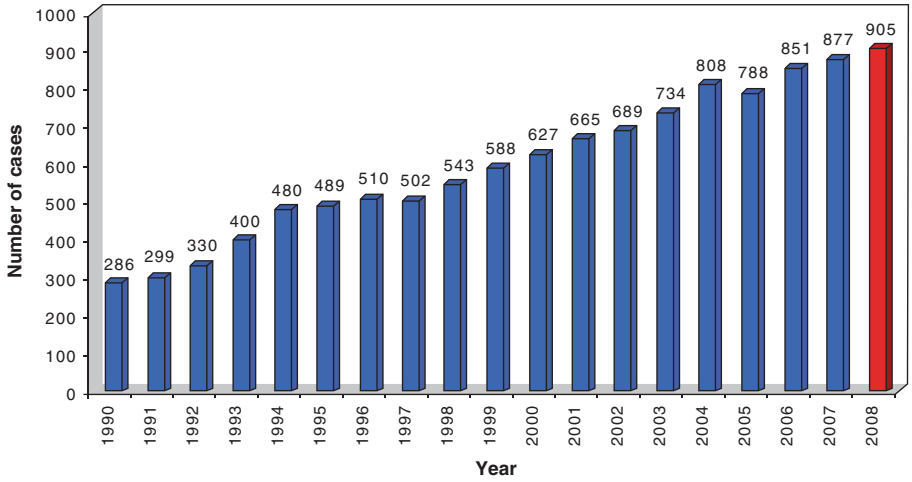



**Fig. 6.2** New recognized occupational disease no. 4105 – malignant mesothelioma

6

human lung. An optimal lung dust analysis is based on representative samples, accurate preparation techniques, and a trained and experienced analyst [37]. Other important variables that determine the quality of the information gained from lung tissue analysis include tissue quantity and the method of analysis.

## 6.2 Techniques for Analysis of Pulmonary Mineral-Fiber Content

There are several established analytical methods for asbestos fiber analysis that differ in their specificity and sensitivity. This diversity is the reason for the poor direct comparability of the results from one laboratory to another. Asbestos fibers are ubiquitous in the air and present in the lung of subjects without any occupational asbestos exposure. So a reliable determination of an elevated pulmonary asbestos fiber content caused by occupational asbestos exposure must be based on the comparison with the so-called normal population. Due to the high variability between different techniques and laboratories, each laboratory has to establish its own reference values for normal lungs in relation to lungs with elevated asbestos burden.

The different analysis techniques can be subdivided into three common operation steps [111]:

1. Dissolving and removal of the organic lung matrix
2. Recovery and concentration of asbestos bodies and mineral fibers
3. Quantification of the asbestos lung tissue burden

### 6.2.1 Lung Tissue Digests

The sampling of the lung tissue for fiber burden analysis is the first relevant step. If possible, for lung dust analysis, tissue from [55] the upper lobe (right and left side) and the lower lobe (right and left side) should be taken. The used lung tissue should be well inflated and without secondary lung alterations (non-tumorous sample, no autolysis and without pneumonia) [14, 55, 93, 94]. There exists a variety of techniques for the extraction of asbestos bodies from lung tissue. Some methods employ chemical digestion, others use low temperature ashing techniques. The tissue digestion must be carefully performed to avoid loss of asbestos bodies, asbestos fibers, or fiber fractions [113]. Any process that may damage fibers by shortening or splitting should be avoided [7, 89]. Drying tissue before digestion leads to fracture of longer asbestos fibers, causing artificial higher results. Introduction of a sonification or hot ashing step of the lung tissue can lead to extended fragmentation of chrysotile fibers and artifactual increase in asbestos fiber numbers [58, 70]. In the German mesothelioma register, we use a direct isolation method without ultrasonification, centrifugation, or drying of the tissue samples.

First step in lung dust analysis procedure is the weighing of the wet lung tissue, followed by a sodium hypochloride-based wet chemical digestion step of the organic lung tissue matrix. Afterward, increasing amounts of the dissolved lung tissue are filtrated through a porous membrane and are concentrated on the filter matrix. These filters are mounted on glass slides and made transparent for light microscopy by acetone vaporization.

The asbestos burden can be given in terms of fibers (or asbestos bodies) per gram of wet tissue, fibers per $cm^3$ of lung tissue, or fibers per gram of dry lung tissue. These values (units) are not precisely comparable and can vary from case to case, but in general one fiber in wet tissue is approximately equivalent to one fiber in $cm^3$, corresponding to a concentration of nearly ten fibers per gram of dry tissue [113].

The concentration of asbestos fibers and asbestos bodies depends partly on the density of lung tissue [7]. Increasing density of lung

tissue – due to fibrosis or pneumonia – will lead to a decrease of fibers or asbestos bodies per unit weight (wet or dry). Decreasing density of lung tissue – due to emphysema – will lead to an increasing number of fibers or asbestos bodies per unit weight (wet or dry). Thus, the use of surface unit in $cm^3$ instead of wet or dry weight is the best method to minimize the influence of tissue density on the results of asbestos burden counts [47].

## 6.3 Methods for Mineral Fiber Analysis

### 6.3.1 Light Microscopy (LM)

Light microscopy analysis of lung tissue burden is characterized by the following pros and cons:

- Allows the detection of low concentrations (1 asbestos body per $cm^3$ or gram of wet or dry lung tissue)
- It is a quick and inexpensive method to confirm asbestos burden.
- Limited resolution (0.2 μm) and magnification (400×).
- Consequently only large fibers with a diameter of >0.2 μm can be detected.
- Asbestos bodies formed primarily on asbestos fibers longer than 8–10 μm, thus asbestos bodies present a selected population of long asbestos fibers.

The first description of asbestos bodies goes back to the work of a German pathologist [77] who called them "pigmented crystals." The term "asbestos bodies" was used for the first time in the 1930s. [31, 80].

The majority of asbestos bodies from human lungs have amphibole asbestos cores. Of these asbestos bodies, only 2–7% [23, 60, 92] consist of a chrysotile core and 98% to 93% enclose an amphibole asbestos core. Chrysotile asbestos fibers cannot be identified by light microscopy due to their very thin diameters. By light microscopy, all structures with a characteristic proteinous envelope containing straight fiber cores that appear colorless, transparent, slight birefringence (under polarized light), and with plan parallel edges [18, 23] are identifiable as asbestos bodies. Most non-asbestos ferruginous bodies or pseudoasbestos bodies can be distinguished from true asbestos bodies at the light microscopic level [18, 26–28, 33, 39, 42]. Therefore, a trained dust analyst can clearly identify asbestos bodies and pseudoasbestos bodies based on the morphological definition of asbestos bodies [39].

The characteristic light microscopical appearance and the identification in histologic sections is an important component of the pathologic diagnosis of asbestosis (I–IV) [94].

So, light microscopy of chemically digested lung tissue at magnifications between 200 and 400× and in combination with polarization techniques is an ideal routine method for the quantification of asbestos bodies and asbestos burden of the lung. [112]. In cases where asbestos bodies cannot be identified by light microscopy and with obvious secondary lung alterations, additional electron microscopic mineral-fiber analysis of digested lung tissue should follow.

### 6.3.2 Electron Microscopy

Electron microscopical methods with high resolutions (Analytical scanning electron microscope (SEM), Analytical Transmission electron microscope (TEM)) were able to detect thin (diameter 0.05–0.01 μm) and small (down to 0.3 μm in length) fibers. The SEM method allows the detection of asbestos bodies and uncoated fibers in parallel, and this technique has the advantage of a relatively simple preparation of the lung tissue. The option to perform EDX-analysis of each single fiber makes it possible to differentiate between non-asbestos and asbestos fibers and also to discriminate and

subtype between different asbestos species. In comparison to TEM-analysis, the SEM method allows the examination of larger proportions of the filter surface and consequently more of the lung tissue. So, the extrapolation of fiber concentration in relation to the total sample volume is more reliable and less prone to over- or underestimation. Due to the more complex preparation techniques and the very small percentage of the sample that can be examined on a single TEM grid, the TEM method is time consuming and only ideal and useful for specialized investigations and where other approaches like light microscopy and SEM techniques have failed.

### 6.3.3 Comparability of Results Generated by Light or Electron Microscopy

There is a good correlation (correlation coefficient = 0.091, $p < 0.0001$) between asbestos bodies concentrations determined by SEM and LM [113]. Also the asbestos bodies concentration of the lung counted light microscopically correlates well (correlation coefficient 0.79, $p < 0.00019$) with the pulmonary burden of uncoated fibers (≥5 μm) measured by SEM [36, 67, 90, 91, 114]. The comparative evaluation of EM and LM lung dust countings has shown, that the ratio of asbestos bodies and asbestos amphibole fibers may range between 1:10 and >1:200 in dependency on tissue preparation and analytical method (SEM/TEM) [28, 30, 49, 97, 101, 108, 110, 113, 126].

### 6.3.4 Reference Population and Background Lung Asbestos Burden

The evaluation of a maximum standard value for a normal or background fiber burden of the lung is a relevant task and an essential assumption to quantitatively define elevated fiber concentrations. The reference population for the "general population" includes subjects without occupational asbestos exposure living in areas without asbestos deposits or asbestos manufacturing industries. Such a "general population" is only exposed to asbestos up to the general and ubiquitous level of environmental contamination with asbestos fibers [37, 41, 43]. The evaluated content of lung asbestos burden of such a reference population can be used to determine an elevated asbestos concentration in disease cases with an occupational asbestos exposure history.

For light microscopical asbestos burden analysis, there are several studies [19, 39, 40, 45, 114, 115] concluding a burden of 0 up to <22 asbestos bodies per gram wet tissue as representative for the general population. On the electron microscopical level, there is no generally applicable and universal asbestos fiber concentration that might be used by every laboratory to distinguish between fiber burden of the normal population and occupationally exposed individuals [49]. Each laboratory has to establish its own reference values. In the German Mesothelioma Register, our reference values for the general population ($n = 50$) were evaluated for the FE-REM method [14]. Based on these values, "normal" asbestos burdens can extent up to $1.0 \times 10^4$ amphibol and $1.8 \times 10^4$ chrysotile asbestos fibers (>5 μm in length) per gram wet tissue.

### 6.3.5 Asbestos Bodies and Fiber Counting

Tissue samples were selected, if possible, from four different locations of both lungs, for the quantification of asbestos body concentrations (asbestos bodies/cm$^3$ lung tissue or g wet tissue). The filter analyses [19, 45] were examined by light microscopy at 200–400 × magnification (differential interference contrast / polarization microscopy). Only characteristic bodies with typical morphology and thin, colorless, and translucent cores were counted as asbestos bodies [18, 113].

Fiber identification and quantification [113, 116, 117] were performed by SEM microscopy 1,000–20,000 magnification. Fibers were defined as particles with a ratio (length / width) of at least 3:1.

## 6.4 Asbestos Lung Tissue Content in Patients with Mesothelioma

### 6.4.1 Light Microscopy

The asbestos burdens of the cases recorded in the German mesothelioma register were determined mainly by light microscopy. The pathologic and demographic data are presented in Table 6.1. In most of the mesothelioma patients (84%), we were able to detect an increased asbestos burden (more than 22 asbestos bodies/cm$^3$ = maximum standard value) of the lung. About 30% of these patients had distinctly elevated concentrations (more than 1,000 asbestos bodies / cm$^3$) in lung tissue and 54% of the examined tissue samples contained a slightly to moderately elevated asbestos burden (>22–1,000 asbestos bodies).

**Table 6.1** Mesothelioma cases: pathologic and demographic data

| | | % |
|---|---|---|
| Sex | | 94 (men)<br>6 (women) |
| Pleura mesothelioma | | 96 |
| Peritoneal mesothelioma | | 3.0 |
| Pericardial mesothelioma | | < 1 |
| Epithelioid subtype | | 36 |
| Biphasic subtype | | 52 |
| Sarcomatoid | | 12 |
| Pleural plaques | Yes | 42 |
| | No | 15 |
| | Unknown | 43 |
| Asbestosis | | 27 |

At least 16% of the mesothelioma patients showed no detectable elevated asbestos burden in light microscopy analysis. In about 10% of this patient group, significant secondary alterations such as pneumonia, autolysis, or tumorous infiltrations were seen. These alterations may cause destruction of the asbestos body coats which subsequently become undetectable by light microscopy. This leads to substantial underestimation of the measured concentration values. After excluding these "false negative" cases, a collective of ca. 6% patients with definitively no measurable elevated asbestos burden on the light microscopical level remained. These cases needed further investigation concerning the background of the etiology of their malignant mesotheliomas.

The total group of mesothelioma patients was divided into two parts {(Group I (1989–1999) and Group II (2000–2009)} in order to assess possible changes of asbestos burden in mesothelioma patients during the respective decades.

In comparison to the older cases in study group I (Table 6.2) there is a significant trend toward lower median asbestos burden (320 to 290 asbestos bodies per cm$^3$) in group II.

Also latency times become significantly longer in group II (38–43 years) and patients in group II are significantly older (mean age 65 years) than the patients of group I (mean age 60 years) at the time of diagnosis.

Our data are in line with results of a recent study by Roggli (2008, Table 6.3). He also showed a time-related significant trend toward lower median asbestos burden and older ages with a median of 480 asbestos bodies and a mean age of 62 years in the period from 1980 to 1992 down to a median of 350 asbestos bodies and a mean age of 65 years for the years 1992–2005.

The median asbestos burden of the lung is significantly ($p < 0.05$) higher for patients with peritoneal mesothelioma than for patients with pleural mesothelioma [Neumann 2001].

**Table 6.2** Mesothelioma and asbestos burden (light microscopy) and latency period

| Light microscopy | | | |
|---|---|---|---|
| | 1989–2009 Asbestos burden (asbestos bodies/cm$^3$ wet tissue) | 1989–1999 Asbestos burden (asbestos bodies/cm$^3$ wet tissue) | 2000–2009 Asbestos burden (asbestos bodies/cm$^3$ wet tissue) |
| Median | 310 | 320 | 290 |
| Minimum | 1 | 1 | 0 |
| Maximum | 990,000 | 990,000 | 410,000 |
| Probability | | <0.05 | |
| Latency period (in years) | 40 | 38 | 43 |
| Mean age at diagnosis | – | 60 | 65 |
| Probability | | <0.05 | |

**Table 6.3** Mesothelioma and asbestos burden (light microscopy) [117]

| Light microscopy | | | |
|---|---|---|---|
| | Total Group 1980–2005 Asbestos burden (asbestos bodies/g wet tissue) | Subgroup I 1980–1992 Asbestos burden (asbestos bodies/g wet tissue) | Subgroup II 1992–2005 Asbestos burden (asbestos bodies/g wet tissue) |
| Median | – | 480 | 350 |
| Minimum | 1 | 1 | 3.3 |
| Maximum | 1,600,000 | 1,600,000 | 207,000 |
| Probability | | $p < 0.05$ | |

### 6.4.2 Electron Microscopy

The predominant fiber type identified by electron microscopy in patients with mesothelioma is amphibole asbestos (crocidolite or amosite) [112]. In a study of 94 cases, about 60% of the analyzed fibers were amosite [111]. Patients with mesothelioma show elevated levels of amphibole but not of chrysotile fibers compared to control groups [56, 57, 111, 116]. The lung SEM dust study [18] based on 409 patients with malignant mesothelioma and the measured (SEM) asbestos contents of patients with malignant mesothelioma are summarized in Table 6.4. As seen in data obtained by light microscopy, SEM analysis of this collective also reflects a significant trend toward lower asbestos bodies and asbestos fiber burden during the decades [18].

The percentage of cases with elevated amphibole fiber burden (over the reference range) in this collective was about 80% [18]. There was a trend for decreasing asbestos fiber burden from group 1 to group 2.

### 6.4.3 Asbestos Content and Fiber Dimensions in Pleural Samples

The vast majority of studies analyses asbestos fiber burden only in lung parenchyma. Only few studies [17, 41, 43, 57] found long amphibole fibers in different samples of the pleura (pleural

**Table 6.4** Mesothelioma and asbestos burden measured by electron microscopy modified from Roggli 2008

| | Total Group 1980–2005 Asbestos burden (asbestos bodies/g wet tissue) | Subgroup I 1980–1992 Asbestos burden (asbestos bodies/g wet tissue) | Subgroup II 1992–2005 Asbestos burden (asbestos bodies/g wet tissue) |
|---|---|---|---|
| *SEM-Analysis Amosite* | | | |
| Median | – | 17,500 | 6,330 |
| Minimum | 120 | 120 | 390 |
| Maximum | 11,900,000 | 11,900,000 | 2,610,000 |
| Probability | | <0.05 | |
| *SEM-Analysis Chrysotile* | | | |
| Median | – | 1,800 | 1,370 |
| Minimum | 580 | 580 | 590 |
| Maximum | 124,000 | 124,000 | 4,180 |
| Probability | | <0.05 | |

plaque, diffuse visceral pleural fibrosis) from asbestos workers. One study [17] found especially long commercial amphibole fibers in black spots of the parietal pleura [17]. Another study [127] reported short chrysotile fibers in pleural and mesothelial tissue. The examination of individuals exposed to mixed amphibole and white asbestos [120] showed that short chrysotile fibers (<5 μm) accumulate primarily in the pleura whereas longer amphibole fibers accumulate primarily in lung tissue. In contrast, several other studies [17, 42, 54, 57, 120, 127] provided evidence that short (<5 μm) and long chrysotile and amphibole asbestos fibers are able to reach the pleural tissue. So, it is especially those fiber types and sizes with the highest carcinogenic potential that can be transported to the pleura [113, 128].

## 6.5 Discussion

The pathogenic response of the lung to inhaled dust depends on the mineral fiber type, exposure conditions (short-time overload or prolonged moderate exposure) and fraction of respirable fibers. The mineral fiber content in the lung reflects the pathogenic fraction of inhaled dust which represents only a minor amount of the total fiber dust exposure prevailing at many workstations [102]. The quantity of mineral fiber asbestos consumption in Europe and other industrial states has changed over the last decades [126]. Therefore, individual asbestos exposure normally changes during lifetime and especially during working life.

### 6.5.1 Asbestos Bodies and Fiber Burden of the Lung

In lung tissue of most mesothelioma patients (85%) [93], elevated levels of asbestos could be detected by light microscopy. Negative results in lung dust analyses (16%) have to be assessed with caution. After excluding such cases with unsuitable lung tissues only 6% of patients revealed definitely no elevated asbestos burden of the lung. The frequency distribution of light microscopically evaluated asbestos body concentrations does not correlate with a special tumor subtype. All asbestos-related tumor entities were seen within the whole range of asbestos lung concentrations [93, 95]. Other investigators

6

[1991], too, found no differences in asbestos body concentrations in relation to different tumor subtypes.

Considering only amphibole fibers, there is a known significant relation of asbestos fiber-concentration and the number of asbestos bodies in lung tissue [1, 51, 67, 110]. The results of most studies, however, show that patients with mesotheliomas and occupational asbestos exposure show increased concentrations of amphibole asbestos, but not of chrysotile [89, 130].

### 6.5.2 Latency Period and Mean Age at Diagnosis of Mesothelioma

As shown in other studies [78, 117], we also observed in our patient group a trend toward longer latency times and an increased average age for the initial diagnosis of mesothelioma. Mesothelioma patients showed an inverse relationship between latency period and pulmonary asbestos burden [117]. So, patients with very high asbestos burdens show significantly shorter latency periods [93]. The observed decrease of the median asbestos burden of the lung from one decade to the other may explain the tendency toward elongated latency periods and higher age of mesothelioma patients.

### 6.5.3 Clearance and Biopersistence of Asbestos Fibers

The geometry of the tracheobronchial tree and the different clearing mechanisms of the respiratory systems are important factors influencing the deposition of particles and fibers. The clearing mechanisms include fine hairs in the nasal cavity, the mucociliary escalator of the tracheobronchial tree and the alveolar macrophages. Long-term inhalation studies demonstrated that the relative retention of amphibole fibers in the lungs is considerably higher than that for chrysotile [24, 25, 34, 131] and that amphibole fibers accumulate within the lungs to a much greater extent than chrysotile fibers.

The average length of fibers – observed for chrysotile and amphibole – retained within the lung increased in parallel with time after exposure. This observation may be explained by a more effective clearance of shorter fibers [81, 82, 84, 85, 89]. As yet, there is no definite reason for the preferential retention of amphibole fibers in the lung; however, various aspects are in discussion. Important factors could be the tendency of chrysotile to split longitudinally into very small individual fibrils [11, 12] or a different biopersistence of chrysotile in comparison to amphibole asbestos. New experimental animal studies provide very different results for the biopersistence of chrysotile asbestos. One study [12] using a rat model showed that one year after asbestos exposure no chrysotile fibers longer than 20 μm remain in the lungs. Another study with monkeys [125] describes the detection of white asbestos fibers and asbestos bodies containing chrysotile fibers 11.5 years after inhalation of chrysotile asbestos. In some cases [49, 50], elevated levels of chrysotile asbestos in the lung were found as late as 60 years after asbestos exposure.

However, chrysotile is less biopersistent than amphibole asbestos fibers [12, 13A, 29, 30]. Only in patients with massive pulmonary asbestos burdens overload, the amounts of both chrysotile and amphibole fibers are increased [23, 29, 107]. After intermediate time of decades elevated chrysotile burden overload of the lung are rare [49, 51]. So, there is no clear correlation between asbestos bodies and chrysotile concentrations [1, 40, 51, 114], and asbestos bodies with chrysotile as a central core are rare [41, 69]. The results of most studies show that patients with mesothelioma after occupational asbestos exposure possess increased concentrations of amphibole asbestos but no elevated levels of chrysotile [46, 52, 82, 86, 115, 130, 133].

### 6.5.4 Carcinogenic Potency of Asbestos Fibers

According to results of a cohort study including 3,072 workers from an asbestos textile plant [124], the carcinogenic potential of the fibers is strongly associated with the exposure to long (>10 μm) and thin fibers (<0.25 μm). The detection of short (<5 μm) white asbestos fibers is of questionable relevance, because a convincing pathogenic potency is not attributable to this subclass of chrysotile fibers [113].

The carcinogenic potency of chrysotile asbestos for mesothelioma is discussed controversially [11–13A, 24, 48, 74, 81, 88, 100, 119, 123]. Some cohort studies stated significant positive relations between estimated chrysotile exposure and lung cancer and asbestosis mortality [62].The tendency of chrysotile asbestos [12, 100] to fragment into shorter fibers and its reduced biopersistence are possibly the reasons for the lower carcinogenic potency in comparison to amphibole asbestos [12]. One meta-analysis [64] comes to the conclusion that the relative specific risks to develop mesothelioma after exposure to the three commercially used asbestos types chrysotile, amosite, and crocidolite, can be described by the ratio of 1:100:500, respectively. Whereas some cohort studies demonstrate significant positive relations between estimated chrysotile exposure and lung cancer or asbestosis mortality [62], the majority of studies stated that amphibole asbestos fibers were the primary reason for an elevated risk to develop mesothelioma [29, 30, 82, 86]. Chrysotile asbestos is often contaminated with low doses of tremolite asbestos, one hypothesis is that the tremolite contaminant is the exclusive substance inducing cancer in chrysotile mine workers [53, 54, 62, 84, 101]. Some suggested that workers exposed to "pure" chrysotile have no increased cancer risk. This speculation has been referred to as the amphibole hypothesis [11–13A, 62, 71, 84, 85].

There is new scientific evidence for the missing fibrogenic potency of chrysotile (exception overload situation) [13A]. Futher chrysotile fibers do not migrate to the pleura cavity, the site of mesothelioma origin [13B].

### 6.5.5 Peritoneal Mesothelioma

Some studies clearly demonstrate a significant relation between the degree of asbestos lung burden and the primary tumor site [8, 73, 93]. Elevated asbestos-concentrations in lung tissue (>5,000 asbestos bodies/cm$^3$) are significantly higher in patients with peritoneal than those with pleural mesotheliomas. Especially, a high number of asbestos bodies can be found in the group of patients with peritoneal mesotheliomas of the most frequent epithelioid subtype. In contrast to one study [68], our data suggest that the amount of asbestos bodies in lung tissue has no prognostic value and does not correlate with the survival time.

### 6.5.6 Asbestos-Associated Mesothelioma and Other Possible Causes of Malignant Mesothelioma

According to other studies [134], the percentage of asbestos-associated mesotheliomas is about 90%. Only 5–10% of the patients have no elevated pulmonary asbestos burden.

Exposure to erionite [10, 98], too, leads to higher incidences of mesothelioma and plays an important role in environmental exposure. For example, in some regions of Central Turkey the development of malignant mesothelioma is associated with a ubiquitous presence of erionite. This mineral is a hydrated aluminum silicate of the zeolith mineral family and shows similar characteristics and cancerogenic potencies as amphibole asbestos.

Apart from this, other mesothelioma-inducing factors are in discussion: Infection with SV-40

virus [5, 21], Wilms tumor [3, 4], recurring inflammations [105], thorotrast [79, 93], ionized radiation [22], Mediterranean fever [76], and genetic factors [118] are suggested to play a role in the development of malignant mesothelioma.

#### 6.5.7 Threshold or Cut-off Level

There is an ongoing discussion about the definition of a cut-off level of asbestos exposure beyond which the exposure to asbestos does not lead to the development of malignant mesothelioma [63, 66, 82, 107, 109, 121, 128]. However, such a specific threshold based on measurements or assumed levels of asbestos exposure has not yet been determined scientifically [16, 63, 82, 93, 108, 117]. In spite of this, every action taken over the last decades resulting in the reduction and prevention of occupational exposure to asbestos fibers was an important and decisive improvement. With the implementation of these exposure prevention measures, a decrease of average concentrations from about 500 fibers/$cm^3$ in the early 1950s to less than 1 fiber/$cm^3$ until the asbestos ban in Germany was achieved [6, 38, 99]. So, the reduction of asbestos doses on different workplaces by effective prevention measures leads to lower asbestos burdens of the lung, resulting in longer latency times, a higher average age of mesothelioma patients, and a shifted peak of mesothelioma development.

## 6.6 Conclusion

The most important causal factor for the development of mesothelioma is still asbestos exposure. In this context, lung dust analyses are a relevant method for the determination of causation in mesothelioma. Quantitative analysis of asbestos burden of the lung and qualitative differentiation of fiber types provide helpful insights into the pathogenesis of malignant mesothelioma.

It is also possible that patients with asbestos bodies or asbestos fibers counts comparable to the "normal population" develop asbestos-associated mesothelioma. But other possible causes of malignant mesothelioma have to be taken into consideration. Patients with no history of occupational asbestos exposure and without elevated asbestos burden of the lung may develop a so-called background or spontaneous mesothelioma. Are these cases a result of other etiological factors than asbestos or the erionite exposure?

## References


1. Albin M, Johansson L, Poley F, Jakobsson K, Attewell R, Mitha R (1990) Mineral fibres, fibrosis and asbestos bodies in lung tissue from deceased asbestos cement workers. Br J Ind Med 47:767–74
2. Albracht G, Schwerdtfeger O (1991) Herausforderung Asbest. Universum Verlag GmbH KG, Wiesbaden
3. Amin KM, Litzky LA, Smythe WR (1995) Wilms tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. Am J Pathol 246:344–56
4. Antman KH, Ruxer RL, Aisner J, Vawter G (1984) Mesothelioma following Wilms' tumor in childhood. Cancer 54:367–69
5. Aoe K, Hiraki A, Murakami T, Toyooka S, Shivapurkar N, Gazdar A, Sueoka N, Taguchi K, Kamei T, Takeyama H, Sugi K, Kishimoto T (2006) Infrequent existence of simian virus 40 large T antigen DNA in malignant mesothelioma in Japan. Cancer Sci 97:292–295
6. Arendt M, Bauer H, Blome H (2007) BK-Report 1/2007 – FaserjahreBerufsgenossenschaftliche Hinweise zur Ermittlung der kumulativen Asbestfaserstaub-Dosis am Arbeitsplatz (Faserjahre) und Bearbeitungshinweise zur Berufskrankheit Nr. 4104 "Lungenkrebs oder Kehlkopfkrebs". In: Deutsche Gesetzliche Unfallversicherung (DGUV), ed. Sankt Augustin, Germany 2007
7. Ashcroft T, Heppleston A (1973) The optical and electron microscopy determination of pulmonary asbestos fibre concentration and its relation to the human pathological reaction. J Clin Pathol 26:224–234

8. Attanoos R, Gibbs AR (1998) Peritoneal Mesothelioma: clinicopathological analysis of 227 cases from the U.K. mesothelioma register. Arch Anat Cyt Path Clin Exp Path 46:376
9. Bang K, Mazurek J, Storey E, Attfiel M, Schleiff P, Wood J (2009) Malignant mesothelioma mortality – United states 1999–2005. MMWR Morb Wkly Rep 58:393–396
10. Baris YI, Simonato L, Artvinli M (1987) Epidemiological and environmental evidence of the health effects of exposure to erionite fibers: a four study in the Cappadocian region of Turkey. Int J Cancer 39:10–17
11. Berman D, Crump K (2008) A meta analysis of asbestos related cancer risk that addressed fiber size and mineral type. Crit Rev Toxicol 38:49–73
12. Bernstein D, Donaldson K, Decker U, Gaering S, Kunzendorf P, Chevallier J, Holm S (2005) A biopersistence study following exposure to chrysotile asbestos alone or in combination with fine particles. Inhal Toxicol 20:1009–1028

13A. Bernstein D, Hoskins J (2006) The health effects of chrysile: current perspective based upon recent data. Regul Toxicol Pharmacol 45:252–264

13B. Bernstein D, Rogers R, Sepulveda R, Donaldson K, Schuler D, Gaering S, Kunzendorf P, Chevalier J, Holm S. (2010) The pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite asbestos following short term inhalation exposure. interim results. Inhal. Toxicol 22:937–962

14. BIA-Arbeitsmappe (2000–2001) Bestimmung von anorganischen Fasern im menschlichen Lungengewebe 26. Lfg 26. III/01 und 24. Lfg. III/00 TEM und REM Methode
15. Bianchi C, Brollo A, Ramani L, Bianchi T (2000) Malignant mesothelioma in Europe. Int J Med Biol Environ 28:103–107
16. Bianchi C, Girelli L, Grandi G, Brillo A, Ramani L, Zuch C (1997) Latency periods in asbestos related mesothelioma of the pleura. Eur J Cancer Prev 6:162–6
17. Boutin C, Dumortiers R, Rey F, Viallat J, DeVuyst P (1996) Black spots concentrate oncogenic asbestos fibers in the parietal pleura. Am J Respir Crit Care Med 153:444–449
18. Brockmann M (1991) Asbestassoziierte Lungen- und Pleuraerkrankungen – pathologische Anatomie. Pneumologie 45:422–428
19. Brockmann M, Fischer M, Müller K (1989) Lungenstaubanalyse bei Bronchialkarzinomen und Mesotheliomen. Atemw Lungenkrankh 6:263–65
20. Browne K, Smither W (1983) Asbestos related mesothelioma factors discriminating between pleural and peritoneal sites. Br J Ind Med 40:145–152
21. Carbone M (1999) New molecular and epidemiological issues in mesothelioma Role of SV40. J Cell Physiol 180:167–72, Review
22. Cavazza A, Travis LB, Travis WD, Wolfe JT, Foo ML, Gillespie DJ, Weidner N, Colby TV (1996) Post irradiation malignant mesothelioma. Cancer 77:1379–1385
23. Churg A (1982) Fiber counting and analysis in the diagnosis of asbestos related diseases. Hum Pathol 13:381–392
24. Churg A (1988) Chrysotile, tremolite and malignant mesothelioma in man. Chest 93:621–28
25. Churg A (1994) Deposition and clearance of chrysotile asbestos. Ann Occup Hyg 38: 625–633
26. Churg A, Warnock M (1981) Asbestos and other ferruginous bodies. Am J Pathol 102:447–456
27. Churg A, Warnock M, Green N (1977) Analysis of the cores ferruginous (asbestos) bodies from the general population. I. Patients with and without lung cancer. Lab Invest 37:280–286
28. Churg A, Warnock M, Green N (1979) Analysis of the core of ferruginous bodies from the general population. II. True asbestos bodies and pseudoasbestos bodies. Lab Invest 40:31–38
29. Churg A, Wiggs H (1984) Fiber size and number of amphibole asbestos induced mesothelioma. Am J Pathol 115:437–442
30. Churg A, Wiggs B, DePaoli L, Kampe B, Stevens B (1984) Lung asbestos content in chrysotile workers with mesothelioma. Am Rev Respir Dis 130:1042–45
31. Cooke W, Hill C (1927) Pulmonary asbestosis. J R Microsc Soc 47:232
32. Craighead J (1987) Current pathogenetic concepts of diffuse malignant mesothelioma. Hum Pathol 18:544–557
33. Crouch E, Churg A (1984) Ferruginous bodies and the histologic evaluation of dust exposure. Am J Surg Pathol 8:109–116
34. Davis J, Beckett S, Bolton R, Collings P, Middleton A (1978) Mass and number of fibers in the pathogenesis of asbestos related lung disease in rats. Br J Cancer 37:673–688

6


35. Dawson A, Gibbs A, Pooley F, Griffiths D, Hoy J (1993) Malignant mesothelioma in women. Thorax 48:269–74
36. DeKlerk N, Musk A, Williams V, Filion P, Whitaker D, Shilkin K (1996) Comparison of measures of exposure to asbestos in former crocidolite workers from Wittenoom Gorge, W. Australia. Am J Ind Med 30:579–587
37. DeVuyst P, Karjalainen A, Dumortier P, Pairon J, Monso E, Brochard P, Teschler H, Tossavainen A, Gibbs A (1998) Guidelines for mineral fibre analysis in biological samples: report of the ERS Working group. Eur Resp J 11:1416–1426
38. DGUV (2008) Occupational cancer statistics www://dguv.de/inhalt/zahlen/bk/index.jsp
39. Dodson F, Aktinson M (2006) Measurements of asbestos burden in tissues. Ann NY Acad Sci 1076:281–291
40. Dodson R, O'Sullivan F, Corn C (1996) Relationships between ferruginous bodies and uncoated asbestos fibers in lung tissue. Arch Environ Health 51:462–6
41. Dodson R, O'Sullivan M, Corn M, McLarty J, Hammar S (1997) Analysis of asbestos fiber burden in lung tissue from mesothelioma patients. Ultrastruct Pathol 21:321–36
42. Dodson R, Williams M, Corn C, Brollo A, Bianchi C (1990) Asbestos content of lung tissue, lymph nodes and pleural plaques from former shipyard workers. Am Rev Respir Dis 142:843–847
43. Dodson R, Williams M, Huang J, Bruce J (1999) Tissue burden of asbestos in non-occupationally exposed individuals from east Texas. Am J Ind Med 35:281–286
44. Edge J, Choudhury S (1978) Malignant mesothelioma of the pleura in Barrow in Furness. Thorax 33:26–30
45. Eitner F, Otto H (1984) Zur Dignität von Asbestkörperzählungen im Lungengewebe. Arbeitsmed Sozialmed Präventivmed 19:1–5
46. Elmes P (1994) Mesothelioma and chrysotile. Ann Occup Hyg 38:547–553
47. Ewers U, Fischer M, Müller K, Seemann J, Theile A, Welge P, Wittig P, Wittsiepe J (1999) Multiple inhalative exposure of the human lung to carcinogens, metalloids and asbestos fibers. BAMAS FP 1947–1469
48. Frank A, Dodson R, Williams M (1998) Carcinogenic implications of the lack of tremolite in UICC reference chrysotile. Am J Ind Med 34:314–17
49. Friedrichs K, Brockmann M, Fischer M, Wick G (1992) Electron microscopy analysis of mineral fibers in human lung tissue. Am J Ind Med 22(49):49–58
50. Friedrichs K, Dykers A, Otto H (1995) Material stability of asbestos fibers in human lung tissue [Materialstabilität von Asbestfasern im Lungengewebe]. Arbeits Sozial Umweltm 30: 18–20
51. Friedrichs KH, Otto H, Fischer M (1992) Gesichtspunkte zur Faseranalyse in Lungenstäuben. Arbeits Soz Prävent 27:228–32
52. Gaudichet A, Janson X, Monchaux G (1988) Assessment by analytical microscopy of the total lung fibre burden in mesothelioma patients matched with four other pathological series. Ann Occup Hyg 32(Suppl):213–23
53. Gibbs G (1970) Qualitative aspects of dust exposure in the Quebec asbestos mining and milling industry. In: Walton WH (ed) Inhalated particles 3, Proceeding of the British occupational hygiene society symposium, London, 1970, pp 783–799
54. Gibbs A (1990) Role of asbestos and other fibres in the development of diffuse malignant mesothelioma. Thorax 45:649–54
55. Gibbs A, Attanonous R (2000) Examination of lung specimens. J Clin Pathol 53:507–12
56. Gibbs A, Pooley F (1996) Analysis and interpretation of inorganic mineral fibers in lung tissue. Thorax 51:327–34
57. Gibbs A, Stephens M, Griffiths D, Blight B, Pooley F (1991) Fibre distribution in the lungs and pleura of subjects with asbestos related diffuse pleural fibrosis. Am Rev Respir Dis 142:843–847
58. Glyseth B, Baumann R, Overaae L (1982) Analysis of fibers in human lung tissue. Br J Ind Med 39:191–195
59. Gold C (1971) Asbestos in tumours. J Clin Pathol 24:481
60. Gross P, Trevillle R, Haller M (1969) Pulmonary ferruginous bodies (asbestos) bodies in city dwellers a study of their central fiber. Arch Environ Health 19:186–191
61. Hammar S, Roggli V, Ovry T, Moffat E (1998) Malignant mesothelioma in women. Lung Cancer 18:38
62. Hein M, Stayner L, Lehmann E, Dement J (2007) Follow up of chrysotile textile workers: cohort mortality and exposure response. Occup Environ Med 64:616–625
63. Hodgson J, Darnton A (2000) The quantitative risk of mesothelioma and lung cancer in relation

to asbestos exposure. Ann Occup Hyg 44: 565–601
64. Hoggson J, McElvenny D, Darnton A, Price M, Peto J (2005) The expected burden of mesothelioma mortality in Great Britain from 2002 to 2050. Br J Cancer 92:587–593
65. Howel D, Gibbs A, Arblaster L, Swinburne L, Schweiger M, Renvoize E, Hatton P, Pooley F (1999) Mineral fibre analysis and routes of exposure to asbestos in the development of mesothelioma in an English region. Occup Environ Med 56:51–58
66. Illgren E, Browne K (1991) Asbestos related mesothelioma: evidence for a threshold in animals and human. Regul Toxicol Pharmacol 13:116–32
67. Karyalainen A, Nurminen M, Vanhala E, Vainio H, Antilla S (1996) Pulmonary asbestos bodies and asbestos fibres as indicators of exposure. Scand J Work Environ Health 22:34–38
68. Kayser K, Becker C, Seeberg N, Gabius HJ (1999) Quantitation of asbestos and asbestos like fibres in human lung tissue by hot and wet ashing and the significance of their presence for survival of lung carcinoma and mesothelioma patients. Lung Cancer 24:89–98
69. Kohyama N, Hiroko K, Kunihiko Y, Yoshizumi S (1992) Evaluation of low level asbestos exposure by transbronchial lung biopsy with analytical electron microscopy. J Electron Microsc 42: 315–327
70. Kohyama N, Suzuki Y (1991) Analysis of asbestos fibres in lung parenchyma, pleural plaques and mesothelioma tissues of north American insulation workers. Ann NY Acad Sci 643:27–52
71. Landrigan P, Nicholson W, Suzuki Y, Ladou J (1999) The hazard of chrysotile asbestos: a critical review. Ind Health 37:271–280
72. Leigh J, Davidson P, Hendrie L, Berry D (2002) Malignant mesothelioma in Australia, 1945–2000. Am J Ind Med 41:188–201
73. Leigh J, Rogers A, Ferguson D, Mulder H, Ackad M, Thompson R (1991) Lung asbestos fiber content and mesothelioma cell type site and survival. Cancer 68:135–141
74. Lidell D (1994) Cancer mortality in chrysotile mining and milling: exposure-response. Ann Occup Hyg 38:519–523
75. Lin R, Takabashi K, Karjalainen A, Hoshuyama T, Wilson D, Kameda T, Chan C, Wen C, Furuya S, Higashi T, Chien L, Ohtaki M (2007) Ecological association between asbestos related diseases and historical asbestos consumption: an international analysis. Lancet 369:844–849
76. Livneh A, Langevitz P, Pras M (1999) Pulmonary associations in familial Mediterranean fever. Curr Opin Pulm Med 5:326–31
77. Marchand F (1906) Über eigentümliche Pigmentkristalle in den Lungen. Verhand Deutsch Pathos Gesell 10:223–228
78. Marinaccio A, Binazzi A, Cauzillo G, Cavone D, DeZottti R, Ferrante P, Gennaro V, Gorini G, Menegozzo M, Mensi C, Merler E, Mirabelli D, Montanaro F, Musti M, Pannelli F, Romanelli A, Scarselli A, Tumino R (2007) Analysis of latency time and its determinants in asbestos related malignant mesothelioma cases of the Italian register. Eur J Cancer 43(18):2722–2728
79. Maurer R, Egloff B (1975) Malignant peritoneal mesothelioma after cholangiography with thorotrast. Cancer 36:1381–85
80. McDonald S (1927) Histology of pulmonary asbestosis. Br Med J 3(2):1025
81. McDonald J (1998) Mineral fibre persistence and carcinogenicity. Ind Health 36:372–5
82. McDonald J, Amstrong B, Case B (1989) Mesothelioma and asbestos fiber type: evidence from lung tissue analyses. Cancer 63:154–1547
83. McDonald J, McDonald A (1996) The epidemiology of mesothelioma in historical context. Eur Respir J 9:1932–42
84. McDonald J, McDonald A (1997) Chrysotile, tremolite and carcinogenicity. Ann Occup Hyg 6:699–705
85. McDonald J, McDonald A, Hughes JM (1999) Chrysotile, tremolite and fibrogenicity. Ann Occup Hyg 43:439–442
86. McDonald AD, McDonald JC, Pooley FC (1982) Mineral fiber content of lung in mesothelioma tumors in North America. Ann Occup Hyg 26:417–422
87. McElvenny D, Darnton A, Price M, Hodgson J (2005) Mesothelioma mortality in Great Britain from 1968 to 2001. Occup Med 55:79–87
88. Mirabelli D, Calisti R, Barone-Adesi F, Foriero E, Merletti F, Magnani C (2008) Excess of mesothelioma after exposure to chrysotile in Balangero, Italy. Occup Environ Med 65:815–819
89. Morgan A, Holmes A (1979) Concentrations and dimensions of coated and uncoated asbestos fibers in the human lung. Br J Ind Med 37:25–32
90. Morgan A, Holmes A (1983) Distribution and characteristics of amphibole asbestos fibres, measured with the light microscope, in the left lung of an insulations worker. Br J Ind Med 40:45–50

6


91. Morgan A, Holmes A (1984) The distribution and characteristics of asbestos fibers in the lungs Finnish anthophyllite mine workers. Environ Res 33:62–75
92. Moulin E, Yourassowsky N, Dumortier P, DeVuyst J, Yernault J (1988) Electron microscopy analysis of asbestos body cores from the Belgian urban population. Eur Respir J 1:818–822
93. Neumann V, Günther S, Müller K, Fischer M (2001) Malignant mesothelioma – German mesothelioma register 1987 to 1999. Int Arch Occup Environ Health 74:383–395
94. Neumann V, Kraus T, Fischer M, Löseke S, Tannapfel A (2009) Relevance of Pathological Examinations and Lung Dust Analyses in the Context of Asbestos-Associated Lung Cancer-No. 4104 of the List of occupational diseases in Germany. Pneumologie 63: 588–593
95. Neumann V, Müller K, Fischer M (1999) Peritoneal mesothelioma – Frequencies and aetiology [Peritoneale Mesotheliome – Häufigkeiten und Ätiologie]. Pathologe 20:169–176
96. Newhouse M, Berry G, Wagner J (1985) Mortality of workers in east London 1933-80. Br J Ind Med 42:4–11
97. Ophus E, Mowe G, Osen K, Glyseth B (1980) Scanning electron microscopy and x-ray microanalysis of mineral deposits in lungs of a patient with pleural mesothelioma. Br J Ind Med 37:375–381
98. Osman E, Hasan B, Meral U, Ercan A, Mehmet T, Nazan B, Ayhan Ö, Erhan E, Öner D (2007) Recent discovery of an old diseases. Malignant pleural mesothelioma in a village in south east turkey. Respirology 12:448–451
99. Pesch B, Taeger D, Johnen G, Gross I, Weber D, Gube M, Müller-Lux A, Heinze E, Wiethege T, Neumann V, Tannapfel A, Raithel H, Brünning T, Kraus T (2010) Cancer mortality in a surveillance cohort of German males formerly exposed to asbestos. Int J Hyg Environ Health 213:44–51
100. Pierce J, McKinley M, Pausenbach D, Finley B (2008) An evaluation of reported no effect chrysotile asbestos exposure for lung cancer and mesothelioma. Crit Rev Toxicol 38: 191–214
101. Pooley F (1976) An examination of the fibrous mineral content of asbestos lung tissue from the Canadian chrysotile mining industry. Environ Res 12:281–298
102. Pooley F, Ranson D (1986) Comparison of the results of asbestos fibers dust counts in lung tissue by analytical electron microscopy and light microscopy. J Clin Pathol 39:313–317
103. Price B, Ware A (2004) Mesothelioma trends in the United states: an update based on surveillance, epidemiology and end results program data for 1973 through 2003. Am J Epidemiol 159:107–12
104. Rees D, Myers J, Goodmann K, Fourie E, Blignaut C, Chapman R, Bachmann M (1999) Case control study of mesothelioma in south Africa. Am Ind Med 35:213–22
105. Ridell RH, Goodman MJ, Moossa AR (1981) Peritoneal malignant mesothelioma in a patient with recurrent peritonitis. Cancer 48:134–139
106. Robinson B, Lake R (2005) Advances in malignant mesothelioma. N Engl J Med 253:1591–603
107. Rödelsperger K, Woitowitz H, Brückel B, Arhelger R, Pohlabeln H, Jöckel K (1999) Dose-response relationship between amphibole fibre lung burden and mesothelioma. Cancer Detect Prev 23:183–93
108. Rogers A (1984) Determination of mineral fiber in human lung tissue by light microscopy and transmission electron microscopy. Ann Occup Hyg 1:1–12
109. Rogers A, Leigh J, Berry G, Fergusond A, Mulder H, Ackad M (1991) Relationship between lung asbestos fiber type and concentration and relative risk of mesothelioma. Cancer 67:1912–20
110. Roggli V (1982) Pulmonary asbestos body counts and electron probe analysis of asbestos body cores in patients with mesothelioma. A study of 25 cases. Cancer 50:2423–2432
111. Roggli V (1992) Quantitative and analytical studies in the diagnosis of mesothelioma. Semin Diagn Pathol 9:162–168
112. Roggli V (2006) The role of analytical SEM in the determination of causation in malignant mesothelioma. Ultrastruct Pathol 30:31–35
113. Roggli V, Oury T, Sporn T (2004) Pathology of asbestos associated diseases, 2nd edn. Springer, New York

114. Roggli V, Pratt P, Brody A (1986) Asbestos content in lung tissue with asbestos associated diseases: a study of 110 cases. Br J Ind Med 43:18–19
115. Roggli V, Pratt P, Brody A (1993) Asbestos fiber type in malignant mesothelioma: an analytical electron microscopy study of 94 cases. Ultrastruct Pathol Am J Ind Med 23:605–614
116. Roggli V, Sanders L (2000) Asbestos content of the lung tissue and carcinoma of the lung: a clinicopathologic correlation and mineral fiber analysis of 234 cases. Ann Occup Hyg 44:109–117
117. Roggli V, Vollmer R (2008) Twenty five years of fiber analysis: what have we learned? Hum Pathol 39:307–15
118. Roushdy-Hammady I, Siegel J, Emri S, Testa J, Carbone M (2001) Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. Lancet 357:444–455
119. Sakai K, Hisanagna N, Huang J, Chibata E, Ono Y, Aoki T, Tarando T, Yokoi T, Takeuchi Y (1994) Asbestos and non asbestos fiber content in lung tissue of Japanese patients with malignant mesothelioma. Cancer 73:1825–1835
120. Sebastien P, Fondimare A, Bignon J, Monchaux G, Desbordes J, Bonnaud G (1977) Topographic distribution of asbestos fibers in human lung in relation to an nonoccupational exposure. In: Walton W, McGovern E (eds) Inhaled particles IV. Pergamon Press, Oxford, pp 435–444
121. Selikoff I (1986) Asbestos associated diseases. In: Rosenau M (ed) Public Health and preventive medicine, 11th edn. Appleton, Century Crofts, New York, pp 568–598
122. Selikoff I, Lee D (1978) Asbestos and disease. Academic, New York
123. Smith A, Wright C (1996) Chrysotile asbestos is the main cause of pleural mesothelioma. Am J Ind Med 30:252–66
124. Stayner L, Kuempel E, Gilbert S, Hein M, Dement J (2008) An epidemiological study of asbestos fibre dimension in determining respiratory disease risk in exposed workers. Occup Environ Med 65:613–19
125. Stettler L, Sharpnack D, Krieg E (2008) Chronic Inhalation of short asbestos: lung fiber burdens and histopathological for monkeys maintained for 11, 5 years after exposure. Inhal Toxicol 20:63–73
126. Survana K, Layton C (2006) What is a significant lung asbestos fibre result? Histopathology 48:200–19
127. Suzuki Y, Yuen S (2001) Asbestos tissue burden study in human malignant mesothelioma. Ind Health 39:150–160
128. Tomatis L, Susanna C, Francessco C, Merler E, Mollo F, Ricci P, Silvestri S, Vineis P, Teracini B (2007) The role of asbestos fiber dimensions in the prevention of mesothelioma. Int J Occup Environ Health 13:64–69
129. Vianna NJ (1978) Non occupational exposure to asbestos and malignant mesothelioma in females. Lancet 1(8073):1061–1063
130. Wagner J, Berry G, Pooley F (1982) Mesothelioma and asbestos type in asbestos textile workers: a study of lung content. Br Med J 285:603–6
131. Wagner J, Berry G, Skidmore J, Timbrell V (1974) The effects of the inhalation of asbestos in rats. Br J Cancer 29:252–269
132. Wagner JC, Sleggs CA, Marchand P (1960) Diffuse pleural mesothelioma and asbestos exposure in the north western cape province. Br J Ind Med 17:260–71
133. Woitowitz HJ, Hillerdal G, Calazos A (1994) Risiko und Einflussfaktoren des diffusen malignen Mesothelioms (DMM). Research Report series “Arbeit und Technik”, Fb 698. Wirtschaftsverlag NW. Federal Institute for occupational Safety and Health, Bremerhaven
134. Yates D, Corrin B, Stidolph P, Browne K (1997) Malignant mesothelioma in south east England: clinicopathological experience of 272 cases. Thorax 52:507–512

STUDY No. 15

# POTENTIAL HEALTH HAZARDS ASSOCIATED WITH EXPOSURE TO ASBESTOS-CONTAINING DRYWALL ACCESSORY PRODUCTS: A STATE-OF-THE-SCIENCE ASSESSMENT

**STUDY No. 15**

# POTENTIAL HEALTH HAZARDS ASSOCIATED WITH EXPOSURE TO ASBESTOS-CONTAINING DRYWALL ACCESSORY PRODUCTS: A STATE-OF-THE-SCIENCE ASSESSMENT

---

The authors provide a well presented and documented review of the potential health hazards associated with exposures to asbestos-containing drywall accessory products.

The one general concern is that throughout the manuscript, the authors should clearly state what measurement techniques were used for fiber counting and or sizing. They seem to range from light microscopy, to SEM and to TEM. When comparing studies with different measurement techniques, the authors should clearly state this and also comment if possible on what the comparable values might be. This can also be incorporated into the data tables.

Reviewers comments

## Potential Health Hazards Associated with Exposures to Asbestos-Containing Drywall Accessory Products: A State-of-the-Science Assessment

The authors provide a well presented and documented review of the potential health hazards associated with exposures to asbestos-containing drywall accessory products.

The one general concern is that throughout the manuscript, the authors should clearly state what measurement techniques were used for fiber counting and or sizing. They seem to range from light microscopy, to SEM and to TEM. When comparing studies with different measurement techniques, the authors should clearly state this and also comment if possible on what the comparable values might be. This can also be incorporated into the data tables.

The following provide specific points which also should be addressed:

Page 6 , Line 25 add “chrysotile”

In the statement that “and an assumption that the **chrysotile** asbestos exposure-response relationship for drywalling activities would be the same as had been observed elsewhere in amosite factory workers (Rohl, 1975)”

Page 14 , Line 34

“It is known that inhaled fibrous (asbestiform) structures can pose a risk of respiratory disease due in part to their ability to reside for long periods in the deep lung.”

It would be more accurate to state that: “It is known that if inhaled fibrous shaped structures are durable (biopersistent) they can pose a risk of respiratory disease due to their ability to reside for long periods in the deep lung.

Page 15, Line 39

It would also be helpful to add that the Agency for Toxic Substances and Disease Registry (2003) in a report ‘Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length’, stated that “Given findings from epidemiologic studies, laboratory animal studies, and in vitro genotoxicity studies, combined with the lung’s ability to clear short fibers, the panelists agreed that there is a strong weight of evidence that asbestos and SVFs (synthetic vitreous fibers) shorter than 5 µm are unlikely to cause cancer in humans”

Page 16 , Line 41

Replace “exposure experiments” with pleural implantation experiments

Page 57, Table 3

When there is no +, ++, or +++ what method was used for analysis of the fiber number? Also, it would be helpful to include in the table whether the analysis was ‘simulated’ ((e.g. National Gypsum Company, 1973b)) or whether the sample was collected in an actual work situation. This should also then be differentiated in the text and discussed.

Page 25, Line 49

In this section, the authors should state by what method the size and morphology were determined for each study. Comparisons between studies can only be made using the same measurement techniques (light, SEM or TEM).

Page 26, Line 25

The original reference for the analysis of the joint compound should also be cited, Brorby et al. 2008, Inhalation Toxicology, 20:1043–1053, 2008. For clarification, the authors should state that in the Bernstein et al. (2010) study, additional chrysotile was added to the joint compound aerosol included in the study in order to achieve the recommendations of having more than 100 fibers longer than 20 µm/cm$^3$ in the exposure aerosol.

Page 28, Line 27

The statement “Bernstein et al. (2008) added chrysotile fibers > 20 µm to both test materials” is incorrect. In that study Bernstein et al. added pure chrysotile to the sanded joint compound exposure. The 2$^{nd}$ exposure group was only pure chrysotile. The pure chrysotile had a distribution of fiber lengths including fibers longer than 20 µm.

Page 29 , Line 8

The statement “both exposure atmospheres” is incorrect. Chrysotile was added only to the sanded joint compound exposure group. It was not added to the amosite exposure group.

Line 20: should read by or at 28 days.

Page 29, Line 49

Wagner used SEM for analysis. Bernstein discussed previously used TEM. This should be discussed. References indicate an approximately 15 fold difference in fiber number between SEM and TEM.

Page 30, Line 10

In Platek they state “The filters were then mounted and counted by phase contrast light microscopy”. Again comparison with SEM or TERM studies should be clarified.

Page 39, line 46

The recommendation that “Nonetheless, it would be helpful if a formal epidemiology study, preferably of a case-control design” should be further clarified that the exposure cohorts should be thoroughly investigated to determine if they had any amphibole exposure.

Page 40, line 15

The references Bernstein et al., 2010, Bernstein et al., 2008 are not the correct references for such a statement. Bernstein did not report the size distribution of the sanded material alone. The article by Brorby would be appropriate.

STUDY No. 16

# SERPENTINE AND AMPHIBOLE ASBESTOS

**STUDY No. 16**

# SERPENTINE AND AMPHIBOLE ASBESTOS

**David M. Bernstein**

The principles of fiber toxicology are based upon three important criteria: dose, dimensions, and durability. This chapter addresses the importance of these criteria for asbestos and provides detaile understanding and support of how these influence the toxicity of what is commonly referred to as asbestos.

To assess these criteria, it is important to realize that asbestos is a term that refers to two minerals, serpentine and amphibole, occurring in fibrous form with very different mineralogical properties, which in the past have often been used and referred to interchangeably. The serpentine form is chrysotile, while the commercial amphibole forms are crocidolite, amosite, and tremolite. In addition, there are, as discussed, several other amphibole forms.

While asbestos has been known for centuries, dating back to Greek and Roman times (Browne and Murray, 1990; Ross and Nolan, 2003), the references did not differentiate the mineral type of asbestos. The first reference to chrysotile, the serpentine form of asbestos, was in 1834 by von Kobell (1834) in which he described that chrysotile is distinguished by its behavior of being decomposed by acid.

The name amphibole (Greek αμφιβολος—amphibolos meaning *ambiguous*) was used by René Just Haüy in 1801 to include tremolite, actinolite, tourmaline, and hornblende. The group was so named by Haüy in allusion to the protean variety, in composition and appearance, assumed by its minerals (Leake, 1978).

# 14 Serpentine and Amphibole Asbestos

*David M. Bernstein*

## CONTENTS



## 14.1 INTRODUCTION

The principles of fiber toxicology are based upon three important criteria: dose, dimensions, and durability. This chapter addresses the importance of these criteria for asbestos and provides detailed understanding and support of how these influence the toxicity of what is commonly referred to as asbestos.

To assess these criteria, it is important to realize that asbestos is a term that refers to two minerals, serpentine and amphibole, occurring in fibrous form with very different mineralogical properties, which in the past have often been used and referred to interchangeably. The serpentine form is chrysotile, while the commercial amphibole forms are crocidolite, amosite, and tremolite. In addition, there are, as discussed, several other amphibole forms.

While asbestos has been known for centuries, dating back to Greek and Roman times (Browne and Murray, 1990; Ross and Nolan, 2003), the references did not differentiate the mineral type of asbestos. The first reference to chrysotile, the serpentine form of asbestos, was in 1834 by von Kobell (1834) in which he described that chrysotile is distinguished by its behavior of being decomposed by acid.

The name amphibole (Greek αμφιβολος—amphibolos meaning *ambiguous*) was used by René Just Haüy in 1801 to include tremolite, actinolite, tourmaline, and hornblende. The group was so named by Haüy in allusion to the protean variety, in composition and appearance, assumed by its minerals (Leake, 1978).

Both serpentine and amphibole asbestos are naturally occurring minerals, which are extracted from the earth in surface, open pit, or underground mines. The fibers are intertwined with adjacent rock and are separated through crushing and milling with subsequent filtration/separation steps. Although the production of chrysotile asbestos has probably always exceeded that of amphibole asbestos by more than 10:1, the amphibole minerals are more abundant. They are frequently constituents of igneous rocks, often major components of metamorphic rocks and thought to account for approximately 20% of the shield area of the earth. However, fortunately for us, most of these rock-forming amphiboles are not at all asbestos like (Whittaker, 1979).

The mineralogical distinction between the two fibrous minerals was blurred, with the commercial mining and use of asbestos starting in the Western Alps of Italy and in England in the 1800s where serpentine and amphibole asbestos was very often found in close proximity. In Canada, the first chrysotile mine was opened in 1879 at Thetford, in the Quebec province. This was followed shortly thereafter with commercial chrysotile mining in Russia.

While both chrysotile and amphibole asbestos are usually of dimensions which can be quite respirable in humans, the mineralogical differences between these two minerals result in a very different formation of the fibers themselves, which has a large impact on the potential toxicity.

In understanding the toxicology of serpentine and amphibole asbestos, the question of dose has an important impact on the evaluation of both toxicology studies and epidemiology studies. As discussed in the following, the early toxicology studies often used very high doses which resulted in a lung overload effect rather than a true evaluation of the fiber. Similarly, many of the epidemiological studies which have been used for evaluation of the relative toxicity of chrysotile versus amphibole asbestos were performed based upon exposures in the first half of the 1900s when there was little understanding and virtually no control of fiber levels in the work environments. It is interesting to note, as discussed in the following, that in a large majority of the mines and plants that have been studied, the dust levels were reduced dramatically due to the implementation of control technology by the time the publications were written.

Based upon lung measurements in humans in various work environments, and more recently on biopersistence and toxicology studies in rats, chrysotile fibers are considerably less biopersistent than amphibole asbestos. This is a result of the very different structural makeup and mineralogical composition of chrysotile versus amphibole asbestos. As with other fiber types, this impacts considerably on the potential of the fibers to cause disease.

### 14.1.1 Chrysotile Characteristics

The words for the golden and fibrous nature of the fibers were used by the Greeks to derive the name chrysotile.

Von Kobell (1934) first described chrysotile stating that it was distinguished by its behavior of being decomposed by acid. As discussed in the following, this is one of the characteristics which differentiate chrysotile from the amphibole asbestos. The other characteristic which differentiate chrysotile is that it is formed of a curved structure of the Mg—analog of kaolinite. This was first suggested by Pauling (1930) due to the misfit between the octahedral and tetrahedral sheets. The crystal structure of chrysotile has been investigated extensively over the years, starting with Warren and Bragg (1930) and subsequently Noll and Kircher (1951) and Bates et al. (1950) who published electron micrographs which showed the cylindrical and apparently hollow chrysotile fibers.

The chrysotile fiber is a sheet silicate, monoclinic in crystalline structure, and has a unique rolled form. The chemistry of chrysotile is composed of a silicate sheet of composition $(Si_2O_5)_n^{-2n}$, in which three of the O atoms in each tetrahedron are shared with adjacent tetrahedra and a nonsilicate sheet of composition $[Mg_3O_2(OH)_4]_n^{2n-}$. In chrysotile, the distances between apical oxygens in a regular (idealized) silicate layer are shorter (0.305 nm) than the O–O distances in the ideal Mg-containing layer (0.342 nm), which may account for the curling of the layers, which results in

the rolling up, like a carpet to form concentric hollow cylinders (Skinner et al., 1988). The walls of the chrysotile fiber are made up of approximately 12–20 of these layers in which there is some mechanical interlocking. It is important to note however that there is no chemical bonding between the layers. Each layer is about 7.3 Å thick, with the magnesium surface facing the outside of the curl and the silica and oxygen tetrahedron inside the curl (Whittaker, 1963, 1957; Tanji et al., 1984). The mineralogical structure is illustrated in Figure 14.1 (adapted from Skinner et al., 1988). The polyhedral model of the chrysotile structure shown in Figure 14.2 illustrates one cylindrical curved layer of a chrysotile fiber (Rakovan, 2011). The Mg atom is on the outside of the curl and is thus exposed to the surrounding environment. This layered construction of chrysotile is illustrated in Figure 14.3 (Bernstein et al., 2013). High-resolution transmission electron photomicrographs of chrysotile are shown in Figure 14.4 (Kiyohara, 1991). Figure 14.5 shows the two forms that occur with chrysotile, one with concentric cylindrically curve layers and the other with apparently rolled layers (Rakovan, 2011).

Table 14.1 summarizes the chemical composition of typical serpentine and the amphiboles tremolite and amosite asbestos. The chemical composition and the structure of chrysotile are notably different from that of amphibole asbestos (Hodgson, 1979).

Commercial chrysotile is usually subdivided into groups using the Canadian Quebec Screening Scale (QSS). These groups are determined using an apparatus with a nest of four rotating trays superimposed one above the other. A known quantity of fiber is placed on the top tray, and the trays are rotated for a fixed time to produce a sifting action. The longest/thickest fibers stay on the top screen (tray) which has the largest openings and the shorter/thinner fibers fall through to lower screens. The grade is determined based upon the weight fractions deposited upon each screen and ranges from 3 to 9, with 3 being the longest (Cossette and Delvaux, 1979).

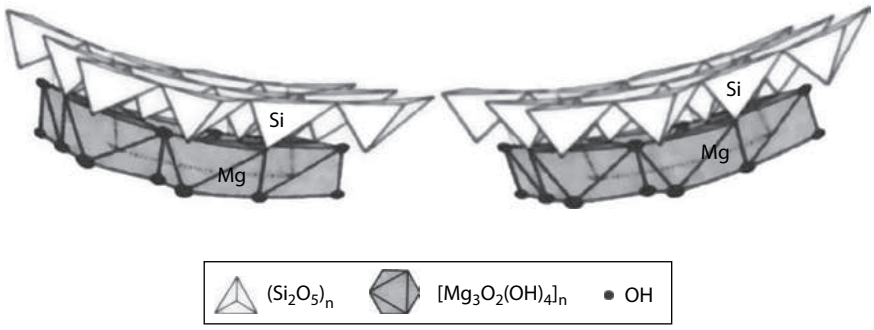



**FIGURE 14.1** Chrysotile structure: thin rolled sheet (7.3 A) with Mg on the outside and Si on the inside. (Reproduced from Bernstein, D.M. et al., *Crit. Rev. Toxicol*., 43(2), 154, 2013.)

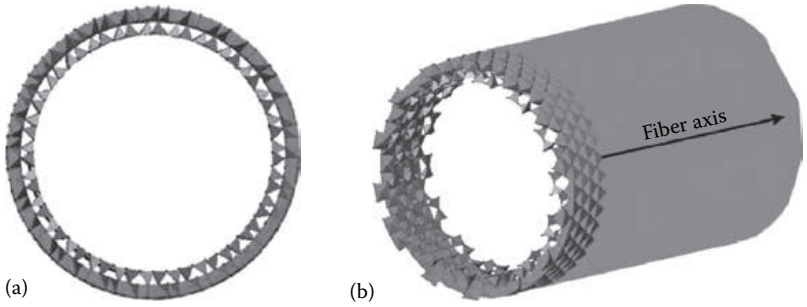



**FIGURE 14.2** (a) Polyhedral model of the chrysotile structure with one cylindrically curved 1:1 layer; viewed down the fiber axis (a-crystal axis). (b) A perspective view of the chrysotile structure. (From Rakovan, J., *J. Rocks Miner.*, 86(1), 63, 2011.)

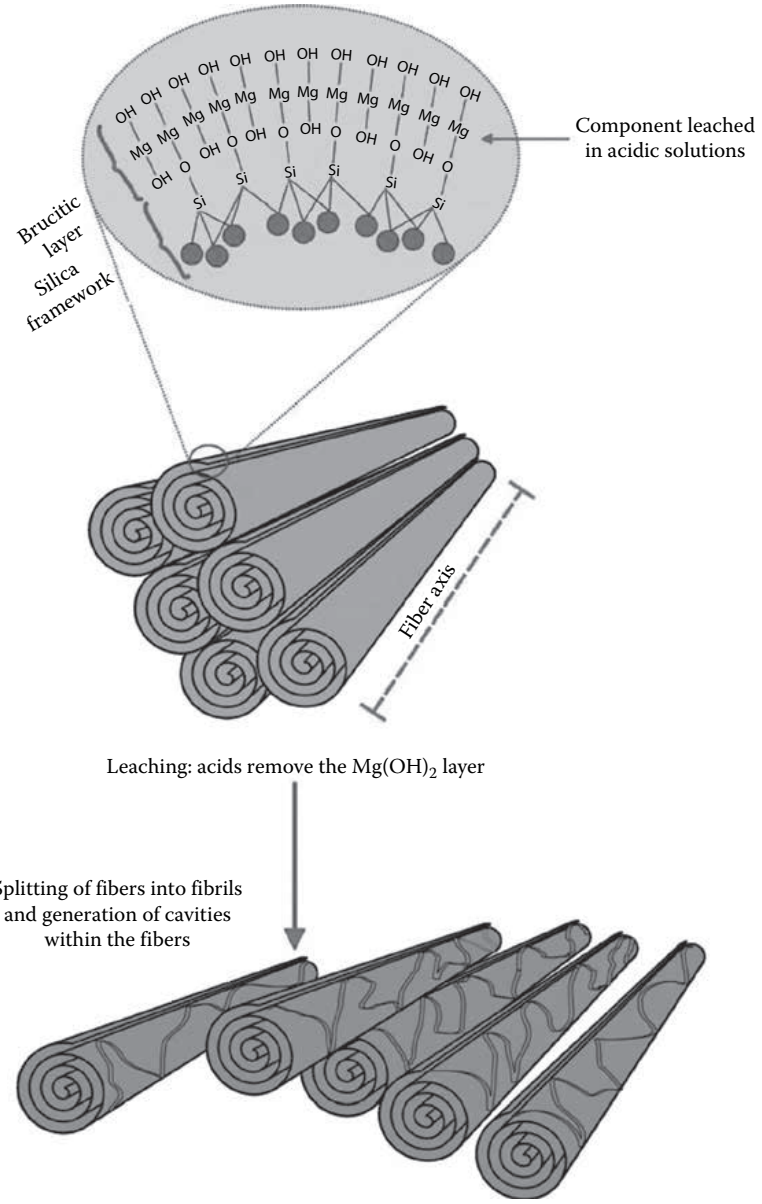



**FIGURE 14.3** Structural formation of the sheet silica chrysotile asbestos. (From Bernstein, D.M. et al., *Crit. Rev. Toxicol.*, 43(2), 154, 2013.)

Nagy and Bates (1952) reporting on the stability of chrysotile, showed that it has a high solubility in hydrochloric acid. They also observed that chrysotile has a relatively low thermal stability compared to other hydrous silicate minerals. The heat of the electron beam of an electron microscope caused a very rapid change in the morphology of the fibers, and prolonged exposure to electron bombardment resulted in complete disintegration of the material (Noll and Kircher, 1952). Hargreaves and Taylor (1946) reported that if fibrous chrysotile is treated with dilute acid, the magnesia can be completely removed, and the hydrated silica remaining, though fibrous in form, completely loses the elasticity characteristic of the original chrysotile and gives an x-ray pattern of one or perhaps two diffuse broadbands, indicating that the structure is "amorphous" or "glassy" in type.

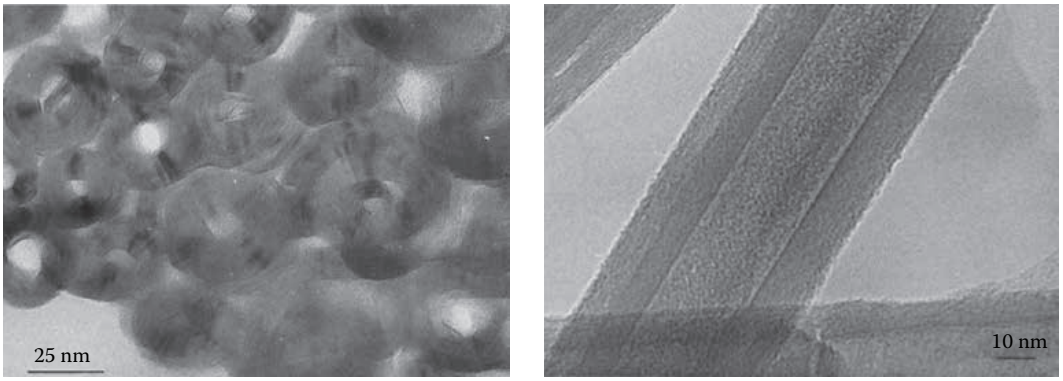
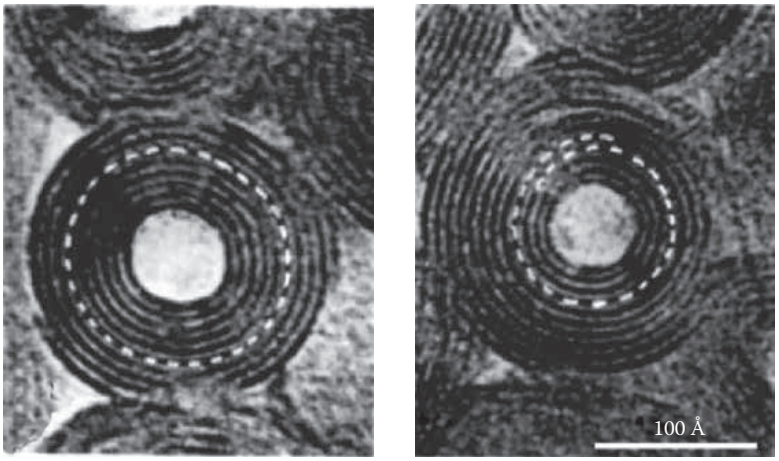



**FIGURE 14.4** Transmission electron micrographs of chrysotile. (From Kiyohara, P.K., Estudo da interface crisotila-cimento Portland em compósitos de fibro-cimento por métodos óptico-eletrônicos, Tese de Doutorado, apres. EPUSP, São Paulo, Brazil, 1991.)




(a) (b)

**FIGURE 14.5** High-resolution transmission electron photomicrographs of chrysotile fibers composed of (a) multiple concentric cylindrically curved 1:1 layers looking directly down the fiber axis, and (b) a 1:1 layer rolled up in a spiral. The dark bands are individual 1:1 layers. Dashed white lines follow a 1:1 layer. (Modified from Yada (1971) by Rakovan, J., *J. Rocks Miner.*, 86(1), 63, 2011.)

### 14.1.2 Amphibole Characteristics

In contrast to chrysotile, with amphibole asbestos, the basic structure is in the form of a double silica chain which appears as an I-beam with corner-linked $(SiO_4)^{-4}$ tetrahedra linked together in a double-tetrahedral chain that sandwiches a layer with the $Ca_2Mg_5$. These chains are paired, "back-to-back," with a layer of hydrated cations in between to satisfy the negative charges of the silica chains. The final structure is formed by the stacking of these sandwich ribbons in an ordered array (Speil and Leineweber, 1969). This amphibole structure is illustrated in Figure 14.6 (Bernstein et al., 2013). As shown below the structure, the fibers can be connected by soluble cations shown as small circles which are located between the double chain silicate fibers. When the soluble cations dissolve, as can happen in the lung, the amphibole fibers in these bundles are released as individual fibers; however, the fibers themselves are not affected. The double chain silicate amphibole fibers themselves are highly insoluble in both the lung fluids and in the macrophages.

**TABLE 14.1**
**Typical Chemical Composition (Percent)**

| Compound | Chrysotile[a] | Tremolite[b] | Amosite[b] |
|---|---|---|---|
| $SiO_2$ | 40.6 | 55.10 | 49.70 |
| $Al_2O_3$ | 0.7 | 1.14 | 0.40 |
| $Fe_2O_3$ | 2.3 | 0.32 | 0.03 |
| FeO | 1.3 | 2.00 | 39.70 |
| MnO | — | 0.10 | 0.22 |
| MgO | 39.8 | 25.65 | 6.44 |
| CaO | 0.6 | 11.45 | 1.04 |
| $K_2O$ | 0.2 | 0.29 | 0.63 |
| $Na_2O$ | — | 0.14 | 0.09 |
| $H_2O$ | — | 3.52 | 1.83 |
| $H_2$ | — | 0.16 | 0.09 |
| $CO_2$ | 0.5 | 0.06 | 0.09 |
| Ignition loss | 14.0 | — | — |
| Total | 100 | 99.93 | 100.26 |

*Source:* Hodgson, A.A., Chemistry and physics of asbestos, in *Asbestos: Properties, Applications and Hazards*, L.M.a.S.S. Chissick (ed.), John Wiley & Sons, New York, 1979, pp. 80–81.

[a] Typical chemical analysis of Canadian chrysotile from the Quebec Eastern.

[b] Townships (LAB Chrysotile, Inc., Quebec, Canada).

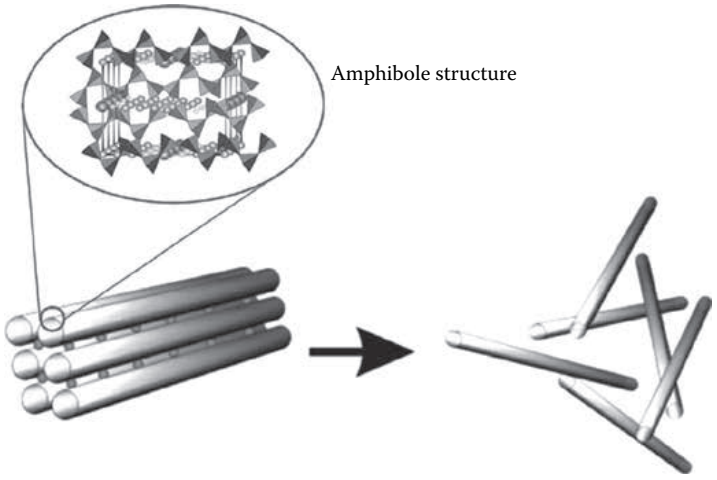



**FIGURE 14.6** **(See color insert.)** With amphiboles, the soluble cations shown as small circles are located between the fibers which are formed with double chain silicate. When the soluble cations dissolve as can happen in the lung, the amphibole fibers in these bundles are released as individual fibers. The double chain silicate amphibole fibers themselves are highly insoluble in both the lung fluids and in the macrophages. (From Bernstein, D.M. et al., *Crit. Rev. Toxicol.*, 43(2), 154, 2013.)

There are five asbestiform varieties of amphiboles: anthophyllite asbestos, grunerite asbestos (amosite), riebeckite asbestos (crocidolite), tremolite asbestos, and actinolite asbestos. Of these, crocidolite and amosite were the only amphiboles with significant industrial uses (Virta, 2002). Tremolite, while not used commercially, has been found as a contaminant in other fibers or in other industrial minerals (e.g., chrysotile and talc).

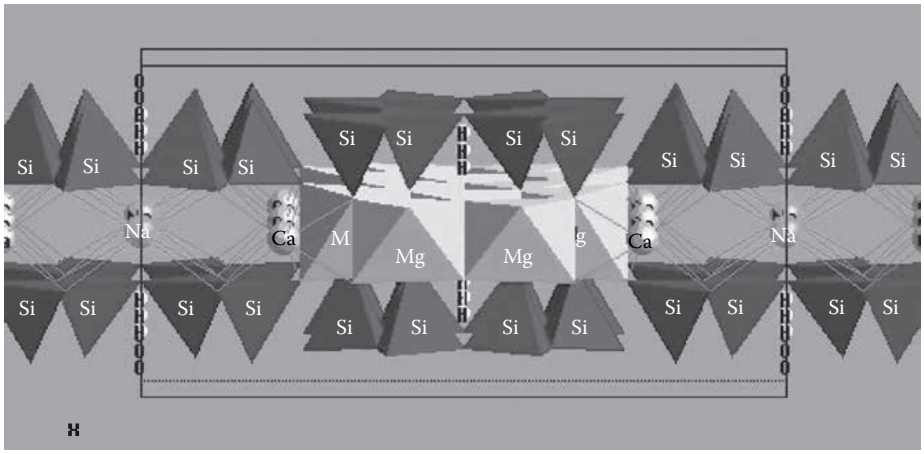



**FIGURE 14.7 (See color insert.)** Illustration of the tightly bound silica-based structure on exterior surfaces of amphibole fibers. (Adapted from Department of Geology and Geophysics, University of Wisconsin, Crystal Structure Movies, http://www.geology.wisc.edu.)

Depending upon the type of amphibole, the principal cations are magnesium, iron, calcium, and sodium. The principal types are as follows:

| | |
|---|---|
| Crocidolite | $(Na_2Fe_3^{2+}Fe_2^{3+})Si_8O_{22}(OH)_2$ |
| Amosite | $(Fe^{2+}, Mg)_7Si_8O_{22}(OH)_2$ |
| Tremolite | $Ca_2Mg_5Si_8O_{22}(OH)_2$ |
| Actinolite | $Ca_2(Mg, Fe^{2+})_5Si_8O_{22}(OH)_2$ |
| Anthophyllite | $(Mg, Fe^{2+})_7Si_8O_{22}(OH)_2$ |

The exterior surfaces of the amphiboles are tightly bound silica-based structures. This is illustrated with tremolite in Figure 14.7.

As a result of their structure, amphibole fibers have negligible solubility at any pH that would be encountered in an organism (Speil and Leineweber, 1969). Some service metals associated with the fibers such as iron can become ionized and released under certain conditions (Aust et al., 2011).

## 14.2 FACTORS INFLUENCING FIBER TOXICOLOGY

As mentioned earlier, mineral fiber toxicology has been associated with three key factors: dose, dimension, and durability. The dose is determined by the fiber's physical characteristics/dimensions, how the fibrous material is used, and the control procedures that are implemented. The thinner and shorter fibers will weigh less and thus can remain suspended in the air longer than thicker and longer fibers. Most asbestos fibers are thinner than commercial insulation fibers; however, they are thicker than the new nanofibers, which are currently being developed. Control procedures in mining and manufacturing have changed dramatically over the years, resulting in markedly reduced exposure concentrations.

The fiber dimensions govern two factors: whether the fiber is respirable, and secondly, if it is respirable, whether the dimensions are also a factor in determining their response in the lung milieu once inhaled. Shorter fibers of the size which can be fully engulfed by the macrophage will be cleared by mechanisms similar to those for nonfibrous particles. These include clearance through the lymphatics and macrophage phagocytosis and clearance. It is only the longer fibers which the macrophage cannot fully engulf; if they are persistent, this will lead to disease.

This leads to the third factor: durability. Those fibers whose chemical structure renders them wholly or partially soluble once deposited in the lung are likely to either dissolve completely,

or dissolve until they are sufficiently weakened focally to undergo breakage into shorter fibers. The remaining short fibers can then be removed through successful phagocytosis and clearance.

In addition, because of the differences in the mineralogical composition and structure of serpentine asbestos (chrysotile) in comparison to amphibole asbestos (e.g., crocidolite and amosite), both the physical structure of the fiber and its ability to dissolve in acid are important criteria in determining the potential to have toxicological effects.

These factors have been shown to be important determinants for both synthetic mineral fibers (Hesterberg et al., 1998a,b; Miller et al., 1999; Oberdöster, 2000; Bernstein et al., 2001a,b) and asbestos (Bernstein et al., 2013).

## 14.3 IN VITRO TOXICOLOGY

In vitro toxicology studies are often very helpful in elucidating possible mechanisms involved in pathogenesis. However, as used in the assessment of fiber toxicology, they are difficult to interpret. This stems from several factors. The in vitro test system is a static system and thus is not sensitive to differences in fiber solubility. High doses of fibers are used to obtain a positive response, and it is difficult to extrapolate from these large short-term cellular exposures to the considerably lower-dose chronic exposures that occur in vivo. In addition, the number of fibers and size distribution are often not quantified. Most important, however, is that these end points have not been validated as screening assays that are predictive of long-term pathological effects in vivo. While in vitro tests may be useful tools to identify and evaluate possible mechanisms, with fibers, these in vitro test systems are of limited use in differentiating different fiber types (Bernstein et al. 2005a).

## 14.4 IN VITRO BIODURABILITY

Within the biological system of the lung, upon deposition, fibers are exposed to two types of environmental conditions. These are the lung surfactant which occurs through the tracheobronchial tree and the alveolar region, and the pulmonary macrophage which is the first line of defense once the fiber is deposited within the lung. The lung surfactant has a pH of 7.4 (neutral), and within the macrophage phagolysosomes, the pH is as low as 4 (acid).

With chrysotile being a thin rolled sheet with the magnesium hydroxide layer on the outside of the fiber, the chrysotile fiber has poor acid resistance compared to the amphibole fibers which is encapsulated by silica. With the amphibole fibers, the silicate oxygen atoms are on the outside of the layers and the hydroxides are masked within the fiber resulting in a fiber, very resistant to solubility at either neutral or acid pH. Von Kobell (1834) was the first to describe the acid solubility of chrysotile as being an important characteristic. Hargreaves and Taylor (1946) described how with treatment of chrysotile fibers with dilute acid, the magnesium can be completely removed, leaving a hydrated silica which has lost the elasticity characteristic of the original fiber. The resulting structure was characterized as amorphous or grassy in type. Similar findings were reported by Wypych et al. (2005) who described how the leached products consisted of layered hydrated disordered silica with a distorted structure similar in form to the original fiber. They also described the removal of the brucite-like (magnesium hydroxide) sheets leaving silica with an eminently amorphous structure. Suquet (1989) also reported that "Acid leaching transformed chrysotile into porous, noncrystalline hydrated silica, which easily fractured into short fragments. If the acid attack was too severe, these fragments converted into shapeless material."

The ability of an acid environment to break apart long chrysotile fibers into shorter fibers in vitro has been reported by Osmon-McLeod et al. (2011). The authors assessed the durability of a number of fibers including long fiber amosite (LFA) and long fiber chrysotile (LFC) in a Gambles solution that was adjusted to a pH of 4.5 to mimic that inside macrophage phagolysosomes, which the authors described as "potentially the most degradative environment that a particle should encounter following lung deposition and macrophage uptake." Figures 14.8 and 14.9 (modified from Figures 3 and

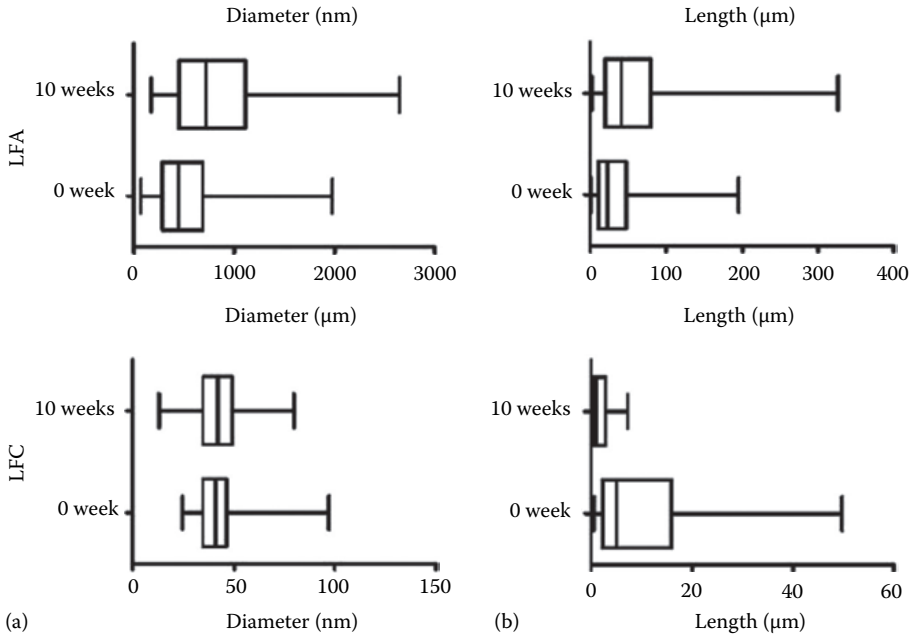



**FIGURE 14.8** Effect of incubation in Gambles solution on (a) fiber widths and (b) lengths. Boxplots showing the distribution of fiber widths and lengths (nm) in samples that had been incubated in Gambles solution for 0 week or 10 weeks. The line in the box represents the median value of measurements from TEM images, and the edges of the box represent the lower and upper quartiles. The ends of the whiskers represent minimum and maximum values. Note that the scale of the horizontal axis is different in the LFA and LFC figures. (Modified from Figure 3 in Osmon-McLeod, M.J. et al., *Part Fibre Toxicol*., 8, 15, May 13, 2011 to show only the results for long fiber chrysotile [LFC] and long fiber amphibole [LFA].)

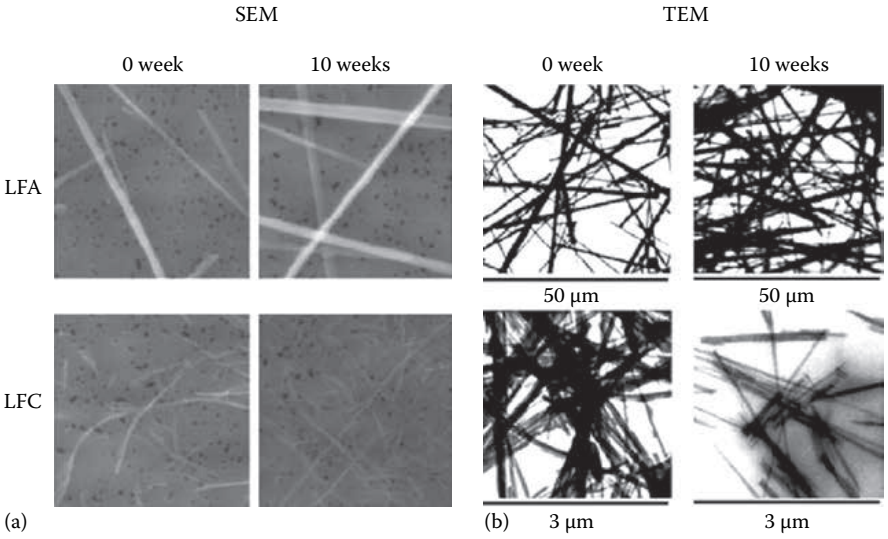



**FIGURE 14.9** Appearance of fibers before and after incubation in Gambles solution. (a) Representative SEM images of samples after 0-week or 10-weeks incubation in Gambles solution are shown at 5.0 K magnification in the two panels on the left. (b) TEM images of equivalent samples, at indicated magnifications, are on the right. Note that the scale of the horizontal axis is different in the LFA and LFC figures. (Modified from Figure 4 in Osmon-McLeod, M.J. et al., *Part Fibre Toxicol*., 8, 15, May 13, 2011 to show only he results for long fiber chrysotile [LFC] and long fiber amphibole [LFA].)

4 in Osmon-McLeod et al. [2011] to show only the results for long fiber chrysotile [LFC] and long fiber amphibole [LFA]) show the marked reduction in length for the long fiber chrysotile following 10 weeks of treatment. The chrysotile fibers following 10 weeks of treatment in vitro break apart so that there are no fibers longer than 10 μm in length. In the lung, such shorter fibers can be readily cleared by the macrophage.

## 14.5 BIOPERSISTENCE

A fiber is unique among inhaled particles in that the fibers aerodynamic diameter is largely related to three times the fiber diameter. Because of this, long thin fibers can penetrate into the deep lung effectively bypassing the filtration which occurs for nonfibrous particles. Within the lung, fibers which can be fully engulfed by the macrophage can be removed as with any other particle. However, those fibers which are too long to be fully engulfed by the macrophage cannot be cleared by this route.

Fibers less than 5 μm in length are effectively not different than nonfibrous particles and are cleared with similar kinetics and mechanism as particles. While longer fibers may also be cleared effectively by the macrophage and as a result not be different kinetically than particles, the 5 μm cut-off was chosen to mirror the use by the WHO of a 5 μm cut-off in their counting schemes for fibers. As is discussed later, recent reviews of these size fibers have concluded that they present very little or no risk to human health (ATSDR, 2003).

Fibers between 5 and 20 μm in length represent the transition range between those fibers which are cleared as particles and the longer fibers that the macrophage cannot fully phagocytize. The actual limit as to what length fiber can be fully phagocytized has been proposed for the rat, as ranging from 15 μm (Miller, 2000) to 20 μm (Morimoto et al., 1994; Luoto et al., 1995).

In the lung, extensive work on modeling the dissolution of synthetic vitreous fibers (SVFs) using in vitro dissolution techniques and inhalation biopersistence has shown that the lung has a very large fluid buffer capacity (Mattson, 1994). These studies have shown that an equivalent in vitro flow rate of up to 1 mL/min is required to provide the same dissolution rate of SVF as that which occurs in the lung. This large fluid flow within the lung results in the dissolution of fibers which are soluble at pH 7.4. Recent publications have shown that the biopersistence of the fibers longer than 20 μm is an excellent predictor of the pathological response to fibers following chronic inhalation studies and chronic intraperitoneal studies (Hesterberg et al., 1998a,b; Bernstein et al., 2001a,b). The value of 20 μm is used as an index for fibers that cannot be fully phagocytized and cleared by the macrophage. The protocol used in these biopersistence studies was developed by a working group for the European Commission and involves a 5-day inhalation exposure, followed by analysis of the lungs at periodic intervals of up to 1 year postexposure (Bernstein and Riego-Sintes, 1999; Bernstein et al., 2005).

The clearance half-time of SVFs longer than 20 μm ranges from a few days to less than 100 days. This is illustrated in Table 14.2. Highlighted in this table are those studies performed on chrysotile using the same protocol which is within the lower range of the SVFs. In contrast, amphiboles have biopersistence half-times considerably longer than the SVFs. For synthetic vitreous fibers, the European Commission has established a directive which states that if the inhalation biopersistence clearance half-time of a fiber is less than 10 days, then it is not classified as a carcinogen.

Clearly, there is a large difference in biopersistence between serpentine and the amphibole asbestos. In addition, as the serpentine asbestos, chrysotile, is a naturally occurring mined fiber, there appears to be some differences in biopersistence, depending upon from where it is mined. However, chrysotile lies on the soluble end of this scale and ranges from the least biopersistent fiber to a fiber with biopersistence in the range of glass and stonewools. It remains less biopersistent than ceramic and special purpose glasses and more than an order of magnitude less biopersistent than amphiboles.

**TABLE 14.2**
**Clearance Half-Time of Synthetic Vitreous Fibers, Chrysotile, and Amphibole Asbestos Longer than 20 μm**

| Fiber | Type | Weighted $T_{1/2}$ Fibers L > 20 μm (Days) | Reference |
|---|---|---|---|
| Calidria chrysotile | Serpentine asbestos | 0.3 | Bernstein et al. (2005b) |
| Brazilian chrysotile | Serpentine asbestos | 2.3 | Bernstein et al. (2004) |
| Fiber B | B01.9 | 2.4 | Bernstein et al. (1996) |
| Fiber A | Glasswool | 3.5 | Bernstein et al. (1996) |
| Fiber C | Glasswool | 4.1 | Bernstein et al. (1996) |
| Fiber G | Stonewool | 5.4 | Bernstein et al. (1996) |
| Chrysotile combined with sanded joint compound | Serpentine asbestos | | Bernstein et al. (2011) |
| MMVF34 | HT stonewool | 6 | Hesterberg et al. (1998a) |
| MMVF22 | Slagwool | 8 | Bernstein et al. (1996) |
| Fiber F | Stonewool | 8.5 | Bernstein et al. (1996) |
| MMVF11 | Glasswool | 9 | Bernstein et al. (1996) |
| Fiber J | X607 | 9.8 | Bernstein et al. (1996) |
| Canadian chrysotile | Serpentine asbestos | 11.4 | Bernstein et al. (2005c) |
| MMVF 11 | Glasswool | 13 | Bernstein et al. (1996) |
| Fiber H | Stonewool | 13 | Bernstein et al. (1996) |
| MMVF10 | Glasswool | 39 | Bernstein et al. (1996) |
| Fiber L | Stonewool | 45 | Bernstein et al. (1996) |
| MMVF33 | Special purpose glass | 49 | Hesterberg et al. (1998a) |
| RCF1a | Refractory ceramic | 55 | Hesterberg et al. (1998a) |
| MMVF21 | Stonewool | 67 | Hesterberg et al. (1998a) |
| MMVF32 | Special purpose glass | 79 | Hesterberg et al. (1998a) |
| MMVF21 | Stonewool | 85 | Bernstein et al. (1996) |
| Amosite | Amphibole asbestos | 418 | Hesterberg et al. (1998a) |
| Crocidolite | Amphibole asbestos | 536 | Bernstein et al. (1996) |
| Tremolite | Amphibole asbestos | ∞ | Bernstein et al. (2005b) |
| Amosite | Amphibole asbestos | >1000 days | Bernstein et al. (2011) |

The rapid clearance of chrysotile is thought to be characterized not by congruent dissolution as with many SVF but rather with the loss of structural integrity of the serpentine sheet silicate and the subsequent disintegration into smaller pieces as a result of the action of the lung surfactant and the acid environment of the macrophage.

This difference between chrysotile and amphiboles is better illustrated, with the actual lung burden data for the fibers longer than 20 μm from the inhalation biopersistence studies. In Figure 14.10, the number of fibers remaining in the rat's lungs is shown as a function of the time in days following cessation of the 5-day exposure (Bernstein et al., 2003a, 2005b, 2011c). Included are the three amphibole asbestos studies including tremolite asbestos and 2 amosite asbestos, a SVF fiber, HT, which has a clearance half-time of 6 days and which showed no tumors or fibrosis in a chronic inhalation toxicology study and 4 chrysotile fibers from Brazil, the United States (Calidria), and Canada and one study on a commercial joint compound mixed with chrysotile fibers. The inhalation exposure aerosol in terms of the number of fibers longer than 20 μm was in the range of 150–200 fibers (L > 20 μm)/cm$^3$ for all fibers except the Brazilian chrysotile which was 400 fibers (L > 20 μm)/cm$^3$.

The amphiboles are very durable with only a small amount of clearance in the days following the cessation of exposure with virtually no further clearance thereafter. In the tremolite biopersistence study,

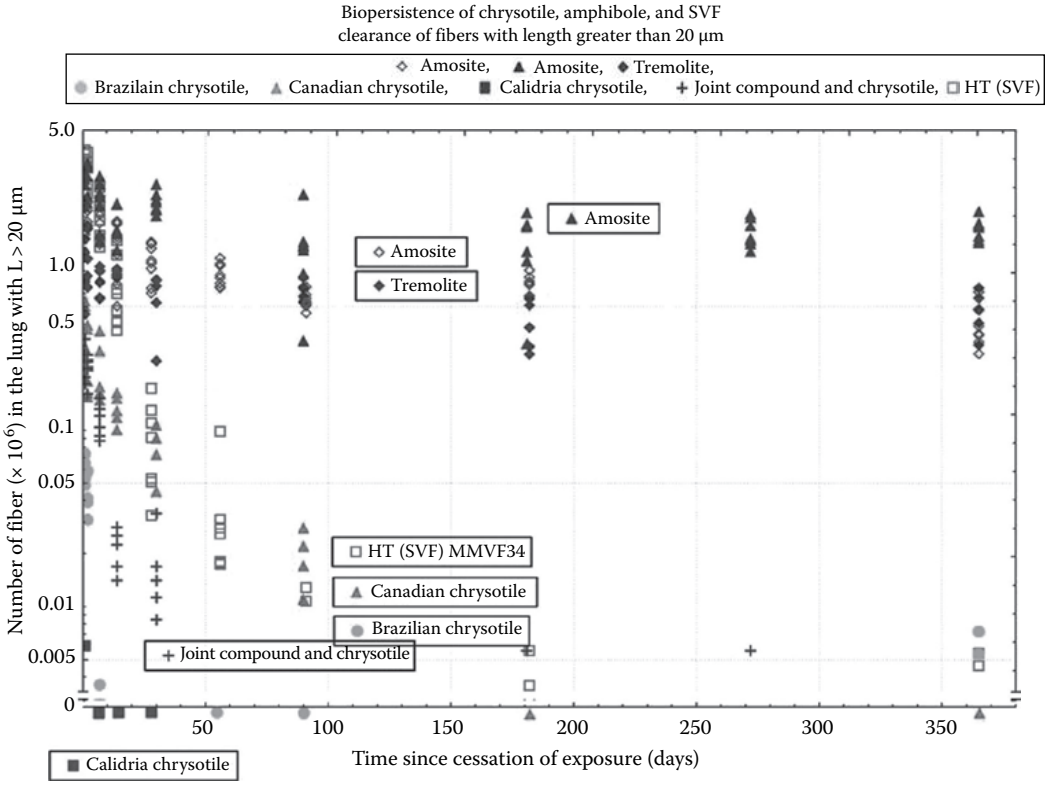



**FIGURE 14.10** **(See color insert.)** Summary of studies showing the number of fibers remaining in the rat's lungs is shown as a function of the time in days following cessation of the 5-day exposure. Included are the three amphibole asbestos studies including tremolite asbestos and 2 amosite asbestos, a soluble synthetic vitreous (SVF) fiber, HT, and 4 chrysotile fibers from Brazil, the United States (Calidria), and Canada and one study on a commercial joint compound mixed with chrysotile fibers. (Data presented are from Bernstein et al., 2003a, 2005c; Bernstein, D.M. et al., *Inhal. Toxicol.*, 23(7), 372, June, 2011.)

the histopathological response of the lungs was examined following the 5-day exposure. A pronounced inflammatory response with the rapid development of granulomas was seen at day 1 postexposure, followed by the development of fibrosis characterized by collagen deposition within these granulomas and by 90 days, even mild interstitial fibrosis. In the same study, chrysotile showed no inflammatory or pathological response following the 5-day exposure (Bernstein et al., 2003b).

While all the chrysotiles cleared relatively quickly, differences were observed between the three types studied. The Calidria chrysotile which is known to be a short fiber chrysotile cleared the fastest, with a clearance half-time of 0.3 days for the fibers longer than 20 µm.

The clearance half time of the Brazilian chrysotile was 2.3 days. At the end of 12 months, 2–3 long fibers were measured following the lung digestion procedure. However, the exposure concentration for the Brazilian chrysotile was 400 fibers L > 20 µm/cm$^3$, rather than the 150–200 fibers L > 20 µm/cm$^3$ for the other fibers evaluated, thus resulting in a very high aerosol concentration of 7 million WHO fibers/cm$^3$ and more than 32 million total fibers/cm$^3$. It certainly is possible that this extremely high total exposure resulted in a response very different from what might be expected at lower exposure concentrations. Even so, the number of fibers observed at 12 months was not statistically different than that which was observed for the HT fiber which had a 6-day clearance half-time for the long fibers.

For the Canadian chrysotile study, textile grade chrysotile was evaluated. This grade was chosen as it was specifically produced to have thin long fibers, which facilitated the production of textiles.

The clearance of the Canadian chrysotile long fibers was 11.4 days. By 365 days, there were no long Canadian chrysotile fibers remaining in the lung.

The pathological response and translocation in the lung and pleura of a commercial chrysotile product similar to that which was used through the mid-1970s in a joint compound intended for sealing the interface between adjacent wall boards was evaluated in comparison to amosite asbestos (Bernstein et al., 2010, 2011). The study design was enhanced to include procedures for the quantitative evaluation of fibers and pathological response not only in the lung but also in the pleural space while limiting procedural artifacts.

In this study, rats were exposed by inhalation for 5 days (6 h/day) to either sanded joint compound consisting of a mixture of added chrysotile fibers and sanded joint compound particles or amosite asbestos. The mean exposure concentration for fibers longer than 20 μm was 295 fibers/cm$^3$ for chrysotile and 201 fibers/cm$^3$ for amosite. The mean number of WHO fibers in the chrysotile fibers and sanded joint compound particle atmosphere was 1496 fibers/cm$^3$. The amosite-exposure atmosphere had fewer shorter fibers, with a mean of 584 WHO fibers/cm$^3$. While the exposure concentrations were controlled to avoid the effect of lung overload, the chrysotile concentration was still more than 10,000 times the OSHA occupational exposure limit of 0.1 fibers/cm$^3$.

The study included examination of the diaphragm as a parietal pleural tissue and the in situ examination of the lungs and pleural space obtained from freeze-substituted tissue in deeply frozen rats. The diaphragm was chosen as a representative parietal pleural tissue because at necropsy, it could be removed within minutes of sacrifice with minimal alteration of the visceral lung surface with the area of the diaphragm chosen for examination that included an important lymphatic drainage site (stomata) on the diaphragmatic surface. Both confocal microscopy and SEM were used to identify fibers as well as examine the pleural space, in situ, for possible inflammatory response. The examination of the pleural space in situ including the lung, visceral pleura, and parietal pleura in rats deeply frozen immediately after termination provided a unique noninvasive method for determining fiber location and inflammatory response.

The results of this study showed that there was no pathological response observed at any time point in the chrysotile fibers and sanded joint compound particles exposure group. As with the other studies reported earlier, the chrysotile long fibers in the lung (L > 20 μm) cleared rapidly ($T_{1/2}$ of 4.5 days) and were not observed in the pleural cavity at any time point. In contrast, following the 5-day exposure to amosite asbestos, a rapid inflammatory response occurred in the lung which resulted in a Wagner grade 4 interstitial fibrosis within 28 days and which persisted through 90 days. (Histopathology was evaluated through 90 days postexposure as the animals were allocated to the confocal microscopic analyses from 181 through 365 days postexposure.) The amosite fibers longer than 20 μm had a biopersistence of $T_{1/2} > 1000$ days in the lung and were observed in the pleural cavity within 7 days postexposure. In the pleural cavity, a marked inflammatory response was observed on the parietal pleural surface by 90 days postexposure. In contrast to the amosite asbestos exposure, this study provides support that exposure to the chrysotile fibers and joint compound particles following short-term inhalation would not initiate any inflammatory response in the lung and that the chrysotile fibers in the lung do not migrate or cause an inflammatory response in the pleural cavity, the site of mesothelioma formation.

### 14.5.1 Clearance Mechanism of HT and Chrysotile

Kamstrup et al. (2001) described possible mechanisms that could account for the rapid clearance half-time of the long HT fibers. He stated that

> The HT fiber is characterized by relatively low silica and high alumina content, with a high dissolution rate at pH 4.5 and relatively low rate at pH 7.4 (Guldberg et al. 2002; Knudsen et al., 1996). Apart from possible exposure to the acidic environment of the phagolysosomes within the macrophages (Oberdörster, 1991), measurements have shown that the microenvironment at the surface of activated

> macrophages is acidic with pH < 5 between attached macrophages and a nonporous glass surface (Etherington et al., 1981). It is therefore probable that long HT fibers, highly soluble at pH 4.5, are subject to extracellular dissolution and consequent breakage when exposed to the acidic environment of attached macrophages without being engulfed completely.

As reviewed earlier, the ability of chrysotile to be broken apart in the acid environment has been known since the publication by von Kobell in 1834. As discussed earlier, a similar process to that described for HT fibers has been demonstrated for chrysotile fibers by Osmond-McLeod et al. (2011) who showed that the long chrysotile fibers break apart into shorter fibers less than 10 μm in length following treatment in Gambles solution that was adjusted to a pH of 4.5.

### 14.5.2 Short Fiber Clearance

For all fiber exposures, there are many more shorter fibers less than 20 μm in length and even more less than 5 μm in length. The clearance of the shorter fibers in these studies has been shown to be either similar to or faster than the clearance of insoluble nuisance dusts (Stoeber et al., 1970; Muhle et al., 1987). In a recent report issued by the Agency for Toxic Substances and Disease Registry entitled "Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length," the experts stated that "Given findings from epidemiologic studies, laboratory animal studies, and in vitro genotoxicity studies, combined with the lung's ability to clear short fibers, the panelists agreed that there is a strong weight of evidence that asbestos and SVFs (synthetic vitreous fibers) shorter than 5 μm are unlikely to cause cancer in humans" (ATSDR, 2003; EPA, 2003). In addition, Berman and Crump (2003) in their technical support document to the EPA on asbestos-related risk also found that shorter fibers do not appear to contribute to disease.

## 14.6 CHRONIC INHALATION TOXICOLOGY STUDIES

The studies presented earlier indicate that there is a large difference in the biopersistence between the serpentine chrysotile and the amphiboles, tremolite, and amosite. These differences appear to be related to the differences in chemical structure between the serpentines and amphiboles and possibly the influence of the acidic pH associated with the macrophage on the chrysotile fiber.

Yet when the chronic inhalation studies that have been performed on chrysotile and amphiboles are examined, these differences are not always apparent.

In an analysis by Berman et al. (1995) of 13 inhalation studies that have been performed on nine different types of asbestos, they concluded that

- Short fibers (less than somewhere between 5 and 10 μm in length) do not appear to contribute to cancer risk.
- Beyond a fixed, minimum length, potency increases with increasing length, at least up to a length of 20 μm (and possibly up to a length of as much as 40 μm).
- The majority of fibers that contribute to cancer risk are thin, with diameters less than 0.5 μm and the most potent fibers may be even thinner. In fact, it appears that the fibers that are most potent are substantially thinner than the upper limit defined by respirability.
- Identifiable components (fibers and bundles) of complex structures (clusters and matrices) that exhibit the requisite size range may contribute to overall cancer risk, because such structures likely disaggregate in the lung. Therefore, such structures should be individually enumerated when analyzing to determine the concentration of asbestos.
- For asbestos analyses to adequately represent biological activity, samples need to be prepared by a direct-transfer procedure.
- Based on animal dose–response studies alone, fiber type (i.e., fiber mineralogy) appears to impart only a modest effect on cancer risk (at least among the various asbestos types).

Concerning the lack of differentiation seen in the dose–response studies, the authors stated that this may be due at least in part to the limited lifetime of the rat relative to the biodurability of the asbestos fiber types evaluated in these studies.

More important in understanding these results are the specifics in the study design of these studies in light of the more recent understanding of the effect of high concentrations of insoluble particles on the rat lung. The majority of the inhalation toxicology studies evaluated by Berman et al. (1995; Table 14.1) were performed at very high exposure concentrations (10 mg/m$^3$).

The chronic inhalation studies that have been performed on asbestos are summarized in Tables 14.3 and 14.4. The exposure regime was similar in most studies and ranged from 5 to 7 h/day, 5 days per week for either 12 months or 24 months. While it is difficult to determine how this was derived, the exposure concentration was set for most studies based upon mass concentration at 10 mg/m$^3$. Davis et al. (1978) referencing Wagner et al. (1974) states that 10 mg/m$^3$ was considered to be high enough to cause significant pathological change; however, there is no rational presented in the Wagner et al. paper as to why 10 mg/m$^3$ was chosen. While the differential toxicity of chrysotile and amphibole asbestos was not well understood at the time, with a common name *asbestos*, a common toxicity may have been considered. Amphibole asbestos produced toxic effects at lower concentrations; however, with the very different mineral chrysotile, it may have been necessary to increase the dose to what is now considered *lung overload* concentrations to produce a similar effect as seen for amphibole asbestos.

Bernstein et al. (2013) have reported on the methods used to prepare the two most commonly used chrysotile samples for toxicology studies, the UICC chrysotile and the NIEHS chrysotile samples. With both of these chrysotile samples, the fibers were extensively ground using large-scale commercial milling devices.

The UICC chrysotile sample was milled using a "Classic Mill designed by R. F. Bourne, at The Asbestos Grading Equipment Company, Johannesburg" (Timbrell et al., 1968). Timbrell and Rendall (1972) describes "The Classic mill is an airswept attrition mill fitted with a disc rotor (16 in. diam.) which carries four beaters and is mounted on a horizontal shaft driven by an electric motor at speeds up to 5000 r.p.m." The patent (Patent number GB 3,490,704) on the mill provides greater detail.

The NIEHS chrysotile was prepared from a grade 4 chrysotile used in the plastics industry which was prepared by passing the material through a hurricane pulverizer (Campbell et al., 1980; Pinkerton et al., 1983). The hurricane pulverizer is a high-speed impact hammer industrial mill with a size classifier which recycled larger fibers/particles back into the device for continued milling (Work, 1962; Perry and Chilton, 1973).

Suquet (1989) has reported that severe grinding of chrysotile fibers "converted them into fragments cemented by a shapeless, noncrystalline material." The authors explained that the comminution treatment apparently broke atomic bonds and produced strong potential reaction sites, which were able to adsorb $CO_2$ and $H_2O$ molecules from the atmosphere."

The issue of using equivalent fiber number for exposure was approached in a study reported by Davis et al. (1978) where chrysotile, crocidolite, and amosite were compared on an equal mass and equal number basis; however, the fiber number was determined by phase contrast optical microscopy (PCOM) and thus, the actual number of the chrysotile fibers was probably greatly underestimated. The 10 mg/m$^3$ exposure to chrysotile was reported by Davis et al. (1978) using PCOM as approximately 2000 fibers/cm$^3$ (length greater than 5 μm) while when a similar mass concentration of another chrysotile was measured by SEM 10,000 fibers/cm$^3$ length greater than 5 μm were reported with a total fiber count of 100,000 fibers/cm$^3$ (Mast et al., 1995). There is little quantitative data presented in these publications on the nonfibrous particle concentration of the test substances to which the animals were exposed. Pinkerton et al. (1983) presents summary tables of length measurements of Calidria chrysotile by SEM in which the number of nonfibrous particles counted is stated; however, from the data presented, the aerosol exposure concentration of nonfibrous particles cannot be extracted. In all studies, the asbestos was ground prior to aerosolization,

**TABLE 14.3**
**Inhalation Toxicology Studies Using Rats**

| Fiber Type | Exposure Time (Hours/Day, Day/Week, Total Months) | Exposure Type, Exposure Concentration ($mg/m^3$) | Fiber Concentration ($Fibers/cm^3$) (Determined by Electron Microscopy Unless Otherwise Noted) | Equivalent Fiber (Concentration/$cm^3$) Based on TEM Evaluation | Type and Total No. of Rats | Number of Pulmonary Tumors | % Pulmonary Tumors | Number of Mesotheliomas | References |
|---|---|---|---|---|---|---|---|---|---|
| Chrysotile Canadian (nickel, cobalt, chromium, and lead contamination) | 6, 5, 14 | w.b. 86 | Nd | 8,600,000 | NS, 41 | 10 | 24 | 1 | Gross et al. (1967) |
| Chrysotile UICC Canadian | 7, 5, 24 | w.b. 10 | Nd | 970,000 | W, 21 | 10 | 48 | 1 | Wagner et al. (1974) |
| Chrysotile UICC Rhodesian | 7, 5, 24 | w.b. 10 | Nd | 1,470,000 | W, 17 | 11 | 65 | 0 | Wagner et al. (1974) |
| Chrysotile Canadian 714–7D (friction linings) | 5, 5, 24 | w.b. 15 | $1.7 \times 10^5$ SEM 9978 > 5 μm | 1,500,000 | W, 45 | 9 | 20 | 0 | Le Bouffant et al. (1984–1987) |
| SFA chrysotile | 7, 5, 24 | w.b. 10 | 430 > 5 μm pcom 669 particles pcom | 1,080,000 | W, 22 | 8 | 36 | 0 | Wagner et al. (1980) |
| Grade 7 chrysotile | 7, 5, 24 | w.b. 10 | 1020 > 5 μm pcom 745 particles pcom | 1,080,000 | W, 24 | 3 | 13 | 0 | Wagner et al. (1980) |
| UICC chrysotile | 7, 5, 24 | w.b. 10 | 3750 > 5 μm pcom 338 particles pcom | 1,080,000 | W, 23 | 5 | 22 | 0 | Wagner et al. (1980) |
| Chrysotile Calidria | 5, 5, 12 | w.b. 6 | 241 131 > 5 μm reported as *thick bundles* | 600,000 | W, 50 | 0 | 0 | 0 | Muhle et al. (1987) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chrysotile long | 7, 5, 12 | w.b. 10 | 1170 > 5 µm pcom<br>33 > 20 µm pcom | 1,000,000 | W, 40 | 20 | 50 | 2 | Davis and Jones (1988) |
| Chrysotile court | 7, 5, 12 | w.b. 10 | 5510 > 5 µm pcom<br>670 > 20 µm pcom | 1,000,000 | W, 40 | 7 | 17 | 1 | Davis and Jones (1988) |
| Chrysotile UICC A | 7, 5, 12 | w.b. 10 | 2560 > 5 µm pcom | 1,000,000 | Included for comparative fiber numbers without animal exposure | | | | Davis and Jones (1988) |
| Chrysotile NIEHS | 6, 5, 24 | n-o 10 | $1.02 \times 10^5$ SEM<br>$1.06 \times 10^4 > 5$ µm | 1,000,000 | F, 69 | 13 | 18 | 1 | Mast et al. (1995) |
| Chrysotile | 7, 5, 12 | w.b. 10 | 1950 > 5 µm pcom<br>360 > 20 µm pcom | 1,000,000 | W, 40 | 15 | 38 | 0 | Davis et al. (1978) |
| Chrysotile | 7, 5, 12 | w.b. 2 | 390 > 5 µm pcom<br>72 > 20 µm pcom | 200,000 | W, 42 | 8 | 19 | 1 | Davis et al. (1978) |
| Chrysotile Calidria | 7, 5, 12 | w.b. 10 | Nd | 778,000 | F, 51 | 2 | 4 | 0 | Ilgren and Chatfield (1997, 1998) and Pinkerton et al. (1983) |
| Chrysotile Jeffrey | 7, 5, 12 | w.b. 10 | Nd | 1,136,000 | F, 49 | 11 | 22 | 0 | Ilgren AND Chatfield (1997, 1998) and Pinkerton et al. (1983) |
| Chrysotile UICC/B | 7, 5, 12 | w.b. 10 | Nd | 1,099,000 | F, 54 | 13 | 24 | 0 | Ilgren and Chatfield (1997, 1998) and Pinkerton et al. (1983) |
| Amosite | 7, 5, 12 | w.b. 10 | 550 > 5 µm pcom<br>6 > 20 µm pcom | — | W, 43 | 2 | 5 | 0 | Davis et al. (1978) |
| Tremolite Korean | 7, 5, 12 | w.b. 10 | 1600 pcom | — | 39 | 18 | 46 | 2 | Davis et al. (1985) |
| Amosite UICC | 7, 5, 24 | w.b. 10 | Nd | — | W, 21 | 13 | 62 | 0 | Wagner et al. (1974) |

(*continued*)

**TABLE 14.3 (continued)**
**Inhalation Toxicology Studies Using Rats**

| Fiber Type | Exposure Time (Hours/Day, Day/Week, Total Months) | Exposure Type, Exposure Concentration ($mg/m^3$) | Fiber Concentration ($Fibers/cm^3$) (Determined by Electron Microscopy Unless Otherwise Noted) | Equivalent Fiber (Concentration/$cm^3$) Based on TEM Evaluation | Type and Total No. of Rats | Number of Pulmonary Tumors | % Pulmonary Tumors | Number of Mesotheliomas | References |
|---|---|---|---|---|---|---|---|---|---|
| Amosite long | 7, 5, 12 | w.b. 10 | 2060 > 5 μm pcom<br>70 > 10 μm pcom | — | W, 40 | 11 | 28 | 3 | Davis et al. (1986) |
| Amosite short | 7, 5, 12 | w.b. 10 | 70 > 5 μm pcom<br>12 > 10 μm pcom | — | W, 42 | 0 | 0 | 1 | Davis et al. (1986) |
| Amosite | | w.b. 300 | Nd | — | SD, 16 | 3 | 19 | 0 | Lee et al. (1981) |
| Crocidolite UICC | 7, 5, 24 | w.b. 10 | Nd | — | W, 18 | 13 | 72 | 0 | Wagner et al. (1974) |
| Crocidolite | 7, 5, 12 | w.b. 10 | 860 > 5 μm pcom<br>estimated figure<br>34 > 20 μm pcom | — | W, 40 | 1 | 3 | 0 | Davis et al. (1978) |
| Crocidolite | 7, 5, 12 | w.b. 5 | 430 > 5 μm pcom<br>17 > 20 μm pcom | — | W, 43 | 2 | 5 | 1 | Davis et al. (1978) |
| Crocidolite | 5, 5, 12 | w.b. 2.2 | 2011<br>162 > 5 μm | — | W, 50 | 1 | 2 | 0 | Muhle et al. (1987) |
| Crocidolite UICC | 6, 5, 24 | w.b. 7 | 3000<br>90 > 10 μm | — | OM, 60 | 3 | 5 | 1 | Smith et al. (1987) |
| Crocidolite exposure truncated | 6, 5, 10 | n-o 10 | $1.6 \times 10^4$ > 5 μm SEM | — | F, 106 | 15 | 14 | 1 | McConnell et al. (1994) |

Exposure types: w.b., whole body; n-o, nose only.
Type of rat: F, Fisher 344; OM, Osborne Mendel; SD, Sprague Dawley; W, Wistar.
ND, not determined.
PCOM, phase contrast optical microscopy.
SEM, scanning electron microscopy.

**TABLE 14.4**
**Inhalation Toxicology Studies Using Hamsters**

| Fiber Type | Exp. Time (Hours/ Day, Day/Week, Max. Months) | Exposure Concentration ($mg/m^3$) | Fiber Concentration ($Fibers/cm^3$) | Total Number of Hamsters | Number of Pulmonary Tumors | % Pulmonary Tumors Adenoma, Carcinomas | Number of Mesotheliomas | References |
|---|---|---|---|---|---|---|---|---|
| Amosite | | w.b. 300 | ND | 7 | 0 | 0 | | Lee et al. (1981) |
| Amosite—low | 6, 5, 18 | n-o 0.8 | 36 > 5 μm<br>10 > 20 μm | 83 | 0 | 0 | 3 | McConnell et al. (1999) |
| Amosite—mid | 6, 5, 18 | n-o 3.7 | 165 > 5 μm<br>38 > 20 μm | 85 | 0 | 0 | 22 | McConnell et al. (1999) |
| Amosite—high | 6, 5, 18 | n-o 7.1 | 263 > 5 μm<br>69 > 20 μm | 87 | 0 | 0 | 17 | McConnell et al. (1999) |
| Crocidolite UICC | 6, 5, 18 | w.b. 7 | 3000<br>90 > 10 μm | 58 | 0 | 0 | 0 | Smith et al. (1987) |
| Chrysotile NIEHS | 6, 5, 18 | n-o 10 | $1.02 \times 10^5$<br>$1.06 \times 10^4 > 5$ μm | Not reported | 0 | 0 | 0 | Mast et al. (1994) |

Exposure types: w.b., whole body; n-o, nose only.

a procedure which when applied to chrysotile would produce a large number of short fibers and particles. Bernstein et al. (2013) have estimated number of chrysotile fibers that would have been present in the aerosol if measured by transmission electron microscope (TEM) in the chrysotile studies listed in Table 14.3. These values have been derived from the gravimetric concentration which was reported for the studies and a conversion based upon the SEM measurements reported by Mast et al. (1995) and Hesterberg et al. (1993), with an extrapolation to TEM (Breysse et al., 1989). The gravimetric exposure concentrations shown in Table 14.3 ranged from 2 to 86 mg/m$^3$, which based upon this extrapolation corresponds to between 200,000 and 8,600,000 fibers/cm$^3$.

In the study reported by Mast et al. (1995) and Hesterberg et al. (1993), the total chrysotile lung burden following 24 months of exposure was $5.5 \times 10^{10}$ fibers/lung, as measured by SEM (Bernstein, 2007). With extrapolation to that which would have been observed by TEM, the lung burden would have been approximately $9.4 \times 10^{11}$ fibers/lung. This would correspond to an average of 2300 fibers per alveoli (assuming 10% deposition) (Bernstein et al., 2013).

In addition, most of the studies prior to Mast et al. (1995) used for aerosolization of the fibers an aerosol generation apparatus based on the design of Timbrell et al. (1968) in which a rotating steel blade pushed/chopped the fibers off a compressed plug into the airstream. As some authors have stated, the steel used in the grinding apparatus and as well the aerosolization apparatus often wore, resulting in sometimes considerable exposure to the metal fragments as well. These factors contribute significantly to the difficulty in interpreting the results of the serpentine chrysotile inhalation exposure studies.

In these studies, a tumorigenic response to amphibole's response is observed as would be expected from the biopersistence results; however, as mentioned, there is also a tumorigenic response to some of the chrysotile exposures even though the biopersistence results would suggest otherwise. Eastes and Hadley (1996) developed a model which related the dose of fibers in the lung to potential pathogenicity.

However, as many studies have now shown, in the rat, another factor can also influence the inflammatory and pathological responses. High concentrations of insoluble nuisance dusts have been shown to compromise the clearance mechanisms of the lung, cause inflammation, and a tumorigenic response in the rat, a phenomenon often referred to as lung overload (Bolton et al., 1983; Morrow, 1988; Muhle et al., 1988; Oberdörster, 1995).

The biopersistence studies conducted at exposure concentrations that did not exceed lung overload conditions elucidate two kinetic patterns with chrysotile. They show that the long fibers are not biopersistent. As a result of the fiber chemistry and structure, the longer fibers are attacked, disintegrating into the smaller pieces. The biopersistence studies also show that these smaller pieces clear at a rate which is similar to the rate of clearance of insoluble nuisance dusts. Chrysotile has also been shown to split longitudinally. In most of the chronic inhalation studies, the total aerosol concentration was probably in the order of $10^6$ particles and fibers/cm$^3$ and if the fibers upon contact with the lung begin to split and break apart, the effective dose in terms of the total number of particles will be increased even further.

With such a breakdown of chrysotile into shorter particles, the question remains as to whether the resulting concentration of particles can result in a nonspecific inflammatory reaction and an overload effect in the rat lung. In a recent study, Bellmann et al. (2003) reported on a calibration study to evaluate the end points in a 90-day subchronic inhalation toxicity study of man-made vitreous fibers with a range of biopersistence and amosite. One of the fibers was a calcium–magnesium–silicate (CMS) fiber for which the stock preparation due to method of preparation had a large concentration of particulate material in addition to the fibers. The aerosol exposure concentration for the CMS fiber was 286 fibers/cm$^3$ length < 5 μm, 990 fibers/cm$^3$ length > 5 μm, and 1793 particles/cm$^3$, a distribution which is not observed in manufacturing. The total CMS exposure concentration was 3069 particles and fibers/cm$^3$. The authors point out that "The particle fraction of CMS that had the same chemical composition as the fibrous fraction seemed to cause significant effects." The number of polymorphonuclear leukocytes (PMN) in the bronchoalveolar lavage fluid

(BALF) was higher, and interstitial fibrosis was more pronounced than had been expected on the basis of biopersistence data. In addition, interstitial fibrosis persisted through the 14-week recovery period following the 90-day exposure. In a separate study on X607, a fiber chemically similar to CMS with, however, considerably fewer particles present in the aerosol was evaluated in a chronic inhalation toxicity study and produced no lung tumors or fibrosis at any time point (Hesterberg et al., 1998b).

This effect attributed to particles in the rat CMS study was observed with an exposure concentration of 3069 particles and fibers/cm$^3$, 50% of which were particles or short fibers. It would follow directly from this and the many publications on overload to expect that a dramatically more pronounced effect would occur if the exposure concentration was 1,000,000 particles and fibers/cm$^3$, 90% of which were particles or short fibers as was the case with chrysotile.

These discrepancies in study design put in question the value of especially the chrysotile studies listed in Tables 14.3 and 14.4. McConnell et al. (1999) reported on a well-designed multiple-dose study of amosite asbestos in the hamster where particle and fiber number were well controlled (Table 14.4). In this study, the aerosol concentration ranged from 10 to 69 fibers/cm$^3$ and were chosen based upon a previous, multi-dose 90-day subchronic longer than 20 μm (Hesterberg et al., 1999).

### 14.6.1 Fiber Length

In an analysis that provided the basis for the European Commission's Directive on synthetic mineral fibers, Bernstein et al. (2001a,b) reported that there exists for SVF an excellent correlation between the biopersistence of fibers longer than 20 μm and the pathological effects following either chronic inhalation or chronic intraperitoneal injection studies. This analysis showed that it was possible using the clearance half-time of the fibers longer than 20 μm as obtained from the inhalation biopersistence studies to predict the number of fibers longer than 20 μm remaining following 24 month chronic inhalation exposure. These studies, however, only included synthetic mineral fibers.

As mentioned earlier, Berman et al. (1995) analyzed statistically 9 different asbestos types in 13 separate studies. Due to limitations in the characterization of asbestos structures in the original studies, new exposure measures were developed from samples of the original dusts that were regenerated and analyzed by transmission electron microscopy. The authors reported that while no univariate model was found to provide an adequate description of the lung tumor responses in the inhalation studies, the measure most highly correlated with tumor incidence was the concentration of structures (fibers) ≥20 μm in length. However, using multivariate techniques, measures of exposure were identified that do adequately describe the lung tumor responses.

The potency appears to increase with increasing length, with structures (fibers) longer than 40 μm being about 500 times more potent than structures between 5 and 40 μm in length. Structures <5 μm in length do not appear to make any contribution to lung tumor risk. As discussed earlier, while this analysis also did not find a difference in the potency of chrysotile and amphibole toward the induction of lung tumors, most of the studies included were performed at very high exposure concentrations.

### 14.6.2 Purity of the Samples

In most inhalation studies on both amphiboles and serpentines, there was no analytical confirmation that the fibers that were aerosolized were uniquely of the type stated.

In addition, an issue which has been discussed at length is whether the presence of tremolite in the chrysotile samples can account for some of its carcinogenic potential as well. This is especially pertinent to the mesotheliomas that have been observed in some of the rat inhalation studies (Churg, 1994; McDonald et al., 1999; Roggli et al., 2002). Using microscopic analysis, Frank et al. (1998) have reported the absence of tremolite in the UICC chrysotile sample which has often been used in

the chronic studies. However, when present with chrysotile, tremolite is usually found in very low concentrations which could be missed during microscopic analysis.

To resolve this issue of method sensitivity, Addison and Davies (1990) developed a method of chemical digestion of chrysotile in which the chrysotile is dissolved using acid, leaving behind the amphiboles such as tremolite. This method was applied to a sample UICC chrysotile obtained from Dr. Fred Pooley who has a repository of the original UICC preparation. In conjunction with Gesellschaft für Schadstoffmessung und Auftragsanalytik GmbH (GSA, Neuss, Germany), 2.13 g of UICC chrysotile was digested in acid, following a procedure similar to that of Addison and Davies (1990). Following digestion, the bivariate size distribution was determined for all residual fibers by transmission electron microscopy and the chemical composition of each fiber was determined by EDAX in order to clearly identify if it is amphibole, chrysotile, or something else.

In the 2 mg sample analyzed, the results indicated that there were 3400 tremolite fibers per mg UICC chrysotile. These fibers ranged in length from 1.7 to 14.4 μm and had a mean diameter of 0.65 μm. Forty one percent of the fibers were longer than 5 μm, with 1394 WHO tremolite fibers per mg of UICC chrysotile. These results indicate that tremolite is present in the UICC sample at low concentrations. As no dose–response studies have been performed at low amphibole concentrations, quantification of the effect of these fibers is not possible in the rat. However, as discussed earlier, amphibole asbestos fibers are very biopersistent in the lung and will persist once inhaled. Davis et al. (1985) performed a chronic inhalation toxicity study on tremolite in order to determine the effect of commercial tremolite in comparison to other asbestos types. The authors reported that tremolite was the most dangerous mineral that they have studied, producing 16 carcinomas and 2 mesotheliomas in a group of 39 animals. As described earlier, even short exposure to tremolite produces a notable response in the lung. Bernstein et al. (2003b) reported that following a 5-day exposure to tremolite, a pronounced inflammatory response was observed, with the rapid development of granulomas, collagen deposition within these granulomas, and by 90 days, even mild interstitial fibrosis.

## 14.7 EPIDEMIOLOGY

Both chrysotile and amphibole asbestos were used extensively, often in uncontrolled situations through a large part of the twentieth century. With the understanding of the danger in the use of amphibole asbestos, governments gradually prohibited its use starting in the 1960s, with France being one of the last countries in 1996 to implement such a prohibition.

While some countries have prohibited chrysotile as well, other countries are still mining and using chrysotile largely for high density cement products such as cement roofing and pipes. The understanding of the importance of industrial hygiene controls often termed *controlled use* has resulted in markedly cleaner work environments in the mines and manufacturing facilities.

Many studies have shown that chrysotile is not of the same potency as the amphiboles and is cleared from the lung more rapidly than amphibole (Howard, 1984; Churg and DePaoli, 1988; Mossman et al., 1990; Churg, 1994; Morgan, 1994; McDonald and McDonald, 1995, 1997; McDonald, 1998; McDonald et al., 1999, 2002, 2004; Rodelsperger et al., 1999; Hodgson and Darnton, 2000; Berman and Crump, 2003). Still other studies have stated the opposite.

Two reviews (Hodgson and Darnton, 2000; Berman and Crump, 2003) have reported on quantification of the potency of chrysotile and amphiboles based upon the statistical analysis of epidemiology studies that were available at the time. However, as discussed in the following, the studies characterized as chrysotile exposure were in actuality studies with *predominately chrysotile* exposure. The authors stated that very small quantities of amphibole fiber were ignored as being important to the findings in some cohorts.

Hodgson and Darnton (2000) provided a review of potency of asbestos for causing lung cancer and mesothelioma in relation to fiber type. They concluded that amosite and crocidolite were,

respectively, on the order of 100 and 500 times more potent for causing mesothelioma than chrysotile. They regarded the evidence for lung cancer to be less clear cut, but concluded nevertheless that amphiboles (amosite and crocidolite) were between 10 and 50 times more potent in causing lung cancer than chrysotile.

Berman and Crump (2003) reviewed and analyzed as part of a technical support document for the USEPA an epidemiology database consisting of approximately 150 studies, of which approximately 35 contained exposure data sufficient to derive quantitative exposure–response relationships.

However, due to the state of occupational hygiene measurements at the time, none of the studies were able to use exposure measurements which included fiber number or fiber type. The associations to disease were attributed to the fiber most used without consideration of the criteria that have been understood more recently to determine fiber potency: fiber mineralogy, biopersistence, and fiber length. In addition, the lack of complete occupational histories is a significant limitation in the early epidemiology studies, resulting at times in improper characterization of fiber-specific exposure. The limitations in these earlier studies have been reviewed by Berman and Crump (2003). However, this review could not take into account the more recent toxicology studies that have been published since, which provide a basis for assessing the importance of even small amounts of amphibole fibers compared to chrysotile. In addition, there has been no systematic analysis of fiber dimensions in these epidemiological studies due to the state of the art at the time. This further compounds understanding the importance of the exposure estimates as the more recent toxicology studies have shown that fiber length is an important determinant in toxicity.

Bernstein et al. (2013) have reviewed the studies characterized as predominately chrysotile and found that amphibole asbestos was often present as well and that other sources of amphibole asbestos were sometimes not considered in the evaluations.

This presence of amphibole asbestos is best supported by fiber lung burden analyses in cohorts such as the Charleston, South Carolina, and Quebec and Italy. Sebastien et al. (1989) reported on the analysis of 161 lung tissue samples taken at necropsy from asbestos textile workers in Charleston, South Carolina, and Quebec miners and millers, both exposed to chrysotile. The authors reported that while chrysotile, tremolite, amosite, crocidolite, talc-anthrophyllite, and other fiber types (including rutile, micas, iron, silica, and unidentified silicates) were found, in both cohorts, tremolite predominated. Churg et al. (1984) analyzed the fiber lung content from 6 cases, with mesothelioma derived from a series of approximately 90 autopsies of long-term workers in the Quebec chrysotile industry. The authors reported that the patients with mesothelioma having only chrysotile ore components had a much higher ratio of tremolite group amphiboles (9.3) than chrysotile fibers (2.8), compared to the control group. Pooley and Mitha (1986) in a report on the determination and interpretation of the levels of chrysotile in lung tissue included result from the South Carolina textile workers. Chrysotile, crocidolite, and amosite fibers were found. In addition, in the lungs from the control population, chrysotile and amosite were also found. Case et al. (2000) evaluated asbestos fiber type and length in lungs of fibers longer than 18 μm in length in chrysotile textile plant from the South Carolina cohort and chrysotile miners/milers from the Thetford Mines portion of the Quebec cohort. The Case et al. (2000) results indicated that the *chrysotile only* textile workers had a high proportion of individuals with lung tissue containing amosite and/or crocidolite. Fornero et al. (2009) assessed fiber lung burden in cattle lungs from two areas in Italy's Western Alps, the Susa Valley and Lanzo Valley. This is the same region in which the chrysotile mine at Balangero is located which was the subject of the epidemiological evaluation by Piolatto et al. (1990), where effect was attributed to chrysotile. Fornero et al. (2009) reported that fibers of tremolite/actinolite, chrysotile, grunerite, and crocidolite were found in the cattle lungs.

The studies included in the reviews by Hodgson and Darnton (2000) and Berman and Crump (2003) were not of chrysotile as used today without amphibole asbestos present and were from

periods when the exposure concentration was very high. Today the situation is remarkably different in that only chrysotile is used commercially. In those chrysotile mines where tremolite veins may be present, the veins can be readily avoided during the mining process as they can be easily differentiated by color (Williams-Jones et al., 2001). As reviewed by Bernstein et al. (2013), the Cana Brava chrysotile mine in Brazil routinely monitors for the presence of amphiboles and has found no detectable amphibole asbestos. Studies on the Calidria (New Idria, California) chrysotile mine have also found only very rarely cleavage fragments away from the ore zone. Reports on the Uralasbest mine in Asbest, Russia, which is the largest mine currently in production, have found no tremolite in air samples.

In chrysotile mines today, the exposure levels have been greatly reduced through the use of water control spraying technology and closed-circuit systems (Williams et al. 2008; 2011).

It is interesting to note that many of the facilities characterized as predominately chrysotile that were studied in the Hodgson and Darnton (2000) and Berman and Crump (2003) evaluations had achieved marked reduction in exposure concentrations prior to their closures. As an example, at the Balangero chrysotile mine in Italy, Silvestri et al. (2001) reported exposure concentrations were reduced from over 100 fibers/mL in the 1930s to 0.19 fibers/mL in the mine; 0.54 fibers/mL in the crushing area; 0.93 fibers/mL in the fiber selection area and 0.78 fibers/mL in the bagging area in the 1980s. In a study of the chrysotile miners and millers in Quebec, Liddell et al. (1998) reported that "On the other hand, modern dust conditions are well below the average even of dust category 1 and so there can be considerable confidence that the risk of lung cancer as a result of such exposure has become vanishingly small."

As mentioned earlier, the toxicology studies indicate that even short-term exposure to amphiboles can lead to important pathological response, with transfer of fibers to the pleural cavity. The importance of amphibole point sources to the induction of mesothelioma has been reported in several studies. Musti et al. (2009) and Barbieri et al. (2012) reported on the relationship of increased mesothelioma risk of individuals who lived near an amphibole asbestos plant for over 50 years. Kurumatani and Kumagai (2008) reported that residents who lived within a 300 m radius of a cement pipe plant that used crocidolite and chrysotile had an standard mortality ratio (SMR) for mesothelioma of 13.9 (5.6–28.7) for men and 41.1 (15.2–90.1) for women. Case and Abraham (2009) reported that an industrial legacy use exposure area in which crocidolite and amosite were used was high in mesothelioma incidence and mortality. Pan et al. (2005) reported that people living in proximity to ultramafic rock deposits had an independent and dose–response association with mesothelioma risk.

Epidemiological studies of workers exposed to chrysotile in high density cement plants have been reviewed (Bernstein et al., 2013). Weill et al. (1979) reported that no excess mortality was observed in asbestos cement manufacturing workers following exposure for 20 years to chrysotile at levels equal to or less than 100 MPPCF·years (corresponding to approximately 15 fibers/cm$^3$ years). Thomas et al. (1982) reported on a cohort within an asbestos-cement factory that used chrysotile. The authors stated that: "Thus the general results of this mortality survey suggest that the population of the chrysotile asbestos-cement factory studied are not at any excess risk in terms of total mortality, all cancer mortality, cancers of the lung and bronchus, or gastrointestinal cancers." Gardner et al. (1986) reported on a cohort study carried out at an asbestos cement factory in England. The authors reported that at mean fiber concentrations below 1 fiber/cm$^3$ (although higher levels had probably occurred in certain areas of the asbestos cement factory), there were no excess of lung cancers or other asbestos-related excess death. Ohlson and Hogstedt (1985) reported on a cohort study of asbestos cement workers in a Swedish plant using chrysotile asbestos in which no excess work-related mortality was observed at cumulative exposures estimated at about 10–20 fibers/cm$^3$ years.

The evaluation of chrysotile should be based upon exposure scenarios which occur in production and use currently. Based upon the science reviewed earlier, in the absence of amphibole asbestos, the use of chrysotile at the current permissible exposure limits in the workplace has not been associated with a statistically detectable increase in risk as observed epidemiologically.

## 14.8 SUMMARY

The mineralogy of the serpentine chrysotile fibers and amphiboles fibers shows distinct differences in the structure and chemistry of these two minerals. The curled layered construction of the sheet silicate chrysotile combined with the susceptibility to acid attack results in the ability for this fiber to be degraded and broken apart in the lung and cleared by the macrophage. In contrast, the amphibole fibers are rigid impermeable structures which are resistant to degradation at any pH encountered in the lung. These differences are reflected in the inhalation biopersistence studies which clearly differentiate chrysotile from the amphiboles and show that longer chrysotile fibers rapidly disintegrate in the lung, while the longer amphiboles once deposited remain.

There is no question that amphibole asbestos is highly carcinogenic. Both animal studies and epidemiology studies indicate the potency of amphibole asbestos. Inhalation toxicology studies on tremolite and amosite asbestos show that even short exposure can produce a pathogenic response in the lung. The recent work with amosite asbestos has shown that after a 5-day exposure, fibers are translocated to the pleural cavity within seven days where they initiate a pathological response. This is in contrast to chrysotile which does not initiate a pathological response in the lung and is not translocated to the pleural cavity.

There is an excellent correlation between the biopersistence of the longer synthetic vitreous fibers and chronic toxicity data. Due to the difficulties in study design and the large particle/fiber exposure concentrations used at the time, the chronic inhalation studies with chrysotile asbestos are difficult to interpret in part due to the nonspecific lung-overload effects of the very large particle concentrations in the exposure aerosols. A 90-day chronic inhalation toxicology study of chrysotile performed at lower doses to minimize lung-overload effects has shown that the chrysotile does not produce pathological response at an exposure concentration 5000 times greater than the US total lung volume (TLV) of 0.1 fibers (WHO)/cm$^3$.

Recent quantitative reviews which analyzed the data of available epidemiological studies to determine potency of asbestos for causing lung cancer and mesothelioma in relation to fiber type also differentiated between chrysotile and amphibole asbestos. The most recent analysis also concluded that it is the longer thinner fibers which have the greatest potency. The quantitative experimental results provide additional support for this differentiation. However, even studies characterized as predominately chrysotile in these evaluations also had amphibole asbestos exposure and were based upon situations in the past where there were high uncontrolled exposures. Many of the same studies showed that exposures can be effectively controlled.

Today chrysotile is used predominantly in manufacturing of high density cement products in situations where the potential exposure is greatly reduced through the implementation of industrial hygiene controls.

With heavy and prolonged occupational exposure to chrysotile there is evidence, as with other respirable particles, such exposure can produce lung cancer. The recent inhalation toxicology studies of chrysotile and the epidemiology studies reporting on use of chrysotile alone in high density cement products and the implementation of controls in mining and manufacturing provide a framework for establishing safe use.

It would be most helpful if future studies on chrysotile and amphiboles asbestos, whether in vitro or in vivo, would be performed using size distributions and at doses approaching those to which humans have been exposed.

## QUESTIONS

1. Explain the mineralogical characteristics of the two minerals families which are called asbestos.
   **Answer:** Asbestos is a generic name which refers to two different mineral families: serpentine and amphibole asbestos.
   - Chrysotile is the most common serpentine asbestos. The chrysotile fiber is a sheet silicate, monoclinic in crystalline structure, and has a unique rolled form resulting from the molecular spacing of the silica and magnesium atoms. The walls of the chrysotile fiber are made up of approximately 12–20 layers in which there is some mechanical interlocking. Each layer is about 7.3 Å thick, with the magnesium surface facing the outside of the curl and the silica and oxygen tetrahedron inside the curl. Chrysotile is distinguished by its behavior of being decomposed by acid. The macrophage which clears foreign material from the lung produces an acidic environment which attacks the chrysotile fiber.
   - Amphibole asbestos has a basic structure consisting of a double silica chain which appears as an I-beam with corner-linked $(SiO_4)_{-4}$ tetrahedra linked together in a double-tetrahedral chain that sandwiches a layer with the $Ca_2Mg_5$. The double chain silicate amphibole fibers themselves are highly insoluble in both the lung fluids and in the acidic environment of the macrophages.
2. What are the principal criteria which determine fiber toxicity?
   **Answer:** Mineral fiber toxicology has been associated with three key factors: dose, dimension, and durability.
   - The dose is determined by the fiber's physical characteristics/dimensions, how the fibrous material is used, and the control procedures that are implemented. The thinner and shorter fibers will weigh less and thus can remain suspended in the air longer than thicker and longer fibers.
   - The fiber dimensions govern two factors: whether the fiber is respirable and, secondly, if it is respirable, the dimensions are also a factor in determining their response in the lung milieu once inhaled. Shorter fibers of the size which can be fully engulfed by the macrophage will be cleared by mechanisms similar to those for nonfibrous particles. These include clearance through the lymphatics and macrophage phagocytosis and clearance. It is only the longer fibers which the macrophage cannot fully engulf that have the potential for causing disease if they are persistent in the lung.
   - The durability is important especially for fibers longer than the macrophage (~20 μm). Fibers longer than the macrophage inhibit the macrophage's mobility, preventing clearance. Fibers which either dissolve completely, or dissolve until they are sufficiently weakened to undergo breakage into shorter fibers can be subsequently cleared. Longer fibers which do not can lead to inflammation, fibrosis, and eventually cancer.
3. How long after a 5-day exposure in rats does it take for serpentine and amphibole asbestos fibers to reach the pleural cavity?
   - The serpentine asbestos fiber chrysotile has not been found to translocate to the pleural cavity in such studies and does initiate any pathological response in the pleural cavity.
   - The amphibole asbestos fiber amosite has been found to translocate to the pleural space of the rat within 7 days following a 5-day exposure. A marked inflammatory response was observed on the parietal pleural surface by 90 days postexposure.
4. What are the primary difficulties in interpreting the older epidemiological studies that attempt to differentiate chrysotile from amphibole asbestos.
   - The occupational hygiene measurements used at the time of exposure in these studies did not use exposure measurements which included fiber number, fiber dimensions, or fiber type (chrysotile, amosite, crocidolite, etc.). The associations to disease were attributed to the fiber most used without consideration of the criteria that have been understood more recently to determine fiber potency: fiber mineralogy, biopersistence, and fiber length.

- The lack of complete occupational histories was also a significant limitation in many of the early epidemiology studies, resulting at times in improper characterization of fiber-specific exposure.

The toxicology studies indicate that even short-term exposure to long fiber (>~20 μm) amphiboles can lead to important pathological responses, with transfer of fibers to the pleural cavity. The importance of amphibole point sources to the induction of mesothelioma has been reported more recently in several studies.

## REFERENCES


Addison, J. and L.S. Davies, 1990. Analysis of amphibole asbestos in chrysotile and other minerals. *Ann Occup Hyg* 34(2):159–175.

ATSDR, 2003. Report on the Expert Panel on Health effects of asbestos and synthetic vitreous fibers: The influence of fiber length. Atlanta, GA: Prepared for Agency for Toxic Substances and Disease Registry Division of Health Assessment and Consultation.

Aust, A.E., P.M. Cook, and R.F. Dodson, 2011. Morphological and chemical mechanisms of elongated mineral particle toxicities. *J Toxicol Environ Health B Crit Rev* January–June; 14(1–4):40–75.

Barbieri, P.G., D. Mirabelli, A. Somigliana, D. Cavone, and E. Merler, 2012. Asbestos fibre burden in the lungs of patients with mesothelioma who lived near asbestos-cement factories. *Ann Occup Hyg* 56(6):660–670.

Bates, T.F., L.B. Sand, and J.F. Mink, 1950. Tubular crystals of chrysotile asbestos. *Science* 3:512.

Bellmann, B., H. Muhle, O. Creutzenberg, H. Ernst, M. Müller, D.M. Bernstein, and J.M. Riego-Sintes, 2003. Calibration study on subchronic inhalation toxicity of man-made vitreous fibers in rats. *Inhal Toxicol* October;15(12):1147–1177.

Berman, D.W. and K.S. Crump, 2003. Draft technical support document for a protocol to assess asbestos-related risk. Washington, DC: Office of Solid Waste and Emergency Response U.S. Environmental Protection Agency.

Berman, D.W., K.S. Crump, E.J. Chatfield, J.M. Davis, and A.D. Jones, 1995. The sizes, shapes, and mineralogy of asbestos structures that induce lung tumors or mesothelioma in AF/HAN rats following inhalation. *Risk Anal* 15(2):181–195.

Bernstein, D., V. Castranova, K. Donaldson, B. Fubini, J. Hadley, T. Hesterberg, A. Kane et al., 2005a. ILSI Risk Science Institute Working Group. Testing of fibrous particles: Short-term assays and strategies. *Inhal Toxicol* September;17(10):497–537.

Bernstein, D.M., J. Chevalier, and P. Smith, 2005b. Comparison of calidria chrysotile asbestos to pure tremolite: Final results of the inhalation biopersistence and histopathology examination following short-term exposure. *Inhal Toxicol* 17(9):427–424.

Bernstein, D.M., R. Rogers, and P. Smith, 2005c. The biopersistence of Canadian chrysotile asbestos following inhalation: Final results through 1 year after cessation of exposure. *Inhal Toxicol* 17(1):1–14.

Bernstein, D.M. and J.M.R. Riego-Sintes, 1999. Methods for the determination of the hazardous properties for human health of man made mineral fibers (MMMF). Vol. EUR 18748 EN, April 93, tsar.jrc.ec.europa.eu/documents/Testing-Methods/mmmfweb.pdf. European Commission Joint Research Centre, Institute for Health and Consumer Protection, Unit: Toxicology and Chemical Substances, European Chemicals Bureau, Ispra, Italy. Accessed May 19, 2014.

Bernstein, D.M., R. Rick, and S. Paul, 2003a. The biopersistence of Canadian chrysotile asbestos following inhalation. *Inhal Toxicol* 15(13):101–128.

Bernstein, D.M., 2003b. Fiber biopersistence, toxicity and asbestos. *J Occup Environ Health (UOEH)* 25(1):237–243.

Bernstein, D.M., 2007. Synthetic vitreous fibers: A review toxicology, epidemiology and regulations. *Crit Rev Toxicol* 37(10):839–886.

Bernstein, D.M., C. Morscheidt, H.G. Grimm, and U. Teichert, 1996. The evaluation of soluble fibers using the inhalation biopersistence model, a nine fiber comparison. *Inhal Toxicol* 8:345–385.

Bernstein, D.M., J. Dunnigan, T. Hesterberg, R. Brown, J.A. Legaspi-Velasco, R. Barrera, J. Hoskins, and A. Gibbs, 2013. Health risk of chrysotile revisited. *Crit Rev Toxicol* 43(2):154–183.

Bernstein, D.M., J.M. Riego-Sintes, B.K. Ersboell, and J. Kunert, 2001a. Biopersistence of synthetic mineral fibers as a predictor of chronic inhalation toxicity in rats. *Inhal Toxicol* 13(10):823–849.

Bernstein, D.M., J.M. Riego-Sintes, B.K. Ersboell, and J. Kunert, 2001b. Biopersistence of synthetic mineral fibers as a predictor of chronic intraperitoneal injection tumor response in rats. *Inhal Toxicol* 13(10):851–875.

Bernstein, D.M., R. Rick, and S. Paul, 2004. The biopersistence of Brazilian chrysotile asbestos following inhalation. *Inhal Toxicol* 16(9):745–761.

Bernstein, D.M., R.A. Rogers, R. Sepulveda, K. Donaldson, D. Schuler, S. Gaering, P. Kunzendorf, J. Chevalier, and S.E. Holm, 2010. The pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite asbestos following short term inhalation exposure—Interim results. *Inhal Toxicol* 22(11):937–962.

Bernstein, D.M., R.A. Rogers, R. Sepulveda, K. Donaldson, D. Schuler, S. Gaering, P. Kunzendorf, J. Chevalier, and S.E. Holm, 2011. Quantification of the pathological response and fate in the lung and pleura of chrysotile in combination with fine particles compared to amosite-asbestos following short-term inhalation exposure. *Inhal Toxicol* June;23(7):372–391.

Bolton, R.E., J.H. Vincent, A.D. Jones, J. Addison, and S.T. Beckett, 1983. An overload hypothesis for pulmonary clearance of UICC amosite fibres inhaled by rats. *Br J Ind Med* 40:264–272.

Bragg, G.M., 2001. Fiber release during the handling of products containing chrysotile asbestos using modern control technology. *Can Mineral* 5(Spec. Publ.):111–114.

Breysse, P.N., J.W. Cherrie, J. Addison, and J. Dodgson, 1989. Evaluation of airborne asbestos concentrations using TEM and SEM during residential water tank removal. *Ann Occup Hyg* 33(2):243–256.

Browne, K. and R. Murray, 1990. Asbestos and the Romans. *Lancet* 336(8712):445.

Campbell, W.J., C.W. Huggins, and A.G. Wylie, 1980. Chemical and physical characterization of amosite, chrysotile, crocidolite, and nonfibrous tremolite for oral ingestion studies by the National Institute of Environmental Health Sciences. Report of Investigations 8452. United States Department of the Interior, U.S. Bureau of Mines, Avondale, MD.

Case, B.W. and J.L. Abraham, 2009. Heterogeneity of exposure and attribution of mesothelioma: Trends and strategies in two American counties. *J Phys Conf Series* 151:012008.

Case, B.W., A. Dufresne, A.D. McDonald, J.C. McDonald, and P. Sebastien, 2000. Asbestos fibre type and length in lungs of chrysotile textile and production workers fibers longer than 18 μm. *Inhal Toxicol* 12(Suppl. 3):411–418.

Churg, A. and L. DePaoli, 1988. Clearance of chrysotile asbestos from human lung. *Exp Lung Res* 14 (5):567–574.

Churg, A., 1994. Deposition and clearance of chrysotile asbestos. *Ann Occup Hyg* 38(4):625–633, 424–425.

Churg, A., B. Wiggs, L. Depaoli, B. Kampe, and B. Stevens, 1984. Lung asbestos content in chrysotile workers with mesothelioma. *Am Rev Respir Dis.* December;130(6):1042–1045.

Cossette, M. and P. Delvaux, 1979. Technical evaluation of chrysotile asbestos ore bodies. In *Short Course in Mineralogical Techniques of Asbestos Determination*, R.C. Ledoux (ed.). Toronto, Ontario, Canada: Mineralogical Association of Canada. pp. 79–110.

Davis, J.M. and A.D. Jones, 1988. Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. *Br J Exp Pathol* 69(5):717–737.

Davis, J.M., J. Addison, R.E. Bolton, K. Donaldson, A.D. Jones, and B.G. Miller, 1985. Inhalation studies on the effects of tremolite and brucite dust in rats. *Carcinogenesis* 6(5):667–674.

Davis, J.M., J. Addison, R.E. Bolton, K. Donaldson, A.D. Jones, and T. Smith, 1986. The pathogenicity of long versus short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. *Br J Exp Pathol* 67(3):415–430.

Davis, J.M., S.T. Beckett, R.E. Bolton, P. Collings, and A.P. Middleton, 1978. Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. *Br J Cancer* 37(5):673–688.

Eastes, W. and J.G. Hadley, 1996. A mathematical model of fiber carcinogenicity and fibrosis in inhalation and intraperitoneal experiments in rats. *Inhal Toxicol* 8:323–342.

EPA, 2003. Report on the Peer Consultation Workshop to Discuss a Proposed Protocol to Assess Asbestos-Related Risk. Prepared for: US Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC 20460. EPA Contract No. 68-C-98-148, Work Assignment 2003–2005. Prepared by: Eastern Research Group, Inc. Lexington, MA 02421. Final Report: May 30, 2003.

Etherington, D.J., D. Pugh, and I.A. Silver, 1981. Collagen degradation in an experimental inflammatory lesion: Studies on the role of the macrophage. *Acta Biol Med Ger* 40(10–11):1625–1636.

Fornero, E., E. Belluso, S. Capella, and D. Bellis, 2009. Environmental exposure to asbestos and other inorganic fibres using animal lung model. *Sci Total Environ* January 15;407(3):1010–1018.

Frank, A.L., R.F. Dodson, and M.G. Williams, 1998. Carcinogenic implications of the lack of tremolite in UICC reference chrysotile. *Am J Ind Med* 34(4):314–317.

Gardner, M.J., P.D. Winter, B. Pannett, and C.A. Powell, 1986. Follow up study of workers manufacturing chrysotile asbestos cement products. *Br J Ind Med* 43:726–732.

Gross, P., R.T. DeTreville, E.B. Tolker, M. Kaschak, and M.A. Babyak, 1967. Experimental asbestosis. The development of lung cancer in rats with pulmonary deposits of chrysotile asbestos dust. *Arch Environ Health* 15(3):343–355.

Guldberg, M., S.L. Jensen, T. Knudsen, T. Steenberg, and O. Kamstrup, April 2002. High-alumina low-silica HT stone wool fibers: A chemical compositional range with high biosolubility. *Regul Toxicol Pharmacol* 35(2 Pt 1):217–226.

Hargreaves, A. and W.H. Taylor, 1946. An X-ray examination of decomposition products of chrysotile (asbestos) and serpentine. *Mineral Mag* 27:204–216.

Hesterberg, T.W., C. Axten, E.E. McConnell, G.A. Hart, W. Müller, J. Chevalier, J. Everitt, P. Thevenaz, and G. Oberdörster, 1999. Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the syrian golden hamster. Part I. Results of a subchronic study and dose selection for a chronic study. *Inhal Toxicol* 11(9):747–784.

Hesterberg, T.W., G. Chase, C. Axten, W.C. Miller, R.P. Musselman, O. Kamstrup, J. Hadley, C. Morscheidt, D.M. Bernstein, and P. Thevenaz, 1998a. Biopersistence of synthetic vitreous fibers and amosite asbestos in the rat lung following inhalation. *Toxicol Appl Pharmacol* 151(2):262–275.

Hesterberg, T.W., G.A. Hart, J. Chevalier, W.C. Müller, R.D. Hamilton, J. Bauer, and P. Thevenaz, 1998b. The importance of fiber biopersistence and lung dose in determining the chronic inhalation effects of X607, RCF1, and chrysotile asbestos in rats. *Toxicol Appl Pharmacol* 153(1):68–82.

Hesterberg, T.W., W.C. Miiller, E.E. McConnell, J. Chevalier, J.G. Hadley, D.M. Bernstein, P. Thevenaz, and R. Anderson, 1993. Chronic inhalation toxicity of size-separated glass fibers in Fischer 344 rats. *Fundam Appl Toxicol* 20(4):464–476.

Hodgson, A.A., 1979. Chemistry and physics of asbestos. In *Asbestos: Properties, Applications and Hazards*, L.M.a.S.S. Chissick (ed.), pp. 80–81. New York: John Wiley & Sons.

Hodgson, J.T. and A. Darnton, 2000. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Ann Occup Hyg* 44(8):565–601.

Howard, J.K., 1984. Relative cancer risks from exposure to different asbestos fibre types. *N Z Med J* 97(764):646–649.

Ilgren, E. and E. Chatfield, 1997. Coalinga fibre—A short, amphibole-free chrysotile. Evidence for lack of fibrogenic activity. *Indoor Built Environ* 6:264–276.

Ilgren, E. and E. Chatfield, 1998. Coalinga fibre—A short, amphibole-free chrysotile. Part 2: Evidence for lack of tumourigenic activity. *Indoor Built Environ* 7:18–31.

Kamstrup, O., A. Ellehauge, J. Chevalier, J.M. Davis, E.E. McConnell, and P. Thévenaz, July 2001. Chronic inhalation studies of two types of stone wool fibers in rats. *Inhal Toxicol* 13(7):603–621.

Kamstrup, O., A. Ellehauge, C.G. Collier, and J.M. Davis, 2002. Carcinogenicity studies after intraperitoneal injection of two types of stone wool fibres in rats. *Ann Occup Hyg* 46(2):135–142.

Kiyohara, P.K., 1991. Estudo da interface crisotila-cimento Portland em compósitos de fibro-cimento por métodos óptico-eletrônicos, Tese de Doutorado, apres. EPUSP, São Paulo, Brazil.

Kurumatani, N. and S. Kumagai, 2008. Mapping the risk of mesothelioma due to neighborhood asbestos exposure. *Am J Respir Crit Care Med* September 15;178(6):624–629.

Leake, B.E., 1978. Nomenclature of amphiboles. *Can Mineral* 16:501–520.

Le Bouffant, L., H. Daniel, J.P. Henin, J.C. Martin, C. Normand, G. Tichoux, and F. Trolard, 1987. Experimental study on long-term effects of inhaled MMMF on the lungs of rats. *Ann Occup Hyg* 31(4B):765–790.

Lee, K.P., C.E. Barras, F.D. Griffith, R.S. Waritz, and C.A. Lapin, 1981. Comparative pulmonary responses to inhaled inorganic fibers with asbestos and fiberglass. *Environ Res* 24(1):167–191.

Leineweber, J.P., 1982. Solubility of fibres in vitro and in vivo. In *Proceedings of WHO//ARC Conference in Biological Effects of Man-Made Mineral Fibers*. Copenhagen, Denmark: World Health Organization. pp. 87–101.

Liddell, F.D.K., A.D. McDonald, and J.C. McDonald, 1998. Dust exposure and lung cancer in quebed chrysotile miners and millers. *Ann Occup Hyg* 42(1):7–20.

Luoto, K., M. Holopainen, J. Kangas, P. Kalliokoski, and K. Savolainen, 1995. The effect of fiber length on the dissolution by macrophages of rockwool and glasswool fibers. *Environ Res* 70(1):51–61.

Mast, R.W., T.W. Hesterberg, L.R. Glass, E.E. McConnell, R. Anderson, and D.M. Bernstein, 1994. Chronic inhalation and biopersistence of refractory ceramic fiber in rats and hamsters. *Environ Health Perspect* 102(Suppl. 5):207–209.

Mast, R.W., E.E. McConnell, R. Anderson, J. Chevalier, P. Kotin, D.M. Bernstein, P. Thevenaz, L.R. Glass, W.C. Miiller, and T.W. Hesterberg, 1995. Studies on the chronic toxicity (inhalation) of four types of refractory ceramic fiber in male Fischer 344 rats. *Inhal Toxicol* 7(4):425–467.

Mattson, S.M., 1994. Glass fibres in simulated lung fluid: Dissolution behavior and analytical requirements. *Ann Occup Hyg* 38:857–877.

McConnell, E.E., 1995. Fibrogenic effect of wollastonite compared with asbestos dust and dusts containing quartz. *Occup Environ Med* 52:621–624.

McConnell, E.E., C. Axten, T.W. Hesterberg, J. Chevalier, W.C. Müller, J. Everitt, G. Oberdörster, G.R. Chase, P. Thevenaz, and P. Kotin, 1999. Studies on the inhalation toxicology of two fiberglasses and amosite asbestos in the Syrian golden hamster. Part II. Results of chronic exposure. *Inhal Toxicol* 11(9):785–835.

McConnell, E.E., O. Kamstrup, R. Musselman, T.W. Hesterberg, J. Chevalier, W.C. Müller, and P. Thievenaz, 1994. Chronic inhalation study of size-separated rock and slag wool insulation fibers in Fischer 344/N rats. *Inhal Toxicol* 6:571–614.

McDonald, J.C., 1998. Mineral fibre persistence and carcinogenicity. *Ind Health* 36(4):372–375.

McDonald, J.C. and A.D. McDonald, 1995. Chrysotile, tremolite, and mesothelioma. *Science* 267(5199):776–777.

McDonald, J.C. and A.D. McDonald, 1997. Chrysotile, tremolite and carcinogenicity. *Ann Occup Hyg* 41(6):699–705.

McDonald, J.C., A.D. McDonald, and J.M. Hughes, 1999. Chrysotile, tremolite and fibrogenicity. *Ann Occup Hyg* 43(7):439–442.

McDonald, J.C., J. Harris, and B. Armstrong, 2002. Cohort mortality study of vermiculite miners exposed to fibrous tremolite: An update. *Ann Occup Hyg* 46(suppl 1):93–94.

McDonald, J.C., J. Harris, and B. Armstrong, April 2004. Mortality in a cohort of vermiculite miners exposed to fibrous amphibole in Libby, Montana. *Occup Environ Med* 61(4):363–366.

Miller, B.G., A.D. Jones, A. Searl, D. Buchanan, R.T. Cullen, C.A. Soutar, J.M. Davis, and K. Donaldson, 1999. Influence of characteristics of inhaled fibres on development of tumours in the rat lung. *Ann Occup Hyg* 43:167–179.

Miller, F.J., 2000. Dosimetry of particles: Critical factors having risk assessment implications. *Inhal Toxicol* 12(Suppl. 3):389–395.

Mohr, U., F. Pott, and F.J. Vonnahme, 1984. Morphological aspects of mesotheliomas after intratracheal instillations of fibrous dusts in Syrian golden hamsters. *Exp Pathol* 26(3):179–183.

Morgan, A., 1994. The removal of fibres of chrysotile asbestos from lung. *Ann Occup Hyg* 38(4):643–646.

Morimoto, Y., H. Yamato, M. Kido, I. Tanaka, T. Higashi, A. Fujino, and Y. Yokosaki, 1994. Effects of inhaled ceramic fibers on macrophage function of rat lungs. *Occup Environ Med* 51(1):62–67.

Morrow, P.E., 1988. Possible mechanisms to explain dust overloading of the lungs. *Fundam Appl Toxicol* 10(3):369–384.

Mossman, B.T., J. Bignon, M. Corn, A. Seaton, and J.B. Gee, 1990. Asbestos: Scientific developments and implications for public policy. *Science* 247(4940):294–301.

Muhle, H. and F. Pott, 2000. Asbestos as reference material for fibre-induced cancer. *Int Arch Occup Environ Health* 73 Suppl.:S53–S59.

Muhle, H. and I. Mangelsdorf, 2003. Inhalation toxicity of mineral particles: Critical appraisal of endpoints and study design. *Toxicol Lett* 140–141:223–228.

Muhle, H., B. Bellman, and U. Heinrich, 1988. Overloading of lung clearance during chronic exposure of experimental animals to particles. *Ann Occup Hyg* 32(Suppl. 1):141–147.

Muhle, H., B. Bellmann, O. Creutzenberg, C. Dasenbrock, H. Ernst, R. Kilpper, J.C. MacKenzie et al., 1991. Pulmonary response to toner upon chronic inhalation exposure in rats. *Fundam Appl Toxicol* 17(2):280–299.

Muhle, H., F. Pott, B. Bellmann, S. Takenaka, and U. Ziem, 1987. Inhalation and injection experiments in rats to test the carcinogenicity of MMMF. *Ann Occup Hyg* 31(4B):755–764.

Musti, M., A. Pollice, D. Cavone, S. Dragonieri, and M. Bilancia, 2009. The relationship between malignant mesothelioma and an asbestos cement plant environmental risk: A spatial case-control study in the city of Bari (Italy). *Int Arch Occup Environ Health* March;82(4):489–497 [Epub 2008 Sep 23].

Nagy, B. and T.F. Bates, 1952. Stability of chrysotile asbestos. *Am Mineral* 37:1055–1058.

Noll, W. and H. Kircher, 1951. Über die Morphologie von Asbesten und ihren Zusammenhang mit der Kristallstruktur. *Neues Jb Mineral Mh* 1951:219–240.

Oberdörster, G., 1991. Deposition, elimination and effects of fibers in the respiratory tract of humans and animals, pp. 17–37. VDI Ber.: Düsseldorf, Germany.

Oberdörster, G., 1995. Lung particle overload: Implications for occupational exposures to particles. *Regul Toxicol Pharmacol* 21(1):123–135.

Oberdörster, G., 2000. Determinants of the pathogenicity of man-made vitreous fibers (MMVF). *Int Arch Occup Environ Health* 73 Suppl.:S60–S68.

Ohlson, C.G. and C. Hogstedt, 1985. Lung cancer among asbestos cement workers. A Swedish cohort study and a review. *Br J Ind Med* 42(6):397–402.

Osmond-McLeod, M.J., C.A. Poland, F. Murphy, L. Waddington, H. Morris, S.C. Hawkins, S. Clark, R. Aitken, M.J. McCall, and K. Donaldson, 2011. Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres. *Part Fibre Toxicol* May 13;8:15.

Pan, X.L., H.W. Day, W. Wang, L.A. Beckett, and M.B. Schenker, 2005. Residential proximity to naturally occurring asbestos and mesothelioma risk in California. *Am J Respir Crit Care Med* October 15;172(8):1019–1025.

Pauling, L., 1930. The structure of chlorites. *Proc Natl Acad Sci* 16:578–582.

Perry, R.H. and C.H. Chilton (eds.), 1973. *Chemical Engineers' Handbook*, 5th edn. New York: McGraw-Hill.

Pinkerton, K.E., A.R. Brody, D.A. McLaurin, B. Adkins, Jr., R.W. O'Connor, P.C. Pratt, and J.D. Crapo, 1983. Characterization of three types of chrysotile asbestos after aerosolization. *Environ Res* 31(1):32–53.

Piolatto, G., E. Negri, C. La Vecchia, E. Pira, A. Decarli, and J. Peto, 1990. An update of cancer mortality among chrysotile asbestos miners in Balangero, northern Italy. *Br J Ind Med* 47(12):810–814.

Pooley, F.D. and R. Mitha, 1986. Determination and interpretation of the levels of chrysotile asbestos in lung tissue. In *Biological Effects of Chrysotile* (Accomplishments in Oncology, Vol. 1, No. 2), Wagner, J.C. (ed.), pp. 12–18. Philadelphia, PA: Lippincott.

Rakovan, J., 2011. Serpentine, California's state rock. *J Rocks Mineral* 86(1):63–68.

Rakovan, J., January/February 2011. Serpentine California's state rock. *Rocks Minerals* 86:63–68.

Rodelsperger, K., H.J. Woitowitz, B. Bruckel, R. Arhelger, H. Pohlabeln, and K.H. Jockel, 1999. Dose–response relationship between amphibole fiber lung burden and mesothelioma. *Cancer Detect Prev* 23(3):183–193.

Roggli, V.L., R.T. Vollmer, K.J. Butnor, and T.A. Sporn, 2002. Tremolite and mesothelioma. *Ann Occup Hyg* 46(5):447–453.

Ross, M. and R.P. Nolan, 2003. History of asbestos discovery and use and asbestos-related disease in context with the occurrence of asbestos within ophiolite complexes. In *Ophiolite Concept and the Evolution of Geological Thought*, Y. Dilek and S. Newcomb (eds.). Boulder, CO: Geological Society of America. pp. 447–470.

Sebastien, P., J.C. McDonald, A.D. McDonald, B. Case, and R. Harley, 1989. Respiratory cancer in chrysotile textile and mining industries: Exposure inferences from lung analysis. *Br J Ind Med* March;46(3):180–187.

Silvestri, S., C. Magnani, R. Calisti, and C. Bruno, 2001. The experience of the Balangero chrysotile asbestos mine in Italy: Health effects among workers mining and milling asbestos and the health experience of persons living nearby, Canadian Mineralogist, pp. 177–186. (The Health Effects of Chrysotile Asbestos: Contribution of Science to Risk-Management Decisions Can. Mineral., Spec. Pub. 5, pp. 177–186 (2001).)

Skinner, H.C.W., M. Ross, and C. Frondel, 1988. *Asbestos and Other Fibrous Materials—Mineralogy, Crystal Chemistry, and Health Effects*, 204pp. New York, Oxford University Press.

Smith, D.M., L.W. Ortiz, R.F. Archuleta, and N.F. Johnson, 1987. Long-term health effects in hamsters and rats exposed chronically to man-made vitreous fibres. *Ann Occup Hyg* 31(4B):731–754.

Speil, S. and J.P. Leineweber, 1969. Asbestos minerals in modern technology. *Environ Res* 2:166–208.

Stoeber, W., H. Flachsbart, and D. Hochrainer, 1970. Der Aerodynamische Durchmesser von Latexaggregaten und Asbestfassern. *Staub-Reinh Luft* 30:277–285.

Suquet, H., 1989. Effects of dry grinding and leaching on the crystal structure of chrysotile. *Clays Clay Mineral* 37: 439–445.

Tanji, T., K. Yada, and Y. Akatsuka, 1984. Note: Alternation of clino- and orthochrysotile in a single fiber as revealed by high-resolution electron microscopy. *Clays Clay Minerals* 32:429–432.

Thomas, H.F., I.T. Benjamin, P.C. Elwood, and P.M. Sweetnam, 1982. Further follow-up study of workers from an asbestos cement factory. *Br J Ind Med* 39(3):273–276.

Timbrell, V. and R.E.G. Rendall, 1972. Preparation of the UICC standard reference samples of asbestos. *Powder Technol* 5:279.

Timbrell, V., A.W. Hyett, and J.W. Skidmore, 1968. A simple dispenser for generating dust clouds from standard reference samples of asbestos. *Ann Occup Hyg* October;11(4):273–281.

Virta, R.L., 2002 USGS Open file 02-149. *Asbestos: Geology, Mineralogy, Mining, and Uses*. Prepared in cooperation with *Kirk-Othmer Encyclopedia of Chemical Technology*, online edition. New York: Wiley-Interscience, a division of John Wiley & Sons, Inc.

von Kobell, F., 1834. Ueber den schillernden Asbest von Reichenstein in Schlesian: Jour. Prakt. *Chemie* 2:297–298.

Wagner, J.C., G. Berry, J.W. Skidmore, and F.D. Pooley, 1980. The comparative effects of three chrysotiles by injection and inhalation in rats. *IARC Sci Publ* 1980(30):363–372.

Wagner, J.C., G. Berry, J.W. Skidmore, and V. Timbrell, 1974. The effects of the inhalation of asbestos in rats. *Br J Cancer* 29(3):252–269.

Warren, B.E. and W.L. Bragg, 1930. The structure of chrysotile, $H_4Mg_3Si_2O_9$. *Z Krystallographie* 76:201–210.

Weill H., J. Hughes, and C. Waggenspack, 1979. Influence of dose and fiber type on respiratory malignancy risk in asbestos cement manufacturing. *Am Rev Respir Dis* 120(2):345–354.

Whittaker, E.J.W., 1957. The structure of chrysotile. V. Diffuse reflexions and fibre texture. *Acta Crystallogr* 10:149.

Whittaker, E.J.W., 1963. Research report: Chrysotile fibers—Filled or hollow tubes? Mathematical interpretation may resolve conflicting evidence, Chem. Eng. News-41(39):34–35, September 30.

Whittaker, E.J.W., 1979. Mineralogy, chemistry and crystallography of amphibole asbestos. In *Short Course in Mineralogical Techniques of Asbestos Determination*, R.C. Ledoux (ed.). Toronto, Ontario, Canada: Mineralogical Association of Canada. pp. 1–34.

Williams, M., P. Larorche, and R. Jauron, 2008. *The Basics of Chrysotile Asbestos Dust Control*, 4th edn., Chrysotile Institute, Montreal, Quebec, Canada.

Williams, M., P. Larorche, and R. Jauron, 2011. *Safe Use of Chrysotile Asbestos: A Manual on Preventive and Control Measures.* Chrysotile Institute, Montreal, Quebec, Canada.

Williams-Jones, A.E., C. Normand, J.R. Clark, H. Vali, R.F. Martin, A. Dufresne, and A. Nayebzadeh, 2001. Controls of amphibole formation in chrysotile deposits evidence from the Jeffrey mine. Canadian Mineralogist, Asbestos, Quebec, Canada, Special Publication, 5, 89–104.

Work, L.T., 1962. Size reduction gets a new stature. *Ind Eng Chem* 54(3):52–54.

Wypych, F., L.B. Adad, N. Mattoso, A.A. Marangon, and W.H. Schreiner, 2005. Synthesis and characterization of disordered layered silica obtained by selective leaching of octahedral sheets from chrysotile. *J Colloid Interface Sci* 283(1):107–112.

Yada, K., 1971. Study of microstructure of chrysotile asbestos by high resolution electron microscopy. *Acta Cryst* A27:659–664.

## NOTES

## NOTES


For environmental
occupational health
safe and responsible use