COPYRIGHT AND CITATION CONSIDERATIONS FOR THIS THESIS/ DISSERTATION

- Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

- NonCommercial — You may not use the material for commercial purposes.

- ShareAlike — If you remix, transform, or build upon the material, you must distribute your contributions under the same license as the original.

How to cite this thesis

Surname, Initial(s). (2012). Title of the thesis or dissertation (Doctoral Thesis / Master's Dissertation). Johannesburg: University of Johannesburg. Available from: http://hdl.handle.net/102000/0002 (Accessed: 22 August 2017).

MALIGNANT PLEURAL MESOTHELIOMA: RETROSPECTIVE ANALYSIS OF THE DEMOGRAPHICS, ASBESTOS LUNG FIBRE BURDEN AND PATHOLOGY

A dissertation submitted to
Faculty of Health Sciences, University of Johannesburg, Johannesburg,
in fulfilment of the requirements for the degree of Master of Technology:
Biomedical Technology
by

Gertruida Susanna Vorster
(Student number: 216057283)

Supervisor: Julian Mthombeni
Co-Supervisor: Prof. J.I Phillips

Johannesburg, 2020

DECLARATION

I, Gertruida Susanna Vorster, hereby declare that this dissertation submitted is my own work. It is being submitted for a Master's degree in Biomedical Technology in the Faculty of Health Sciences at the University of Johannesburg. It has not been submitted to any other institution for obtaining any qualification.

_____	30 November 2020
Gertruida Susanna Vorster	**Date**

ABSTRACT

Background: Asbestos mining operations have left South Africa with a legacy of asbestos contamination. Hence asbestos-related diseases continue to be a problem. Currently there are no South African studies that have determined the relationship between asbestos type, fibre sizes or asbestos burden and the development of histological subtypes of mesothelioma.

Objectives: The aim of this study was to describe the demographics and asbestos fibre burden of individuals with mesothelioma. The associations between the asbestos type, fibre sizes and asbestos burden and the histological subtype of mesothelioma were also determined.

Methods: The records of all deceased miners, ex-miners, Asbestos Relief Trust and Kgalagadi Relief Trust compensation Trust claimants in the PATHAUT database who were histologically diagnosed with mesothelioma for the period Jan 2006 – Dec 2016 (11 years) were reviewed.

Results: In all, 270 cases of mesothelioma were reported from 2006 to 2016. The mean age of the mesothelioma cases was 64.0± 10.8 years. About 89.3% were occupationally exposed to asbestos while 10.7% were environmentally exposed. The prevalence of the histological types of mesothelioma was 64.4%, 23.3%, 12.2% for epithelioid, biphasic and sarcomatous subtypes respectively. Our study demonstrated that on average, individuals with the sarcomatous subtype appeared to be about five years older at diagnosis than individuals diagnosed with the other two histological subtypes. Asbestos fibre analysis showed crocidolite was present in most of the lungs (n=155; 94.5%) that contained asbestos fibres, with 85.4% (n=140) of mesothelioma cases having only crocidolite in their lungs. The epithelioid subtype was a major histological subtype among both occupational (63.9%) and environmental (69.0%) cases.

Conclusion: No relationship was established between the subtypes of mesothelioma and the asbestos types, fibre sizes or asbestos burden.

Keywords: Histological subtype, PATHAUT, ART, KRT, South Africa

DEDICATION

This study is dedicated to JJ de Bruyn, my husband, who has stood by me through my studying years and career. He is always ready to motivate and support me. He never complained when I worked late nights, just loved and assisted me in everything so that I will be able to reach my dreams. Your love has given me endless courage.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my heavenly Father for providing me with enormous strength, courage and support. This opportunity has taught me patience, humbleness and appreciation.

Secondly, I would like to thank my supervisors, Ms Julian Mthombeni and Prof Jim Phillips for their support. Julian, thank you for standing by me till the very end, your support has meant so much to me. Prof Phillips has taught me everything I know in the field of Pathology and asbestos. With my first job and even after, he was my mentor and has expressed patience and passion in teaching especially in research and writing. I would not be the researcher or writer I am today if it had not been for him.

Dr Gbenga Olorunfemi, thank you from the bottom of my heart for all your assistance. Your support in the dire time of need has meant so much to me.

The Asbestos Relief Trust through Dr Jim teWater Naude for assisting me with my data requirements and always answering all my questions patiently. The Kgalagadi Relief Trust through Brian Gibson for answering all my questions. Prof Jill Murray for her brilliance in constructing a research project completely changed my way of thinking. Prof Tony Davies who was always there providing informed criticism and suggestions. Prof David Rees and Prof Gill Nelson for their help in constructing my proposal. Dr Naseema Vorajee for her support in my research project. All of the NIOH Pathology staff for their help and assistance. Mr AG Kuhudzai from UJ Statistical consulting services for his statistical assistance.

Ms Conita van Rensburg for providing me with all her love, support and understanding and Ambledown Financial Services for their financial assistance.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

ABBREVIATIONS

NIOH – National Institute for Occupational Health

PATHAUT Database – Pathology Automation Database

ART – Asbestos Relief Trust

KRT – Kgalagadi Relief Trust

RPI – rehabilitation prioritization index

ODMWA – Occupational Disease in Mines and Works Act

KBCA – Kuruman Cape Blue Asbestos

DCBA – Danielskuil Cape Blue Asbestos

SEM – Scanning Electron Microscopy

EDS – Energy Dispersive Spectroscopy

LM – Light Microscopy

PCM – Phase Contrast Microscopy

TEM – Transmission Electron Microscopy

CHAPTER ONE – INTRODUCTION

1.1 Background

South Africa is a mineral-rich country and its economy was underpinned by mining. Asbestos was one mineral that was mined for more than 100 years (Murray *et al*., 2015). South Africa was the world's third-largest producer of asbestos, with production peaking in 1977 (Virta, 2006). Uniquely, three different types of asbestos, namely amosite, crocidolite and chrysotile were mined on a large commercial scale (Hart, 1988). Most of South Africa's asbestos production was exported, but some was used to manufacture goods within the country (Harrington & McGlashan 1988).

Asbestos has useful properties and confers strength and durability to manufactured products in which it is incorporated to (Hart, 1988). However, asbestos is associated with adverse health effects. Inhalation of asbestos fibres causes diffuse pleural thickening, pleural plaques, asbestosis, lung cancer and malignant mesothelioma of the pleura (mesothelioma) (Churg & Green 1998). The association between crocidolite asbestos and mesothelioma was established by Wagner and colleagues in 1960 (Wagner *et al*.,1960). Mesothelioma is an invariably fatal tumour which usually occurs about 20 to 40 years after exposure to asbestos (Bianchi & Bianchi, 2007). The diagnosis is confirmed histologically and classified under three main histological types of mesothelioma namely epithelioid, sarcomatous and a mixed or biphasic type with both epithelioid and sarcomatous elements (Franklin *et al.,* 2016). Current treatment modalities are ineffective (Orenstein & Schenker, 2000).

Because of activities such as mining, milling and transporting of asbestos, there is widespread contamination of the environment. In addition, there are large amounts of asbestos in manufactured items in the built

environment such as asbestos cement roofs (Phillips *et al.,* 2016). This legacy of asbestos in the South African environment means that there is a potential for exposure and disease for many years to come.

The large-scale mining of three types of asbestos along with the detailed data captured at the NIOH in the PATHAUT database presents a unique opportunity to study mesothelioma in South Africa. This also enables us to study asbestos lung fibre burden, mesothelioma subtypes and any relationships between asbestos lung fibre burden and the development of the mesothelioma subtypes.

1.1.1 Asbestos in South Africa

Asbestos is a generic name given to a group of rock-forming, fibrous, silicate minerals (Hart, 1988). The asbestos group is divided into an amphibole and a serpentine class as shown in Table 1.1. The six members of the two groups differ in their structure, chemical composition and biological effect (Roggli, 1990). The sole member of the serpentine class is chrysotile, also known as white asbestos, which is a hydrated magnesium silicate (Phillips *et al.,* 2012) displayed in Figure 1.1a. Chrysotile consists of curved fibres and does not contain any iron. The amphiboles have straight fibres and contain iron with combinations of other cations. The two types of amphiboles that were mined in South Africa are amosite, also known as grunerite or brown asbestos, and crocidolite also known as riebeckite or blue asbestos (Roggli, 1990) displayed in Figure 1.1b and c. Crocidolite contains sodium, magnesium and iron, whereas amosite contains magnesium and iron (Phillips *et al.,* 2012). The other three amphiboles are tremolite, actinolite and anthophyllite but these were not mined on a large commercial scale in South Africa (Phillips *et al.,* 2012). The asbestos types are structurally and chemically different and may be expected to trigger different health effects.

Table 1.1: Asbestos chemical structures and common uses (adapted from Hart, 1988).

Group	Type	Chemical structure	Common uses
Serpentine	Chrysotile	$Mg_6[(OH)_4 Si_2O_5]_2$	Asbestos textiles, friction linings, asbestos cement and insulation products.
Amphibole	Crocidolite	$Na_2Fe_5[(OH)Si_4O_{11}]$	Boiler lagging, acid-resistant packages, gaskets and asbestos cement products.
Amphibole	Amosite	$MgFe_6[(OH)Si_4O_{11}]_2$	Felted insulation, covering for marine turbines, jet engines and asbestos cement products.

It is estimated that, worldwide, asbestos was used in the manufacture of more than 3000 products (Liddell 1997). These products include brake linings, roofing sheets, floor tiles, ropes, insulation materials, prefabricated wall sections, pipes and heat resistant clothing. Asbestos products were used in a broad range of industries such as construction, transport and the electricity, gas and water supply sector (Milne *et al.,* 2013). The demand for all types of asbestos was due to its unique properties. These properties include durability, flexibility, high tensile strength, incombustibility and resistance to heat and various chemical solutions (Hart, 1988).

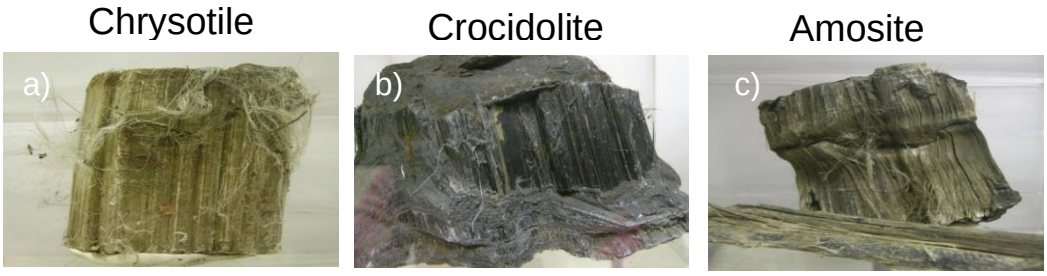


Figure 1.1: The three principal types of asbestos, from the left a) chrysotile, b) crocidolite and c) amosite. Photographs courtesy of the National Institute for Occupational Health.

1.1.2 History of asbestos mining in South Africa

South Africa is a uniquely mineral-rich country and its economy was built on the mining of minerals (Phillips *et al.,* 2012). Asbestos was one of these minerals and South Africa was the only country to mine all three commercial types of asbestos (Hart 1988).

Crocidolite asbestos mining operations began in 1893 and in the period from 1910 to 1920 several small operations were started by farm owners who obtained mining rights. Over time these small mines were bought by large companies such as the Griqualand Exploration and Finance Company Limited (GEFCO) (van Zyl, 2017). In 1920 to 1930, the exportation of asbestos increased (Felix *et al*., 1994) from 7 567 to 10 928 tons (Virta, 2006). In 1977, South Africa was the world's third-largest producer of asbestos (Phillips *et al.,* 2012) behind Russia and Canada (Virta, 2005). South African asbestos was milled locally, and more than 75% of the production was exported after 1960 (Virta, 2006). The fibre was first exported to Europe, North America, South America and later to other parts of Africa, the Middle East, Far East and Oceania (Hart, 1988; Virta, 2006). In the 1970s, some 350 000 tons of asbestos were exported annually (Kielkowski *et al.,* 2011).

Awareness concerning the adverse health effects of asbestos also increased in the 1970s and countries started imposing restrictions on the use of asbestos (Kazan-Allen, 2019; Virta, 2006). By 1977, South African crocidolite production peaked and production surpassed demand (Virta, 2006). This affected the profitability of asbestos mining (Phillips *et al.,* 2016). As the demand for asbestos further declined, mines began to close (Nelson & teWater Naude 2016). The last asbestos mine in South Africa, a chrysotile mine at Msauli, in Mpumalanga Province, closed in 2002 (McCulloch, 2003) and the asbestos cement manufacturer, Everite, used

its last bag of asbestos in its manufacturing process at the end of 2002 (Gibson, 2019).

1.1.2.1 Chrysotile

In 1905, chrysotile deposits were discovered in the Barberton area in Mpumalanga, close to the Swaziland border (Felix *et al*., 1994). Later in 1937, chrysotile was discovered in the Msauli River valley area, near Barberton, in what was the Eastern Transvaal, and which is now Mpumalanga Province. The Msauli mine was the most productive South African chrysotile mine, with an annual production capacity of 110 000 tons and a workforce of approximately 1 650 employees (Felix *et al*., 1994). Production of chrysotile peaked in 1989 at about 115 000 tons (Virta, 2006). Chrysotile was also mined in other provinces including Limpopo and Kwazulu-Natal (Phillips *et al.,* 2012).

1.1.2.2 Crocidolite

Crocidolite was discovered in the 1800s in the town of Prieska in the Northern Cape Province (Felix *et al*., 1994). The crocidolite belt in the Northern Cape Province extends from Prieska, past Griquatown and Kuruman, to Pomfret, an approximate distance of 450 km (Hart, 1988). The mining of Cape Crocidolite started in 1893 in Prieska and escalated in the Second World War (Felix *et al*., 1994). The production of crocidolite peaked in 1977 at 200 000 tons and dropped to 12 000 tons in 1992 (Hart, 1988; Felix *et al*., 1994). The crocidolite mines employed between 12 000 and 14 000 workers and mining ended in 1997 (Virta, 2006).

1.1.2.3 Amosite

The name amosite is an acronym for “asbestos mines of South Africa”. Amosite was first discovered in 1907 in the Lydenburg fields in Mpumalanga, on the farm Penge, near Burgersfort (Felix *et al.,* 1994; van Zyl, 2017). Commercial mining started in 1914. Amosite deposits were also found in the Pietersburg asbestos fields where seams of Transvaal crocidolite were reported to overlap with amosite (Felix *et al.,* 1994; Phillips *et al*., 2012). The British Navy together with other companies used asbestos to manufacture amosite insulation, fireproof clothing and brake pads for armoured vehicles (McCulloch, 2003). Amosite production peaked in 1973 at 106 000 tons (Hart, 1988) and with 7 000 employees. South Africa was the only producer of amosite (Webster, 1973). Penge was the only amosite-asbestos mine in the world (Hart, 1988). The Penge mine closed in 1992 (Felix *et al.,* 1994).

1.1.3 Asbestos contamination

Asbestos mining operations in South Africa have left a legacy of environmental contamination. This contamination is still present around mines and tailings dump. It is also present around roads and railways that were used to transport asbestos across the country (Braun & Kisting 2006; Milne *et al.,* 2013). The responsibility for ensuring contaminated areas, where mines operated, are rehabilitated, lies with the Department of Minerals and Energy. Other contaminated areas outside of the mining sites fall under the governance of the Department of Environmental Affairs and Tourism. In addition to mining and environment contamination, South Africa has a legacy of durable asbestos-containing products in the environment (Phillips *et al.,* 2016) mainly in buildings in the form of cement roofs, vinyl floor tiles and cement materials (Vorster *et al*., 2018). The asbestos regulations make provision for the safe disposal of asbestos

products at specifically designated asbestos disposal sites (Department of Labour, 2002). Examples of these disposal sites in South Africa include Holfontein and Chloorkop in Gauteng. Some countries have recognised the need for spatial planning of designated asbestos waste sites in eradicating asbestos-containing products from the environment (Wilk *et al.,* 2017).

1.1.3.1 Rehabilitation of contaminated areas

Some of the asbestos contaminated areas have been rehabilitated such as the Prieska mill site, part of Penge, Mafefe and Heuningvlei (Liebenberg-Weyers, 2010). The Department of Mineral Resources appointed Mintek, South Africa's national mineral research organisation, to rehabilitate asbestos mines (Cornelissen *et al*., 2019). Rehabilitation is a complex and expensive process and many asbestos contaminated areas remain unrehabilitated in South Africa (Ndlovu *et al.,* 2013). A rehabilitation prioritization index (RPI) for South Africa was developed in 2007 (Liebenberg *et al*.,2012). This index was developed to provide a scientifically-based sequence, from high risk to low risk, in which asbestos mines should be rehabilitated. The RPI was later implemented by the South African Department of Minerals and Energy (Van Rensburg, 2009). One hundred and forty-five asbestos contaminated areas were identified as a priority to rehabilitate, of which in 2009, 84 still needed to be rehabilitated (Van Rensburg, 2009). Data on the number of asbestos mines that still need to be rehabilitated seems to be inconsistent. Cornelissen and colleagues (2019) reported 40 of 249 asbestos mines have been rehabilitated.

Unrehabilitated asbestos contaminated areas continue to pose health risks to nearby communities. Especially communities in the Northern Cape, Limpopo and Mpumalanga provinces, where asbestos was mined. Although environmental exposure to asbestos fibres is at a lower level

than occupational exposures, it has been shown to cause asbestos-related diseases including mesothelioma (Orenstein & Schenker, 2000).

1.1.4 Asbestos exposure in South Africa

Asbestos fibres that enter the lungs through inhalation may cause asbestos-related diseases (Meintjes *et al*., 2008). These fibres which enter the lung are known as respirable fibres and there are many sources of exposures to such fibres. Although asbestos exposures no longer occur in asbestos mines, mills and the manufacturing of asbestos products, asbestos exposures still occur occupationally and environmentally. Asbestos fibres in contaminated areas still pose a health risk to nearby communities (Braun & Kisting 2006). Contaminated areas include unrehabilitated or partially rehabilitated asbestos dumps, dried riverbeds, roads and railways where asbestos spillage took place (Braun & Kisting 2006). Other exposures include asbestos fibres liberated from asbestos products when the products age or are not kept in good condition (Vorster *et al*., 2018) Fibres can also be released during maintenance on or demolition of asbestos building materials (Phillips *et al*., 2016).

1.1.4.1 Occupational exposure

In South Africa, there are large populations with previous occupational exposure, the majority being asbestos miners, millers and workers involved in the manufacturing of asbestos products. Boilermakers, plumbers, shipyard workers, construction workers, metal smelter workers and mechanics may still be exposed to asbestos (Davies *et al*., 1987; Martin 2001; Rice & Heineman 2003 and Roggli *et al*., 2002). There are also some unexpected high-risk occupations where asbestos exposure may occur, such as farmworkers, policemen and teachers (Rees *et al.,*

1999). In certain occupations, mesothelioma cases that had substantial asbestos exposure, such as the manufacturing of asbestos building materials and the installation of asbestos insulation, have declined due to the asbestos ban, whereas we continue to see mesothelioma cases in construction workers (Gilham *et al*.,2016; Rudd *et al*., 2010; Vimercati *et al*., 2019). This may be due to the exposure of workers while repairing, renovating or demolishing materials that contain asbestos.

1.1.4.2 Non-occupational exposure

Aside from occupational exposure, there are other non-occupational situations where persons can be exposed. Non-occupational exposures may be grouped into domestic, neighbourhood and true environmental exposure (Orenstein & Schenker, 2000). Domestic exposure can also be referred to as para-occupational or familial exposure (Orenstein & Schenker, 2000). This can occur when asbestos workers carry asbestos fibres home on their working clothes which usually affects their family members (Ndlovu *et al.,* 2013). In some instances, asbestos was also used in hobby or leisure activities (Marinaccio *et al*., 2015). Neighbourhood exposure, also referred to as environmental exposure, affects residents living close to mine tailings or other asbestos contaminated areas (Ndlovu *et al.,* 2013). True environmental exposure arises from naturally occurring asbestos contaminated soil (Ndlovu *et al.,* 2013).

1.1.5 Asbestos-related diseases

The inhalation of asbestos fibres can cause specific asbestos-related diseases and these are pleural plaques, diffuse pleural thickening, asbestosis, lung cancer and malignant pleural mesothelioma (Ndlovu *et*

al., 2013). Pleural plaques are thickened fibrous areas of the pleural due to collagen deposition in response to injury to the pleural membrane that lines the lungs (Mutsaers *et al*., 2004). Over time the collagen may calcify. Pleural plaques may be markers of asbestos exposure and are associated with lower levels of asbestos exposure (Chauhan, 2005; O'Reilly *et al*., 2007). Diffuse pleural thickening is a result of scar tissue thickening the pleura or lining of the lungs (Wolff *et al.,* 2015). Asbestosis, a fibrosis of the parenchyma of the lung, is typically associated with high levels of asbestos exposure (Ndlovu *et al.,* 2013). Lung cancer is also associated with high levels of asbestos exposure but cannot be distinguished histologically from lung cancer caused by other factors such as cigarette smoking (Chauhan, 2005). While lung cancer has multiple other causes, mesothelioma appears to be a fibre specific tumour (Rees *et al.,* 1999). South Africa is amongst 10 countries with the highest burden of mesothelioma in the world (Murray *et al*., 2015). Mesothelioma continues to be a legacy of asbestos mining in South Africa as asbestos was mined for more than 100 years and due to the high intensities of exposure (Murray *et al*., 2015).

1.1.5.1 Malignant mesothelioma

Malignant pleural mesothelioma is a relatively rare tumour compared to lung cancer (Perez-Guzman *et al*., 2016). The tumour arises from the pleura which covers the lung and chest wall (Chauhan, 2005). Mesothelioma is almost always associated with exposure to asbestos fibres. The link between exposure to crocidolite asbestos fibres and the development of mesothelioma was made in 1960 in South Africa by Wagner, Sleggs and Marchand (Wagner *et al*., 1960).

Mesothelioma does not develop immediately after exposure but has a long latency period typically reported as 20 to 40 years (Bianchi and Bianchi,

2007). In a review of 21 articles, documenting a total of 1105 histologically confirmed cases of mesothelioma in occupationally exposed workers (Lanphear & Buchner, 1992), it was reported that there was a median latency period of 32 years after initial exposure until death, with a range of 13 to 70 years. Differences in reported latency periods were addressed by Bianchi and Bianchi (2007). The authors highlighted that the intensity of exposure to asbestos has to be taken into account as a relationship between the intensity of exposure and the length of the latency period exists (Bianchi and Bianchi, 2007). It has been suggested that a minimum of 10 years from first exposure to asbestos is required to attribute the development of mesothelioma to asbestos exposure (Wolff *et al*., 2015). Because of the long latency period of this type of tumour ex-mine workers and individuals that were exposed to asbestos many years ago continue to present with mesothelioma.

Patients diagnosed with mesothelioma have a very poor prognosis (Ndlovu *et al.,* 2013). There are no effective clinical treatment options and patients usually die within 18 months from the time of diagnosis (Orenstein & Schenker, 2000). Some studies have reported even lower median survival periods of 9.4 months (Neragi-Miandoab *et al*., 2008) and 10.5 months (Ceresoli *et al*.,2001).

Patients with mesothelioma typically present with chest pains, shortness of breath and a cough (Wagner *et al*., 1960). Mesothelioma rarely metastasises but is locally aggressive and grows rapidly around the lung, encasing the lung and compressing viable lung parenchyma (Campbell, 1950; Tertemiz *et al*., 2014). The diagnosis is confirmed histologically (Røe & Stella, 2015) and a panel of immunohistochemistry stains are useful for making an accurate diagnosis (Geltner *et al*., 2016; Husain *et al*., 2018).

Immunohistochemical stains assist with differentiation between epithelioid mesothelioma and lung adenocarcinoma. It has become the standard to use a panel of positive and negative immunohistochemical stains in the diagnosis of mesothelioma. The Guidelines for Pathologic Diagnosis of Malignant Mesothelioma by International Mesothelioma Interest Group of 2017 stated that there is variability between staining and laboratories and that there is no recommended panel of stains (Husain *et al*., 2018). Some of the stains used include the calretinin stain that is a calcium-binding protein that can be demonstrated in benign and malignant mesothelial cells. This stain is most commonly used to differentiate the epithelioid mesothelioma subtype, which would stain positive, from lung adenocarcinoma, which would stain negative (Chhieng *et al*., 2000). Other stains used in diagnosing mesothelioma include Cytokeratin 5 or 5/6, Wilms tumor-1 (WT1), Podoplanin (D2-40), Claudin 4, MOC31, carcinoembryonic antigen (CEA), thyroid transcription factor-1 (TTF-1), B72.3, BER-EP4 and BG8 (Husain *et al*., 2018).

1.1.5.1.1 Mesothelioma subtypes

There are three main histological types of mesothelioma namely epithelioid, sarcomatous and a mixed or biphasic type with both epithelioid and sarcomatous elements (Franklin *et al*., 2016). The World Health Organization (WHO) classification of tumours of the lung, pleura, thymus and heart (Travis *et al*., 2015) are used to classify mesotheliomas into their respective subtypes (Husain *et al*., 2018). Epithelioid subtypes are described to compose of polygonal, oval or cuboidal cells. The sarcomatous subtype is described to consist of spindle cells and the biphasic subtype can contain both epithelioid and sarcomatous areas within the same tumour (Husain *et al*., 2018).

Patients diagnosed with the sarcomatous subtype, in particular, have a shorter median survival period of around 8 months, compared to 18 and

11 months for the epithelioid and the biphasic subtype, respectively (De Assis *et al.,* 2014). It has been reported that patients with the epithelioid type respond better to treatment (Franklin *et al*., 2016). Studies have also reported (Bitchatchi *et al*., 2010; Thomas *et al*., 2015) an improved overall survival in younger patients diagnosed with mesothelioma.

1.1.6 South African asbestos regulations

In 2002, SA promulgated regulations to stipulate how to work safely with asbestos (Department of Labour, 2002). It was only in 2008 that South Africa prohibited the use, manufacturing, import and export of asbestos as well as asbestos-containing materials (Department of Environmental Affairs and Tourism, 2008). In so doing, South Africa joined 67 other countries that had banned asbestos (Kazan-Allen, 2019).

1.1.7 Compensation systems

Despite the banning of asbestos in South Africa in 2008 and the provision of regulations on how to work safely with asbestos, we continue to see cases of asbestos-related diseases, including mesothelioma (Murray *et al.,* 2015; Phillips *et al*., 2016). The law makes provision for compensation of occupationally acquired asbestos-related diseases, including mesothelioma, under the Occupational Disease in Mines and Works Act, 1973 (ODMWA) (Department of Health, 1973).

The compensation system facilitates the submission of cardio-respiratory organs of deceased patients to the National Institute for Occupational Health (NIOH) for examination and diagnoses to assist with the compensation process. Pathological findings, which includes macroscopic and microscopic findings from the examination are stored in the Pathology

Automation Database (PATHAUT). The examinations are performed in accordance with a standard procedure. The lungs are cut in 1cm thick slices to examine macroscopically. Thereafter, a representative section of each of the zones of the lung is taken, together with sections of the main bronchus and lymph nodes, for microscopic examination. The sections undergo a fixation process and are embedded in wax. Sectioning is performed using microtomy and 4-micron thick sections are mounted on glass slides. Thereafter, routine stains are performed on these sections to determine fibrosis and detect ferruginous bodies. The pathologist will request immunohistochemistry to be performed on certain sections. Pleural malignant mesothelioma is diagnosed based on morphology and supported by immunohistochemical stains. The calretinin stain is most commonly used to differentiate the epithelioid mesothelioma subtype, which would stain positive, from lung adenocarcinoma, which would stain negative. This process was also described by Ndlovu and colleagues (Ndlovu *et al*.,2017).

Part of the examination also includes determining the lung fibre burden of individuals with asbestos-related diseases. There are various methods to establish lung fibre burden such as light microscopy (LM), phase contrast microscopy (PCM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Asbestos bodies are also seen during the lung fibre burden analysis. Asbestos bodies are formed when macrophages attempt to phagocytize amphibole asbestos fibres (Wenk, 1976). As a result, the asbestos fibres are coated with complexes of hemosiderin, ferritin and glycoproteins and bodies with a characteristic drumstick appearance are formed around the asbestos fibre (Wenk, 1976). The methods used to analyse the lung fibre burden have advantages and disadvantages in detecting and analysing asbestos fibres as shown in Table1.2.

Table 1.2: Advantages and disadvantages of methods used for detection and analysis of asbestos fibres in lung tissue (adapted from De Vuyst *et al*., 1998 & Roggli *et al*., 2010)

Method	Advantages	Disadvantages
Light microscopy	Quick and easy Inexpensive Widely available Possible to detect low concentrations AB/ml or 3 AB/g	Low resolution (only fibres thicker than 0,20 µm can be detected). Consequently, limited only to asbestos bodies and large fibres Does not enable identification of the fibre type
Polarized light microscopy	Quick and little preparation needed	More operator skills required than for Light microscopy
Interference microscopy	Relatively inexpensive Fibre types can be identified	Low resolution Some skill is required to identify different fibre types
Phase contrast microscopy	Relatively easy Inexpensive method	Cannot distinguish between fibre types Resolution limit for fibres of 0,20 mm diameter
Scanning electron microscopy	More sensitive High resolution Identification of the fibre type and measurement of the fibre dimensions Allows for larger areas of tissue	Time consuming Expensive Detection of concentrations below 5 fibres/ml or 5 000 fibres/g is very time consuming Needs EDS to identify and distinguish the fibre types
Transmission electron microscopy	Most sensitive Identification of fibre type Fibres as thin as 0,01 µm can be detected	Time consuming with more operator skills required than SEM. More expensive than SEM Not widely available Needs EDS or X-ray diffraction to identify and distinguish the fibre types

*AB – Asbestos bodies

At the NIOH, the lung fibre burden is determined by extracting the asbestos fibres from the lungs. The asbestos fibre types, fibre sizes and asbestos body concentration are determined by SEM together with Energy Dispersive Spectroscopy (EDS). This method is described in detail in Appendix A. The different types of asbestos display different EDS peaks upon analysis. When analysing asbestos bodies with EDS, peaks of the asbestos fibre with a much higher iron peak are displayed (Wenk, 1976). The findings on asbestos fibre type, fibre sizes and asbestos body concentrations are stored in the Electron Microscopy (EM) database. Figure 1.2 a) shows the characteristic asbestos body and Figure 1.2 b) displays asbestos fibres identified in lung tissue under SEM.

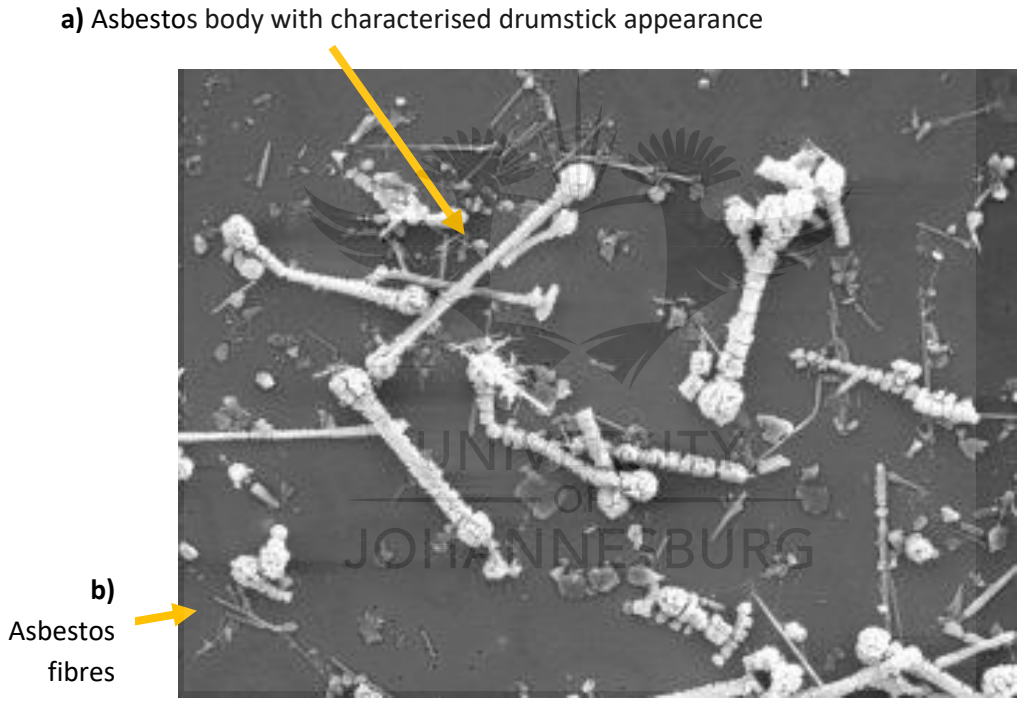


Figure 1.2: Asbestos fibres and bodies identified in lung tissue under Scanning Electron Microscopy. a) Asbestos body with characterised drumstick appearance and b) Asbestos fibres. Photo courtesy of NIOH archives.

Analysis of asbestos fibres retained in the lung can show the number of fibres, fibre types and fibre sizes that individuals have been exposed to (asbestos burden) (Phillips *et al.,* 2012). Although chrysotile fibres are cleared from the lungs, amphiboles such as crocidolite appear to persist (Bernstein *et al*., 2015;

Doll & Peto, 1985; Rees *et al*., 2001). The fibre burden of amphiboles reflects the cumulative number of fibres that individuals have been exposed to. The lungs can, therefore, be considered as a type of personal air sampler.

Compensation for environmental asbestos-related diseases is a neglected issue (Ndlovu *et al.,* 2013). Environmentally contracted asbestos-related diseases can only receive compensation through the ART/KRT if the criteria set out by the Trusts are met (Ndlovu *et al.,* 2013).

1.1.7.1 Asbestos Relief Trust and Kgalagadi Relief Trust

The ART was formed in 2003, through a class action settlement against the Cape Public Limited Company (Plc) and the General Mining Corporation (Gencor) (teWater Naude, 2014a). This settlement made provision for claimants and environmental rehabilitation (teWater Naude, 2014a). The compensation that claimants receive is in addition to compensation received by mineworkers through the ODMWA (teWater Naude, 2014a). The KRT was formed in 2006, through a legal settlement against the Swiss Eternit Group. The Trust has enabled ex-miners of the Kuruman and Danielskuil Cape Blue Asbestos (KCBA and DCBA) mines to apply for compensation (teWater Naude, 2014b).

The ART/KRT require environmental claimants with an asbestos-related disease to fulfil certain criteria to claim. In addition to having an asbestos-related disease caused by environmental exposures, these criteria include having a domestic or neighbourhood exposure within 10km of an asbestos mine or mill. The claimants had to be exposed during the qualifying periods (1955 – 2002) and should have had no prior occupational exposure to asbestos (teWater Naude, 2014b). Outcomes of the compensation claims and where the exposure occurred are stored in the ART/KRT database.

The ART, from when it was established, was pro-active in their efforts. Trustees actively sought work records from founding companies, recognising claimants lack of resources and difficulty accessing these documentations needed for proof to claim (teWater Naude 2014b). The Trust also had enormous success in administering claims. In 2007, it was reported that 27% of claimants receive their awards within 6 months and 36% within 7 to 12 months, 33% within 13 to 24 month and only 4% within more than 25 months. Given these successes, future Trusts may be able to learn from the ART/KRT model and develop a similar structure to ensure similar success. Compensation under the ODMW Act, on the other hand, has not had the same success. The compensation system does not serve its intended beneficiaries and calls for the fund to be managed more efficiently (Baker, 1998; Ehrlich 2012a; Ehrlich 2012b; Maiphetlho & Ehrlich, 2010).

1.2 Literature Review

1.2.1 Asbestos lung fibre burden

Lung fibre burden analysis provides objective information about past exposures to asbestos (Kraynie *et al.,* 2016). The presence of asbestos bodies is also indicative of the inhalation of asbestos fibres (Roggli *et al*., 2010). In South Africa, there are a few studies that examined the asbestos lung fibre burden and typed the asbestos, using electron microscopy and EDS, in patients diagnosed with mesothelioma (Hiroshima *et al*., 1993; Nolan *et al*., 2006; Phillips and Murray 2010; Rees *et al*., 2001).

Hiroshima and colleagues (1993) characterised asbestos fibres in mesothelioma tissues. The authors exposed baboons to the three types of asbestos and focused specifically on the translocation of fibres from the lung to other tissue (Hiroshima *et al*., 1993). Rees and colleagues (2001) studied asbestos lung fibre concentrations in chrysotile miners. The researchers found low fibre contents in the lungs of chrysotile mine workers and concluded that South African chrysotile is not heavily contaminated with tremolite. Nevertheless, contamination with the amphibole asbestos has been suggested to cause mesothelioma (Rees *et al.,* 2001).

The persistency of chrysotile in the lungs and the role it plays in causing mesothelioma, remain controversial. Research has shown that chrysotile fibres are cleared from the lungs by macrophages (Bernstein *et al*., 2015) however, they may still cause lung disease (Landrigan *et al.,* 1999; Martinez-Alier, 2001).

Nolan and colleagues (2006) studied the lung content of forty-three mesothelioma cases from South Africa. The study characterised the asbestos fibres linked to mesothelioma. The study showed the major contribution of crocidolite and the smaller role of amosite and chrysotile in the etiology of mesothelioma cases (Nolan *et al*., 2006). Phillips and Murray 2010 described

a rare case of mesothelioma attributed to anthophyllite exposure, which was not commercially mined in South Africa.

In other countries, there are studies on the analysis of asbestos lung fibre burden in mesothelioma cases. Gilham and colleagues (2016) found that due to the high consumption of amosite in Britain, amosite is a major contributor to mesothelioma incidences in the UK. Roggli and colleagues (2002) results illustrate mesothelioma in the USA are mostly attributable to the commercial amphiboles, amosite and crocidolite. In Australia, mesothelioma cases are due to crocidolite exposure from the Wittenoom area, the site of a crocidolite mine (de Klerk et al., 1996). Berman and Crump (2008) performed a meta-analysis and concluded that amphiboles were more potent in causing mesothelioma than chrysotile, but still rejected the hypothesis that pure chrysotile cannot cause mesothelioma.

1.2.2 Mesotheliomagenic potential

Research has indicated that certain types of asbestos fibres are more likely to cause mesothelioma than others (Rees *et al.,* 1999; Nolan *et al.,* 2006; White *et al.,* 2008). Crocidolite is more likely to cause mesothelioma than amosite and chrysotile, and the mesotheliomagenic potential fibre gradient for South Africa has been proposed as crocidolite>amosite>chrysotile (Rees *et al.,* 1999) and supported by other studies (Nolan *et al.,* 2006; Murray & Nelson, 2008; White *et al.,* 2008).

1.2.3 Length of fibres and pathogenicity

There is controversy regarding the fibre length and the role it plays in potential pathogenicity of asbestos-related diseases (Roggli 2015; Phillips *et al*., 2016; Eligman & Tran, 2016). Several *in vitro*, animal and epidemiological studies have been conducted to determine which fibre sizes are more pathogenic

(Tilkes & Beck 1980; Stanton *et al*., 1981; Davis & Jones 1988; Suzuki *et al*., 2005; Berman & Crump 2008). Although the pathogenic role of asbestos fibres longer than 5 µm is well-established, Authors have expressed their concern not to disregard short fibres in the pathogenicity of asbestos-related diseases (Dodson *et al*., 2003; Lemen *et al*., 2006; Suzuki *et al*., 2005). These and other studies suggested not to disregard short fibres until further research is done to fully understand the role of fibre length in the pathogenicity of asbestos-related diseases (Phillips *et al*., 2016; Barlow, Grespin & Best 2017; Boulanger *et al*., 2014).

1.2.4 Association between mesothelioma subtype and asbestos

There are very few publications on the relationship between exposure characteristics, such as asbestos fibre type, and mesothelioma subtypes (Franklin *et al*., 2016). A study by Klebe and colleagues (2010) reported that patients with the sarcomatous subtype had significantly more amosite fibres in their lungs. However, neither the exposure information was provided, nor the number of amosite fibres within the lungs. Furthermore, Haber & Haber (2011) concluded that there was no association between exposure frequency or intensity and histological subtype. The researchers, however, did not report on fibre types. A more recent study in Australia found no significant relationship between exposure characteristics and histological subtypes of mesothelioma (Franklin *et al*., 2016). The analyses were only performed on the proportion of the study population that was from Wittenoom, the site of a crocidolite mine in Australia, where crocidolite exposure dominated as evident in their results showing that 98.3% of lung tissue samples from mesothelioma patients contained crocidolite.

1.3 Problem statement

Evidence has shown that exposure to asbestos is the major cause of mesothelioma. However, exposure to various types and burden of asbestos fibres may lead to varying clinical manifestation and prognosis of mesothelioma (Brcic & Kern 2020). Furthermore, the modality of treatment, prognosis and survival from mesothelioma is partly related to the histological types. Although asbestos is the leading cause of all mesothelioma types, however, it is not clear if exposure to a particular type of asbestos fibres can be linked with a specific histological type of mesothelioma.

Given South Africa's unique asbestos mining history, three types of asbestos can be studied. This enables us to study any relationships between asbestos lung fibre burden and the development of the mesothelioma subtypes.

There are no South African studies determining the relationship between asbestos exposure characteristics and the development of the three main histological subtypes of mesothelioma. Understanding these relationships may provide insight into how mesothelioma subtypes develop and assist with future treatment strategies.

1.4 Aim

The aim of this study was to describe data collected on individuals diagnosed with mesothelioma at the NIOH. The associations between the asbestos type, fibre size and burden and mesothelioma subtype were also analysed.

1.5 Objectives

1. To describe and compare the environmental and occupational cases of mesothelioma in terms of demographic characteristics and ART/KRT compensation outcomes.
2. To describe the asbestos fibre burden in terms of the number, type and size of the asbestos fibres, as well as the number of asbestos bodies.
3. To describe the association between the histological morphology of mesothelioma and the type, size and number of asbestos fibres.

CHAPTER TWO – METHODS

This chapter describes the research design and the process used to collect, analyse and interpret data. Secondary data on mesothelioma cases were extracted from three different databases namely PATHAUT, ART/KRT and the Electron Microscopy (EM) database of the NIOH.

2.1 Study Design

This study was a retrospective cross-sectional secondary analysis of three administrative databases. These databases contained information on individuals with mesothelioma diagnosed at autopsy at the NIOH for the 11-year period from 2006 – 2016. The period was chosen as both autopsy data and data for fibre analysis were available for this time.

2.2 Methodology

2.2.1 Study population

This study comprised all deceased miners, ex-miners and ART/KRT claimants histologically diagnosed with mesothelioma at autopsy, whose data had been captured on the PATHAUT database for the period Jan 2006 – Dec 2016 (11 years).

2.2.2 Data collection

All data were extracted electronically. Data of individuals pathologically diagnosed with mesothelioma were extracted from the PATHAUT database. This data included demographics such as age, sex, employment histories and detailed pathology findings of the respiratory organs examined. Records

retrieved from the ART/KRT database was linked against the records extracted from the PATHAUT database to obtain additional information regarding the ART/KRT compensation outcomes. The key variable or unique identifier for the data linkage was the patient's South African Identification Number.

Data concerning the asbestos lung fibre burden, asbestos fibre counts, asbestos types and fibre sizes and concentrations were obtained from the EM Unit database within the NIOH Pathology Department.

PATHAUT was the main source of data and the ART/KRT and EM records were linked to the main source to obtain additional information on compensation outcomes and asbestos lung fibre burden. The variables used in each data set are shown in Appendix B.

2.2.3 Data management

The deceased individuals' information from the PATHAUT database were linked to records from the ART/KRT database. The records were linked by using the South African identification number. Only those records that linked to records from the PATHAUT database were used. Records that did not have a South African ID could not be linked and were therefore excluded from the ART/KRT compensation outcome analysis. Records from the EM database were also linked to records from the PATHAUT database. These records were linked using the laboratory number assigned to each autopsy case. After linking the data frothed three databases and completing data validations, a unique code was created for each case.

The variable name *milemeso* in the PATHAUT database was utilised to identify the 270 cases of mesothelioma that were recorded in the study period (2006 -2016).

The variable name *milmesar* in the PATHAUT database represented all the mesothelioma cases with sarcomatous histological type. The variable *milmesep* represented all epithelioid histology subtype. The third histology subtype is Biphasic (both types). A new variable containing the three categories of the histology subtype was generated from "*milmesep*" and "*milmesar*" and utilized for further analysis.

2.2.3.1 Variables descriptions

For objective one, the demographic characteristics such as age, sex, region, employment history and commodity exposure were described. The compensation outcomes, (whether the family received compensation or not for the ART/KRT cases) was also described. For objective two, the asbestos lung fibre burden was described in terms of the asbestos types and fibres per gram of dry weight of lung tissue. The asbestos fibre sizes were described in the ranges of 1-5 µm, ≥5-10 µm and >10 µm. Asbestos burden or concentration of fibres per gram of dry weight of lung tissue was described in ranges (1 – 999 999, 1 000 000 – 2 999 999, ≥3 000 000). For objective three, the histological morphology of mesothelioma was described for the subtypes epithelioid, sarcomatous or biphasic (mixed). The histological subtype was the dependent variable and the explanatory variables were the asbestos types, fibre size and the asbestos fibre burden. The missing values in the datasets were coded as "unknown".

2.2.3.2 Data validation

Validation was performed on the data. The SA ID number was only used to check for consistencies in sex as well as age. Where discrepancies were found, sex and/or age was changed as indicated by the SA identification number. Data were also checked for duplications and any missing values.

2.2.4 Data analysis

The merged data in Excel was imported into Stata version 16 (StataCorp, USA) statistical software for further analysis. For objective one, descriptive statistics and tabulation were conducted for categorical and continuous variables. Bivariate analysis was conducted to determine the relationship between demographic and regional variables and the asbestos exposure types (occupational or environmental). Analysis involving categorical variables such as sex, region and exposure types was conducted using the Pearson's Chi-square (Fischer's exact test was utilised when the expected frequency was less than 5 in more than 25% of the cells). The mean difference of continuous variables (such as age) across exposure types (occupational or environmental) was assessed using the Student's independent t-test. Similarly, Chi-square and Student's t-test was utilised to respectively assess the relationship between categorical and continuous variables and the compensation outcome.

For objective two, mean and standard deviation, range, frequency and percentages were utilised to describe the type, fibre size and burden of asbestos in the lungs. The relationship between the aforementioned explanatory variables and asbestos exposure (occupational and environmental) was respectively conducted using the Student's t-test and Chi-Square (or Fischer's exact in appropriate condition) for the continuous and categorical variable. Equality of variance was also assessed before conducting either student's t-test for equal or unequal student's t-test as appropriate.

For objective three, the relationship between mesothelioma subtypes (categorical) and asbestos types (categorical), fibre sizes (categorical) and asbestos burden ranges (categorical) was assessed using the Pearson's Chi-square tests. Furthermore, one-way analysis of variance (ANOVA) was conducted to assess the differences in the mean levels of continuous

variables such as asbestos fibres across the three categories of histological types of mesothelioma. A post hoc Bonferroni test was conducted when the p-value of ANOVA was statistically significant to determine where the pairwise difference(s) lie. A two-tailed test of the hypothesis was assumed and a P-value < 0.05 was assumed to be statistically significant.

2.3 Ethical Considerations

The Occupational Disease in Mines and Works Act No. 78 of 1973 makes provision for the cardio-respiratory organ to be sent to the NIOH only if the relatives of deceased individuals give consent (Appendix C). The Human Tissue Act of 1983 and the National Health Act of 2003 together with the consent, make provision for the tissue to be kept for diagnostics, medical education, research and scientific purposes. Ethical approval was obtained from the University of Johannesburg prior to the commencement of the study. The certificate, with the NHREC registration number 241112-035 is included as Appendix D.

In addition, ethical clearance from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand to study the demographic, exposure and pathological data collected on deceased miners and ex-miners in the PATHAUT database was obtained and renewed every five years. The clearance certificate number is M170879 (Appendix E).

Written permission was obtained from the data keeper of the ART/KRT before the retrieval of data from the ART/KRT database. A unique study code was created to represent the identification of the deceased. Access to the datasets was password protected to prevent any other person from accessing it. Only the main investigator had access to the data.

CHAPTER THREE – RESULTS

This chapter describes the study population demographics and other statistical results obtained after analysing the data from the PATHAUT, ART/KRT and the EM databases. For the study period Jan 2006 – Dec 2016 (11 years), 270 cases of mesothelioma were identified in the PATHAUT database.

3.1 Demographic Data

Of the 270 mesothelioma cases identified in the PATHAUT database, 89.3% (95% CI: 84.9% - 92.4%, n=241) were occupationally exposed to asbestos and 10.7% (95% CI: 7.6% - 15.1%, n=29) were environmentally exposed as presented in Figure 3.1.

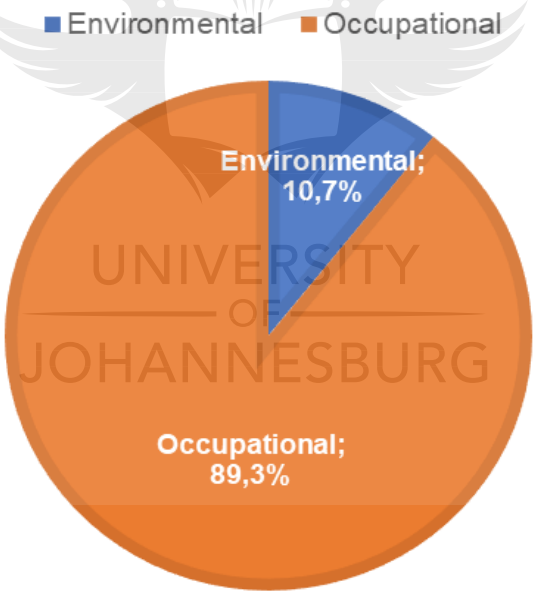


Figure 3.1. Pie chart showing the pattern of Asbestos exposure of the mesothelioma cases.

The mean age of all the individual mesothelioma cases was 64.0± 10.8years. Furthermore, the mean age of the occupationally exposed cases was similar to the mean age of the environmentally exposed cases (occupation vs environment: 64.09 ± 10.8) years vs 64.3 ± 10.8 years, P-value = 0.8787). Nearly four-fifths of the mesothelioma cases were males (n= 214, 79.3%). The majority of the occupationally exposed cases where male patients (86.3%, n=208), while the majority of the environmentally exposed cases were female (65.5%, n=19) Table 3.1.1. There was no statistically significant difference between the mean age among male and female mesothelioma patients (Male vs Female: 63.8 ± 10.9 years vs 64.9 ± 10.3 years, P-value = 0.516).

More than half of the occupational (62.2%,n=150) and environmental (58.6% ,n=17) cases came from the Northern Cape Province and the highest proportion of the deceased patients (29.9%, n=72) last worked for GEFCO followed by Impala Platinum (6.2%, n=15) and Iscor 4.6% (n=11). There was an increase in autopsies for miners who were employed between the years 1970 to 1979. The increase went from 15.6% in the 1960s to 35.6% in the 1970s. The mean years of exposure were 13.5 ± 11.3 years. The mean years of exposure among the environmentally exposed cases (23.5 ± 2.1 years) was higher than the mean years of exposure among the occupationally exposed cases (13.4 ± 11.3 years) as shown in Table 3.1.1.

Table 3.1.1 Demographic characteristics, region and annual mesothelioma diagnosis stratified by asbestos exposure.

Characteristics	Asbestos exposure		Total	P-value
	Environmental, N= 29, (%)	Occupational, N = 241 (%)	N= 270, (%)	
Age (mean ±SD) years	64.3 ± 10.8	64.0 (± 10.8)	64.0 ± 10.8	0.8787[$]
40-49	2 (8.3)	15 (6.3)	17 (6.5)	0.749 [Ω]
50-59	5 (20.8)	81 (34.2)	86 (33.0)	
60-69	8 (33.36)	66 (27.9)	74 (28.4)	
70-79	7 (29.2)	54 (22.8)	61 (23.4)	
80 and above	2 (8.3)	21 (8.9)	23 (8.8)	
Sex				
Female	19 (65.5)	30 (12.5)	49 (18.2)	< 0.001^*
Male	6 (20.7)	208 (86.3)	214 (79.3)	
Unknown	4 (13.8)	3 (1.2)	7 (2.6)	
Province				
Northern Cape	17 (58.)	150 (626.2)	167 (61.9)	0.279^
Gauteng	5 (17.2)	30 (12.5)	35 (13.0)	
North West	0 (0.0)	25 (10.4)	25 (9.3)	
Eastern Cape	0 (0.0)	1 (0.4)	1 (0.4)	
Free State	0 (0.0)	6 (2.5)	6 (2.22)	
Limpopo	0 (0.0)	1 (0.4)	1 (0.4)	
Mpumalanga	1 (3.5)	4 (1.7)	5 (1.9)	
Western Cape	0 (0.0)	2 (0.8)	2 (0.7)	
Foreign	0 (0.0)	1 (0.4)	1 (0.4)	
Unknown	6 (20.7)	21 (8.7)	27 (10.0)	
Miners Job Commencement date				
1940-1949	0 (0.0)	5 (2.1)	5 (1.9)	< 0.001^*
1950-1959	0 (0.0)	27 (11.2)	27 (10.0)	
1960-1969	1 (3.4)	41 (17.0)	42 (15.6)	
1970-1979	1 (3.4)	95 (39.4)	96 (35.6)	
1980-1989	0 (0.0)	45 (18.7)	45 (16.7)	
1990-1999	0 (0.0)	2 (0.8)	2 (0.7)	
≥2000	0 (0.0)	3 (1.2)	3 (1.1)	
Unknown	27 (93.1)	23 (9.5)	50 (18.5)	
Year of diagnosis				
2006	0 (0.0)	2 (0.8)	2 (0.7)	0.386^
2007	1 (3.5)	3 (1.2)	4 (1.5)	
2008	7(24.1)	30 (12.5)	37(13.7)	
2009	0 (0.0)	9 (3.7)	9 (3.33)	
2010	4 (13.8)	24 (10.0)	28 (10.4)	
2011	2 (6.9)	32 (13.3)	34 (12.6)	
2012	6 (20.7)	36 (14.9)	42 (15.6)	
2013	2 (6.9)	30 (12.5)	32 (11.9)	
2014	5 (17.2)	30 (12.5)	35 (13.0)	
2015	2 (6.9)	25 (10.4)	27 (10.0)	
2016	0 (0.0)	20 (8.3)	20 (7.4)	

Last mining site				
GEFCO	-	72 (29.9)		
Impala Platinum	-	15 (6.2)		
Iscor	-	11 (4.6)		
Cape Blue	-	10 (4.1)		
Industry	-	10 (4.1)		
Pomfret Asbestos Mine	-	9 (3.7)		
Hotazel Manganese Mine	-	8 (3.3)		
Gencor	-	7 (2.9)		
Associated Manganese	-	6 (2.5)		
Asbestos Mine	-	5 (2.1)		
ESKOM	-	5 (2.1)		
¥Others	-	69 (28.6)		
Unknown	-	14 (5.8)		
Length of exposure (Mean ± SD) years	23.5 ± 2.1	13.4±11.3	13.5 ± 11.3	< 0.0001$

¥Others are companies with less than 5 cases. *Statistically significant at P-value < 0.05. SD: Standard deviation. $Student's t-test, ^Fischer's exact test, ΩPearson's Chi-square

3.2 Exposure Data

Of the 270 mesothelioma cases, 10.7% (n=29) were environmentally exposed. The remaining 89.3% (n=241) occupational cases mostly worked in different mineral mines which resulted in mixed exposures. The exposure data is, however, based on the longest service history. Nearly half of the occupational cases, (46.5%, n=112) had their longest service in the asbestos mining industry with a mean length of service of 5.8 ± 6.5 years as presented in Table 3.2.1.

Table 3.2.1 Number of occupational cases by commodity most exposed to and mean exposure years.

Commodity most exposed to	Total number of cases (%)	Length of exposure (Mean ± SD), years
Asbestos	112 (46.5)	5.8 ± 6.5
Gold	25 (10.4)	20.6 ± 11.5
Platinum	25 (10.4)	17.9 ± 9.9
Manganese	21 (8.7)	17.9 ± 10.7
Industry	10 (4.1)	20.6 ± 14.1
Iscor	8 (3.3)	17.4 ± 8.8
Coal	7 (2.9)	18.9 ± 13.0
Diamond	7 (2.9)	14.2 ± 15.9
Eskom	5 (2.1)	31.6 ± 6.5
Iron	3 (1.2)	16.7 ± 11.0
SA Railways	3 (1.2)	22.0 ± 12.5
Unknown	7 (2.9)	0.0
*Other	8 (3.3)	12.9 ± 12.3

3.3 Compensation Outcomes

Of the 270 mesothelioma cases identified in the PATHAUT database, 54.4% (n=147) were referred by the ART/KRT to the NIOH. Of the referred cases, 87.8% (n=129) qualified to claim compensation, while 12.2% (n=18) did not meet the compensation criteria. Furthermore, Table 3.3.1 showed that a higher percentage of patients who had occupational asbestos exposure were paid as compared to the percentage of environmentally exposed patients that were paid (51.5% vs 17.2%; P-value = 0.001).

Table 3.3.1 Compensation outcome by type of asbestos exposure.

ART/KRT compensation outcomes	**Asbestos exposure**		**Total N= 270, (%)**	**P-value**
	Environmental N= 29, (%)	**Occupational, N = 241 (%)**		
Paid	5 (17.2)	124 (51.5)	129 (47.8)	0.001*
Not paid	5 (17.2)	13 (5.4)	18 (6.7)	
Unknown	19 (65.5)	104 (43.2)	123 (45.6)	

From Table 3.3.2 below, there was no statistically significant difference in the pattern of payment of compensation according to age (P-value = 0.2132), sex (P-value = 0.237) and year period of diagnosis (P-value = 0.119). However, there was a statistically significant relationship between the province of diagnosis and compensation outcome (P-value = 0.004). Northern Cape province had the highest mesothelioma cases with a higher percentage of patients that received compensation as compared to patients who were not compensated in the Province (89.2% vs 77.8, P-value = 0.004).

Table 3.3.2 ART/KRT compensation outcomes by demographic and provincial characteristics.

Characteristics	Compensation outcome		Total	P-value
	Not Paid N, (%)	Paid N, (%)		
Age (mean ± SD) years	64.7± 8.9	61.6 ± 9.8	62.0 ± 9.7	0.2132[£]
Sex				
Female	6 (33.3)	27 (20.9)	33 (22.5)	0.237[$]
Male	12 (66.7)	102 (79.1)	114 (77.6)	
Province				
Northern Cape	14 (77.8)	115 (89.2)	129 (87.8)	0.004*^
North West	0 (0.0)	10 (7.8)	10 (6.8)	
Gauteng	2 (11.1)	1 (0.8)	3 (2.0)	
Free State	0 (0.0)	3 (2.3)	3 (2.0)	
Eastern Cape	1 (5.6)	0 (0.0)	1 (0.7)	
Mpumalanga	1 (5.6)	0 (0.0)	1 (0.7)	
Year period of diagnosis				
2006 – 2009	1 (5.6)	24 (18.6)	25 (17.0)	0.381[$]
2010 – 2013	11 (61.1)	66 (51.2)	77 (52.4)	
2014 – 2016	6 (33.3)	39 (30.2)	45 (30.6)	

[£:] Student's t-test; [$] Pearson's Chi-square. ^ Fischer's exact test·
*Statistically significant at P-value< 0.05

3.4 Asbestos Fibre Burden

Of the 270 mesothelioma cases identified, 97.0% (n=262) were sent for asbestos fibre analyses. Of these, 90.8% (n=238) were occupational cases and 9.2% (n=24) were environmental cases. Only Crocidolite was found in the lungs of about half (n= 140, 53.4%) of the mesothelioma cases and no asbestos fibre was identified in about one-third of cases (n= 98, 37.4%). There was no statistically significant difference in the pattern of asbestos fibres that were found among the environmentally and occupationally exposed cases (P-value = 0.507) Table 3.4.1. Of the cases that contained only crocidolite fibres, 92.1% (n=129/140) were occupational and 7.9% (n=11) were environmental cases.

Table 3.4.1. Type of asbestos fibre among mesothelioma cases based on the pattern of exposure.

Asbestos type	Asbestos exposure		Total N= 262, (%)	P-value
	Environmental N= 24, (%)	Occupational N = 238 (%)		
Crocidolite	11 (45.8)	129 (54.2)	140 (53.4)	0.419^
Amosite and Crocidolite	0 (0.0)	15 (6.3)	15 (5.7)	
Amosite	1 (4.2)	7 (2.9)	8 (3.1)	
Amosite and Chrysotile	0 (0.0)	1 (0.4)	1 (0.4)	
No Asbestos fibres identified	12 (50.1)	86 (36.1)	98 (37.4)	

^ Fischer's exact

3.5 Histological Features of Mesothelioma

The histological features of the mesothelioma cases are presented in Table 3.5.1. Nearly two-thirds (n=64.4%, n=174/270) of the Mesothelioma cases were of the Epithelioid histological type. The next common histological type was the Biphasic type (23.3%, n=63/270) (Table 3.5.1) Furthermore, the predominant histological type among the environmentally exposed (69.0%) and occupationally (63.9%) exposed patients was the epithelioid subtype. There was no statistically significant difference in the histological pattern among the environmentally exposed cases as compared to the occupationally exposed cases. (P-value = 0.649) as presented in Table 3.5.1

Table 3.5.1 Histological features of mesothelioma cases by exposure type.

Histological type	Exposure type		Total N=270, (%)	P-value
	Environmental	Occupational		
Epithelioid	20 (69.0)	154 (63.9)	174 (64.4)	0.649$
Biphasic	7 (24.1)	56 (23.2)	63 (23.3)	
Sarcomatous	2 (6.9)	31 (12.9)	33 (12.2)	

$ Pearson's Chi-square test

Table 3.5.2 Pattern of age and sex across the histological types of mesothelioma.

Characteristics	Histological types			P-value
	Epithelioid	Biphasic	Sarcomatous	
Age (mean ± SD) Years	63.6 ± 11.2	62.8 ± 9.9	68.8 ± 9.4	0.0237¥
Sex				
Female	33 (19.0)	13 (20.6)	3 (9.1)	0.654^
Male	136 (78.2)	49 (77.8)	29 (87.9)	
Unknown	5 (2.9)	1 (1.6)	1 (3.0)	

¥One-way Analysis of variance test; ^Fischer's exact test

The mean age of individuals with different histological types of mesothelioma is displayed in Table 3.5.2. Individuals with the sarcomatous subtype had the highest mean age of 68.8 ± 9.4 years while individuals with the Biphasic type had the lowest mean age of 62.8 ± 9.9 years. The one-way analysis of variance showed that there was a statistically significant difference in mean age across the histological subtypes, P-value = 0.0237. The post-hoc Bonferroni test showed that the difference was between the mean age of individuals who had Sarcomatous and Biphasic (68.8 ± 9.4 vs 62.8 ± 9.9, P-value = 0.030); and between individuals with Sarcomatous and Epithelioid histological subtype (68.8 ± 9.4 vs 63.6 ± 11.2, P-value = 0.034). There was no statistically significant difference in the mean age of individuals who had Biphasic and Epithelioid histological types (62.8 ± 9.9 vs 63.6 ± 11.2, P-value = 1.00). Furthermore, there was no statistically significant difference in the pattern of histological subtypes of mesothelioma among females as compared to males (P-value = 0.654) as shown in Table 3.5.2

3.6 Fibre Burden per Mesothelioma Subtype

The mean concentration and range of fibres by exposure type is presented in Table 3.6.1. The mean concentration of asbestos fibres in the lungs of mesothelioma patients was 2939321 ± 9964.4 million per gram of dried lungs and was the highest concentration of asbestos fibres in the lungs. Generally, the mean concentration of the various types of asbestos bodies and fibers was higher among the lungs of mesothelioma patients that were designated as occupationally exposed as compared to the lungs of patients with environmental exposure (Table 3.6.1).

Table 3.6.1 Asbestos bodies and fibre counts by exposure type (millions per gm of dried lung)

Asbestos characteristics	Occupational Mean ± SD, (Range)	Environmental (Mean ± SD), (Range)	Total (Mean ± SD), (Range)	£P-value
Asbestos bodies	339730.9 ± 11664.5 (0 - 12600000)	45388.92 ± 94847.7 (0 - 386645)	312768.3 ± 1115.1 (0 - 12600000)	0.0002
Asbestos fibres	3180292 ±10400000 (0 - 92100000)	549698 ± 1824964 (0 - 8791489)	2939321 ± 9964.4 (0 - 92100000)	0.0008
Crocidolite	1122159 ± 4086234 (0 - 47000000)	268395.7± 942343.2 (0 – 4576008)	1043952 ± 3911657 (0 - 47000000)	0.0101
Amosite	60502.2 ± 488711.2 (0 - 6442069)	423.7 ± 2075.7 (0 - 10169)	54998.83 ± 466024 (0 - 6442069)	0.0591
Chrysotile	2.6 ± 40.2 (0 - 620)	0.0	2.6 ± 40.2 -	-

£Student's t-test

Table 3.6.2 shows the pattern of asbestos bodies and fibre counts by histological type among the environmentally - and occupationally exposed patients. Of the occupational cases, the epithelioid subtype had the highest mean number of asbestos fibres, asbestos bodies and crocidolite fibres. The highest mean number of amosite fibres occurred among the biphasic subtype. However, the one-way analysis of variance showed that the mean concentration of the various asbestos fibres was not statistically different across the histological types. Of the environmental cases, the epithelioid subtype had the highest average number of asbestos fibres and the highest average number of crocidolite fibres. Asbestos bodies were only identified in the epithelioid subtype.

Table 3.6.2 Asbestos bodies and fibres counts per mesothelioma subtype (millions per gm of dried lung).

Asbestos fibres	**Occupational**			**P-value**	**Environmental**			**¥P-value**
	Epithelioid (Mean ± SD)	**Biphasic (Mean ± SD)**	**Sarcomatous (Mean ± SD)**		**Epithelioid (Mean ± SD)**	**Biphasic (Mean ± SD)**	**Sarcomatous (Mean ± SD)**	
Asbestos bodies	418617.3 ± 1369760.7 (0 - 12 604 909.0)	277 991.2 ± 804634.86 (0 - 4 288 916.0)	62 471.1 ± 147019.4 (0 - 584 099.0)	0.2735	64 078.5 ± 107978.81 (0 - 386 645.0)	0(0-0)	0(0-0)	-
Asbestos fibres	4 305 486.7 ± 12732351 (0 - 92 100 633.0)	1 248 759.7 ± 3234998.9 0 - 16833 994.0	1 090 117.3 ± 1911436.7 (0 - 6 124 898.0)	0.0851	728 206.4 ± 2158220.1 (0 - 8 791 489.0)	92 718.3 ± 197146.3 (0 - 491 665.0)	256 936.0 ± 0.0 (0 - 256 936.0)	0.7703
Crocidolite	1542616.1 5029988.6 0 - 46 955 632.0	395855.2 ± 937251.32 (0 - 5523 175.0)	349166.4 ± 707094.82 (0 - 2 372 658.0)	0.1075	359574.2± 1115324 0 - 4 576 008.0	40033.0 ± 83075.646 0 - 207 495.0	88 536.0 ± 0 0 - 88 536.0	0.7763
Amosite	44927.0 ± 318188.2 (0 - 3 226 637.0)	124272.0 ± 868509.6 (0 - 6442 069.0)	23731.0 ± 96924.8 (0 - 508 216.0)	0.5329	598.2 ± 2466.3 (0 - 10169.0)	0.0	0.0	-
Chrysotile	4.1 ± 50.3 (0 - 620.0)	0.0	0.0	-	0.0	0.0	0.0	-

¥ ANOVA test

Table 3.6.3 Mean concentration of fibres sizes per mesothelioma subtype (millions per gm of dried lung µg).

Occupational cases									
Asbestos type	**Crocidolite***			**Amosite***			**Chrysotile***		
Fibre size (µm)	**1-5 Mean** ± SD	**>5 - 10 Mean** ± SD	**>10 Mean** ± SD	**1-5 Mean** ± SD	**>5 - 10 Mean** ± SD	**>10 Mean** ± SD	**1-5 Mean** ± SD	**>5 - 10 Mean** ± SD	**>10 Mean** ± SD
Epithelioid	443972.0 ± 1578011.3	644023.1± 2236788.7	454606.1± 1493316	20485.3± 161395.2	16383.6± 126438.8	8058.0± 49272.1	0.0	0.0	4.1
Biphasic	101575.8± 359481.4	142661.3± 335642.6	151607.7± 342878.6	41450.0± 302087	32538.7± 226611.1	50283.3± 340045.6	0.0	0.0	0.0
Sarcomatous	52238.1 ± 127165.2	139176.0± 299715.2	157740.4± 327522.5	0.0	3050.8± 14619.5	20679.3± 93056.8	0.0	0.0	0.0
¥P-value	0.1133	0.1207	0.1874	0.6206	0.6523	0.2935	-	-	-
Environmental cases									
	Crocidolite*			**Amosite***					
Fibre size (µm)	**1-5 Mean** ± SD	**>5 - 10 Mean** ± SD	**>10 Mean** ± SD	**1-5 Mean** ± SD	**>5 - 10 Mean** ± SD	**>10 Mean** ± SD			
Epithelioid	27415.7 ± 90113.0	109149.6± 306629.9	223005.9± 806913	0.0	598.1	0.0			
Biphasic	3555.7 ± 8709.6	20963.0± 37671.9	15512.7 ± 37998.1	0.0	0.0	0.0			
Sarcomatous	0.0	59024.0 ± 0	29512.0 ± 0	0.0	0.0	0.0			
¥P-value	0.7902	0.7853	0.8114	-	-	-			

*** Fibres per µg dry weight; ¥P-value of ANOVA**

The mean concentrations of fibres categorised in sizes are displayed in Table 3.6.3. The analysis of variance showed that there were no statistically significant differences in the sizes of the asbestos fibres across histological types.

3.7 Relationship Between Asbestos Type, Fibre Sizes, Burden and Mesothelioma Subtypes

Of the 270 mesothelioma cases in this study, 164 cases were used to establish the relationship between mesothelioma subtype and asbestos type. These cases had both mesothelioma subtype and asbestos type identified. The relationship between asbestos fibres and the histological type of mesothelioma is displayed in Table 3.7.1. There was no statistically significant relationship between the asbestos fibres and the histological types (P-value = 0.508). The majority of the lungs of the mesothelioma cases had similarly high proportions of crocidolite across the histological subtypes (epithelioid vs biphasic vs sarcomatous: 85.3% vs 88.9% vs 78.9%, P-value = 0.508).

Table 3.7.1 Relationship between histological mesothelioma cases by asbestos fibre type and mesothelioma subtype.

Asbestos fibres	Epithelioid n, %	Biphasic n, %	Sarcomatous n, %	Total n, %	P-value
Crocidolite	93 (85.3)	32 (88.9)	15 (78.9)	140 (85.4)	0.508^
Amosite and Crocidolite	8 (7.3)	4 (11.1)	3 (15.8)	15 (9.2)	
Amosite	7 (6.4)	0 (0.0)	1 (5.3)	8 (4.9)	
Amosite and Chrysotile	1 (0.9)	0 (0.0)	0 (0.0)	1 (0.6)	
Total	109	36	19	164	

^ Fischer's exact test

The relationship between asbestos fibre sizes, the number of fibres and mesothelioma subtype are presented in Table 3.7.2. For crocidolite fibre size 1-5 µm and asbestos fibre amounts: 0, 1 – 999 999, 1 000 000 – 2 999 999, ≥3 000 000, there was no statistically significant association across the histological subtypes (P-value =0.642). The same was observed for crocidolite >5-10 µm (P-value =0.458), crocidolite >10 µm (P-value = 0.836).

For amosite 1-5 µm and asbestos fibre amount: 0, 1 – 999 999, 1 000 000 – 2 999 999, ≥3 000 000, no significant association was found between the histological subtypes (P-value =0.675). Similarly, there was no statistically significant relationship for amosite >5-10 µm (P-value =0.749) and amosite >10 µm (P-value =0.343) and the histological types.

Table 3.7.2. Relationship between asbestos fibre sizes ranges and mesothelioma subtypes.

Number of Asbestos fibre size	Epithelioid N, %	Biphasic N, %	Sarcomatous N, %	Total	P-value^
Crocidolite 1-5 µm					
0	104 (61.5)	40 (65.6)	23 (71.9)	167 (63.7)	0.642
1 - 999 999	52 (30.8)	20 (32.8)	9 (28.1)	81 (30.9)	
1 000 000 - 2 999 999	6 (3.6)	1 (1.6)	0 (0.0)	7 (2.7)	
≥3 000 000	7 (4.1)	0 (0.0)	0 (0.0)	7 (2.7)	
Crocidolite >5-10 µm					
0	86 (50.9)	35 (57.4)	18 (56.3)	139 (53.1)	0.458
1 - 999 999	64 (37.9)	24 (39.3)	13 (40.6)	101 (38.6)	
1 000 000 - 2 999 999	9 (5.3)	2 (3.3)	1 (3.1)	12 (4.6)	
≥3 000 000	10 (5.9)	0 (0.0)	0 (0.0)	10 (3.8)	
Crocidolite >10 µm					
0	93 (55.0)	33 (54.1)	19 (59.4)	145 (55.3)	0.836
1 - 999 999	60 (35.5)	25 (41.0)	11 (34.4)	96 (36.6)	
1 000 000 - 2 999 999	10 (5.9)	3 (4.9)	2 (6.3)	15 (5.7)	
≥3 000 000	6 (3.6)	0 (0.0)	0 (0.0)	6 (2.3)	
Amosite 1-5 µm					
0	162 (95.9)	59 (96.7)	32 (100.0)	253 (96.6)	0.675
1 - 999 999	6 (3.6)	1 (1.6)	0 (0.0)	7 (2.7)	
1 000 000 - 2 999 999	1 (0.6)	1 (1.6)	0 (0.0)	2 (0.8)	
≥3 000 000	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Amosite >5-10 µm					
0	159 (94.1)	58 (95.1)	30 (93.8)	247 (94.3)	0.749
1 - 999 999	9 (5.3)	2 (3.3)	2 (6.3)	13 (5.0)	
1 000 000 - 2 999 999	1 (0.6)	1 (1.6)	0 (0.0)	2 (0.8)	
≥3 000 000	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Amosite > 10µm					
0	160 (94.7)	57 (93.4)	29 (90.6)	246 (93.9)	0.343
1 - 999 999	9 (5.3)	3 (4.9)	3 (9.4)	15 (5.7)	
1 000 000 - 2 999 999	0 (0.0)	1 (1.6)	0 (0.0)	1 (0.4)	
≥3 000 000	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Chrysotile 1-5 µm					
0	169 (100.0)	61 (100.0)	32 (100.0)	262 (100.0)	-
1 - 999 999	-	-	-	-	
1 000 000 - 2 999 999	-	-	-	-	

≥3 000 000	-	-	-	-	
Chrysotile >5-10 µm					
0	169 (100.0)	61 (100.0)	32 (100.0)	262 (100.0)	
1 - 999 999	-	-	-	-	
1 000 000 - 2 999 999	-	-	-	-	
≥3 000 000	-	-	-	-	
Chrysotile >10 µm					
0	168 (99.4)	61 (100.0)	32 (100.0)	261 (99.6)	1.000
1 - 999 999	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.4)	
1 000 000 - 2 999 999					
≥3 000 000					

^ Fischer's exact test was conducted

CHAPTER FOUR – DISCUSSION

4.1 Discussion

The aim of this study was to describe data collected on individuals diagnosed with mesothelioma at the NIOH from 2006 to 2016. This was achieved by describing and comparing the environmental and occupational cases of mesothelioma in terms of demographic characteristics, ART/KRT compensation outcomes and the asbestos fibre burden. The associations between mesothelioma subtypes and asbestos types, burden and fibre sizes were also analysed.

Of the cases diagnosed with mesothelioma in the PATHAUT database over the 11-year study period, there were more occupational than environmental mesothelioma cases. In the occupational setting, more than 80% of the cases were male. McCulloch (2003) described that even though women comprised up to half of the South African asbestos mine workforce, they were invisible in the industry as women were never formally employed in asbestos mines. This could explain why so few women were identified in this study, especially in the occupational setting.

In the environmental setting more than 60% of cases were seen in women. The overall male to female ratio for occupational cases was 7:1, while the overall female to male ratio for environmental cases was 3:1. These findings agree with the study by Haber & Haber (2011) that found a higher male to female ratio among occupational cases and a higher female to male ratio among non-occupational cases. These findings were also in agreement with reports from other South African studies which also found that females with mesothelioma predominate in the environmental setting (Nelson & teWater Naude, 2016; Phillips et al., 2012). The predominance of females in the environmental setting could be as a result

of domestic exposure to asbestos containing dust at home or in the neighbourhood.

We found that the mean age of individuals who had mesothelioma was about 64 years and the peak prevalence of mesothelioma among our study population was 50-59 years. Our result was in agreement with the findings of a global study by Delgermaa *et al* that reported a mean age of 63.4 years among mesothelioma patients in South Africa (Delgermaa *et al.,* 2011). However, Delgermaa and colleagues reported a higher global mean age of mesothelioma deaths of 70.1 years. (Delgermaa *et al.,* 2011). Delgermaa and colleagues ascribed the lower mean age of mesothelioma cases in South Africa to a background lower life expectancy as compared to the life expectancy indices in high income countries. Likewise, Kielkowski and colleagues suggested that the lower mean age of South Africa's mesothelioma cases can be attributed to competing causes of death, as fewer people reach the age at which clinical symptoms of the disease manifest (Kielkowski et al., 2011).

Although mesothelioma is described as a disease of the elderly (Thomas et al., 2015), younger patients have also developed the disease. Our study found that the youngest mesothelioma case was 40 years old. However, younger mesothelioma cases were also reported elsewhere by Perez-Guzman and colleagues (2016) who documented malignant pleural mesothelioma in a 17-year-old boy in Mexico. The higher risk of diagnosing mesothelioma among young adults in South Africa may be partly related to the common practice of early exposure when children play on asbestos waste dumps or used these dumps as sandpits (Braun and Kisting (2006). Being environmentally exposed at such a young age would lead to the disease developing earlier in life. For occupational cases, the overall mean age was 64.0 years. The overall mean age for environmental cases was 64.3 years. The mean age for males was 60.6 years and for females 65.3 years. No statistical differences were found between the

mean ages of males and females or between ages in the occupational and environmental setting.

More than half of the cases in this study, (both occupational and environmental), came from the Northern Cape Province. Although Muteba (Muteba 2018) showed that Gauteng province had the highest mesothelioma cases and deaths as respectively reported by the National Cancer Registry (NCR) and Statistics South Africa (Stats SA), the majority of ART/KRT cases came from the Northern Cape province. The majority of crocidolite asbestos was mined and milled in the Northern Cape Province along the asbestos belt where small operations and larger companies mined Cape Crocidolite asbestos (van Zyl, 2017). The high prevalence of environmental cases of mesothelioma also confirmed the environmental contamination in the Northern Cape Province caused by big operations, such as mines operated by GEFCO, Gencor and small independent mines on farms (van Zyl, 2017). Our findings show a provincial disparity of mesothelioma cases as compared to Stats SA and NCR. This suggests that mesothelioma sufferers (who are likely to be staff of asbestos mining companies) and their families in the Northern Cape usually prioritise reporting to the PATHAUT or other Trusts (ART/KRT) because of the financial benefits that may ensue.

Almost a third of the miners in the study worked for GEFCO mines. Others worked in asbestos mines such as Cape Blue, Pomfret and Gencor. This may suggest that GEFCO mines were the predominant company. Around 4% of our study population of mesothelioma cases were employed at Iscor corporation. Ordinarily, the staff of Iscor were not expected to be occupationally exposed to asbestos However, the boilers and pipes that were utilised by Iscor for the majority of their operations were covered in asbestos lagging to retain heat. The lagging consisted of long asbestos fibres woven as a fabric (NEDLAC, 2002). This may have resulted in exposure to asbestos for Iscor workers.

Looking at service years, there was an increase in mesothelioma cases from 16% to 35% amongst those who began service between the years 1970 to 1979, a period when South African asbestos production was at its peak of 350 000 tons per annum (Kielkowski et al., 2011; Virta, 2006). This possibly reflects high exposures due to high production.

Of the cases that applied for compensation through the ART/KRT, more than 85% were compensated. Our study further showed that about one-third of the unsuccessful claims were environmentally related. The concern regarding compensation for environmental asbestos-related diseases was previously addressed by Ndlovu and colleagues (2013). They concluded that exposure to asbestos outside of the workplace contributed to asbestos-related diseases and that most future asbestos-related diseases will be as a result of neighbourhood exposures in South Africa. The other two thirds of unsuccessful claimants were working for non-compensable operations, such as Iscor and Eskom but will still be able to apply for compensation under the statutory compensation acts. The rest might not have been compensated as they did not meet some of the criteria required by the ART/KRT.

To assist the compensation process in terms of the ODMW Act, cardio-respiratory organs are examined by pathologists who utilize histopathological techniques to make the diagnosis of malignant mesothelioma. Thereafter, the pathologist request samples of the lung to be sent to the EM department for asbestos fibre count analysis. Fibre count analysis was performed on more than 95% of all cases in this study. The remainder were either not sent for analysis or not entered into the EM database.

Asbestos fibre analysis showed crocidolite was present in more than half (53.4%) of the lungs. Only one case contained chrysotile fibres and these were found together with amosite fibres. No cases were reported where

only chrysotile fibres were present in the lungs. These findings are in keeping with a study by Nolan and colleagues (2006) who found that most cases contained crocidolite, and chrysotile was never found alone. Studies showed that chrysotile is cleared by macrophages from the lungs even though it may still cause damage to the lungs (Landrigan *et al*., 1999; Martinez-Alier, 2001; Gibbs *et al*., 1998). A more recent study by Bernstein and colleagues (2015) also showed that chrysotile is cleared from lungs which would explain why so few chrysotile fibres were found when performing the fibre analysis.

Overall, fibre analyses revealed that occupational mesothelioma cases contained higher concentration of asbestos fibres and bodies compared to environmental cases. This was anticipated as higher exposures were expected for occupational cases. Asbestos bodies are normally associated with high asbestos exposure. In mesothelioma, asbestos bodies can also be histologically present (Wolff *et al.,* 2015).

The epithelioid subtype of mesothelioma was the predominant histological type with a prevalence of 64.4% among the mesothelioma cases. Furthermore, the epithelioid subtype was also a major histological subtype among both occupational (63.9%) and environmental (69.0%) cases. This histological pattern that was observed among our study population is similar to the findings of Haber & Haber 2011, Franklin *et al*., 2016 and Brcic & Kern 2020 who found that epithelioid histological type was the major subtype of mesothelioma. Only three female cases (9.1%) were diagnosed with the sarcomatous subtype compared to 20.6% and 19.0% for biphasic and epithelioid, respectively. A study by Klebe and colleagues (2010) had similar findings where only 4% of female cases were diagnosed with the sarcomatous subtype of mesothelioma.

Our study demonstrated that on the average, individuals with the sarcomatous subtype appeared to be about five years older at diagnosis

than individuals diagnosed with the other two histological subtypes. Similarly, Franklin and colleagues (2016), showed that patients diagnosed with sarcomatous mesothelioma were slightly older at diagnosis, thereby suggesting a longer latency period. Haber & Haber (2011) found a significant difference between the survival periods when comparing the epithelioid and sarcomatous subtype. The mean survival period for the epithelioid type was 12.2 months versus 7.3 months for the sarcomatous subtype.

Expectedly, we found that the concentration of asbestos fibres was generally higher among the occupational cases of mesothelioma as compared to the environmental cases. The most commonly identified fibre in the lung (occupational and environmental cases) was crocidolite. However, there was no statistically significant difference of the asbestos fibres across the mesothelioma subtype. This finding is similar to the report by Franklin and colleagues (2016). In contrast, Klebe and colleagues (2010) observed that the lungs of sarcomatous cases contained more amosite fibres. Thus, exposure to crocidolite is a major risk factor for the evolution of mesothelioma in South Africa, (even among the environmentally exposed cases) (Nolan et al., 2006).

We found that there was no statistically significant relationship between mesothelioma subtype, asbestos types, burden and fibre sizes. Franklin and colleagues (2016) also found no association between mesothelioma subtypes and asbestos exposure characteristics. However, the conclusions by Franklin and co-workers may not be valid as fibre analysis was only performed on 7% (122/1656) of their study population. Additionally, Franklin *et al* stated that it was difficult to investigate the different fibre types as their mixed fibre exposure group were likely to have had some crocidolite exposure. The study concluded that the possibility that fibre type affects the development of a particular mesothelioma subtype remains unknown (Franklin *et al*., 2016). Likewise, Haber & Haber

(2011) also found that the intensity of exposure and exposure frequency does not influence the development of the mesothelioma subtype. However, Klebe and colleagues (2010) found a significantly higher amosite concentration in sarcomatous subtypes.

4.2 Limitation of the study

Some limitations of this study are related to secondary data analysis, as routinely collected administrative databases that were created primarily for compensation purposes were utilised. These databases had incomplete records such as occupational histories and other demographic information that may impact on the study conclusions.

The PATHAUT database provides data on ex-miners or workers that worked at a controlled mine or works. The data, therefore, were primarily of workers that were occupationally exposed. Environmental cases were only recorded if the ART/KRT sent such cases to the NIOH. The ART/KRT were the only Trusts that provides compensation for environmental cases of asbestos-related disease. However, to qualify for compensation from the Trusts, environmental cases must fulfil certain criteria. Not all cases of mesothelioma fulfil these criteria and will therefore not be eligible for compensation nor autopsy at the NIOH.

PATHAUT data only captured individuals who had an autopsy-confirmed diagnosis of Mesothelioma. However, there are several barriers to requesting and providing consent for autopsy by relations of deceased miners. (Mthombeni 2017). Ignorance, distrust of the compensation system, traditions and religious beliefs and poor access to an autopsy facilities were some identified barriers to autopsy of potential mesothelioma cases. (Mthombeni 2017). Moreover, racial and regional disparity also impact on request and consent for autopsy. Invariably, the incidence of mesothelioma in South Africa is under-reported by the PATHAUT register. (Ndlovu *et al*., 2017).

Cases that were diagnosed with asbestosis or pleural plaques could have served as a comparison group to improve the validity of our findings and conclusions. Additionally, fibre burden analyses may not reflect true

exposure to chrysotile asbestos as chrysotile fibres can be cleared from the lung, thereby leading to lower concentrations of fibres found in the lung at autopsy.

CHAPTER FIVE – CONCLUSION AND RECOMMENDATION

5.1 Conclusion

No strong evidence was found to support any relationship between mesothelioma subtype and asbestos type, fibre size or asbestos burden. However, there was a statistically significant difference in mean age between the sarcomatous and biphasic subtype and between the sarcomatous and epithelioid subtype.

This study supports the mesotheliomagenic potential fibre gradient for South Africa as crocidolite> amosite> chrysotile. Although South Africa mined crocidolite, amosite and chrysotile on a commercial scale, crocidolite is the main cause of mesothelioma in South Africa.

Mesothelioma subtypes have different characteristics, prognoses and outcomes. The aetiology and evolution of these subtypes remain unknown. Research to further characterise aetiopathology of these histological subtypes is necessary.

5.2 Recommendations

Not all cases in the PATHAUT database could be linked to the EM database. Stricter quality control regarding samples sent for fibre analysis and stricter quality controls for the EM database is recommended. Capturing all information collected and validation of data is critically important for reliable and accurate results.

Further research is needed to validate the findings of this study and to further explore the development of the different subtypes of mesothelioma.

REFERENCES

Baker, M. (1998) Is there a case to partially privatise the state compensation fund for occupational injuries and diseases? *Occupational Health Southern Africa* 4(1):15-23.

Barlow, C.A., Grespin, M. and Best, E.A. (2017) Asbestos fiber length and its relation to disease risk. *Inhalation Toxicology*, 29(12-14), pp.541-554.

Berman, D.W. and Crump, K.S. (2008) A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. *Critical Reviews in Toxicology*, 38(sup1), pp.49-73.

Bernstein, D.M., Rogers, R.A., Sepulveda, R., Kunzendorf, P., Bellmann, B., Ernst, H., Creutzenberg, O. and Phillips, J.I. (2015) 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(1), pp.20-34.

Bianchi, C. and Bianchi, T. (2007) Malignant mesothelioma: global incidence and relationship with asbestos. *Industrial Health*, 45(3), pp.379-387.

Bitchatchi, E., Kayser, K., Perelman, M. and Richter, E.D. (2010) Mesothelioma and asbestosis in a young woman following occupational asbestos exposure: Short latency and long survival: Case Report. *Diagnostic Pathology*, 5(1), p.81.

Boulanger, G., Andujar, P., Pairon, J.C., Billon-Galland, M.A., Dion, C., Dumortier, P., Brochard, P., Sobaszek, A., Bartsch, P., Paris, C. and Jaurand, M.C. (2014) Quantification of short and long asbestos fibers to assess asbestos exposure: a review of fiber size toxicity. *Environmental Health*, 13(1), p.59.

Braun, L. and Kisting, S. (2006) Asbestos-related disease in South Africa: the social production of an invisible epidemic. *American Journal of Public Health*, 96(8), pp.1386-1396.

Brcic, L. and Kern, I. (2020). Clinical significance of histologic subtyping of malignant pleural mesothelioma. *Translational lung cancer research*, 9(3), p.924.

Campbell, W.N. (1950) Pleural mesothelioma. *The American Jjournal of Pathology*, 26(3), p.473.

Ceresoli, G.L., Locati, L.D., Ferreri, A.J.M., Cozzarini, C., Passoni, P., Melloni, G., Zannini, P., Bolognesi, A. and Villa, E. (2001) Therapeutic outcome according to histologic subtype in 121 patients with malignant pleural mesothelioma. *Lung Cancer*, 34(2), pp.279-287.

Chauhan, S. (2005) Attribution of lung cancer to asbestos exposure in miners South Africa, Masters dissertation, University of the Witwatersrand, Johannesburg, 2005.

Chhieng, D.C., Yee, H., Schaefer, D., Cangiarella, J.F., Jagirdar, J., Chiriboga, L.A. and Cohen, J.M. (2000) Calretinin staining pattern aids in the differentiation of mesothelioma from adenocarcinoma in serous effusions. *Cancer Cytopathology: Interdisciplinary International Journal of the American Cancer Society*, 90(3), pp.194-200.

Churg, A., Green, F.H.Y. (1998) *Pathology of Occupational Lung Disease*. 2nd ed. Baltimore: Williams and Wilkins.

Cornelissen, H., Watson, I., Adam, E. and Malefetse, T. (2019). Challenges and strategies of abandoned mine rehabilitation in South Africa: The case of asbestos mine rehabilitation. *Journal of Geochemical Exploration*, p.106354.

Davies, J.C.A., Landau, S.P., Goldsmith, C., Langton M.E. (1987) Mesothelioma risk among gold mine workers. *South African Journal of Science*. 83(1),pp.184-185.

Davis, J.M. and Jones, A.D. (1988) Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. *British Journal of Experimental Pathology*, 69(5), p.717.

De Assis, L.V.M., Locatelli, J. and Isoldi, M.C. (2014) The role of key genes and pathways involved in the tumorigenesis of Malignant Mesothelioma. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1845(2), pp.232-247.

de Klerk, N.H., Musk, A.W., Williams, V., Filion, P.R., Whitaker, D. and Shilkin, K.B. (1996) Comparison of measures of exposure to asbestos in former crocidolite workers from Wittenoom Gorge, W. Australia. *American Journal of Industrial Medicine*, 30(5), pp.579-587.

De Vuyst, P., Karjalainen, A., Dumortier, P., Pairon, J.C., Monso, E., Brochard, P., Teschler, H., Tossavainen, A. and Gibbs, A. (1998) Guidelines for mineral fibre analyses in biological samples: report of the ERS Working Group. European Respiratory Society. *European Respiratory Journal,* 11(6), pp.1416-1426.

Delgermaa, V., Takahashi, K., Park, E.K., Le, G.V., Hara, T. and Sorahan, T. (2011) Global mesothelioma deaths reported to the World Health Organization between 1994 and 2008. *Bulletin of the World Health Organization*, 89, pp.716-724.

Dodson, R.F., Atkinson, M.A. and Levin, J.L. (2003) Asbestos fiber length as related to potential pathogenicity: a critical review. *American Journal of Industrial Medicine*, 44(3), pp.291-297.

Doll, R. and Peto, J. (1985) Effects on health of exposure to asbestos. Health & Safety Commission. [Online], Available: http://www.hse.gov.uk/Asbestos/assets/docs/exposure.pdf.

Egilman, D. and Tran, T., 2016. A commentary on Roggli's "The So-Called Short-Fiber Controversy". *International Journal of Occupational and Environmental Health,* 22(3), pp.181-186.

Ehrlich, R. (2012a). Persistent failure of the COIDA system to compensate occupational disease in South Africa. *South African Medical Journal*, 102(2).

Ehrlich, R. (2012b). A century of miners' compensation in South Africa. *American Journal of Industrial Medicine*, 55(6), pp.560-569.

Felix, M.A., Leger, J.P. and Ehrlich, R.I. (1994) Three minerals, three epidemics-asbestos mining and disease in South Africa. *Advances in Modern Environmental Toxicology*, 22, pp.265-265.

Franklin, P., Alfonso, H., Reid, A., Olsen, N., Shilkin, K.B., Brims, F., de Klerk, N. and Musk, A.W. (2016) Asbestos exposure and histological subtype of malignant mesothelioma. *Occupational and Environmental Medicine*, 73(11), pp.749-752.

Geltner, C., Errhalt, P., Baumgartner, B., Ambrosch, G., Machan, B., Eckmayr, J., Klikovits, T., Hoda, M.A., Popper, H. and Klepetko, W. (2016) Management of malignant pleural mesothelioma–part 1: Epidemiology, diagnosis, and staging. *Wiener Klinische Wochenschrift*, 128(17-18), pp.611-617.

Gibson, B. (2019) Everite's last bag of asbestos. Personal discussion, 15 October 2019. (Trustee: Kgalagadi Relief Trust. Tel. +27 (011) 482 1000)

Gilham, C., Rake, C., Burdett, G., Nicholson, A.G., Davison, L., Franchini, A., Carpenter, J., Hodgson, J., Darnton, A. and Peto, J. (2016) Pleural mesothelioma and lung cancer risks in relation to occupational history and asbestos lung burden. *Occupational and Environmental Medicine*, 73(5), pp.290-299.

Haber, S.E. and Haber, J.M. (2011) Malignant mesothelioma: a clinical study of 238 cases. *Industrial Health*, 49(2), pp.166-172.

Goldberg, M. and Luce, D. (2009) The health impact of nonoccupational exposure to asbestos: what do we know?. *European Journal of Cancer Prevention*, 18(6), p.489.

Harington, J.S. and McGlashan, N.D. (1998) South African asbestos: production, exports, and destinations, 1959–1993. *American Journal of Industrial Medicine*, 33(4), pp.321-326.

Hart, H.P. (1988) Asbestos in South Africa. *Journal of the Southern African Institute of Mining and Metallurgy*, 88(6), pp.185-198.

Hiroshima, K., Murai, Y., Suzuki, Y., Goldstein, B. and Webster, I. (1993) Characterization of asbestos fibers in lungs and mesotheliomatous tissues of baboons following long‐term inhalation. *American Journal of Industrial Medicine*, 23(6), pp.883-901.

Husain, A.N., Colby, T.V., Ordóñez, N.G., Allen, T.C., Attanoos, R.L., Beasley, M.B., Butnor, K.J., Chirieac, L.R., Churg, A.M., Dacic, S. and Galateau-Sallé, F. (2017) Guidelines for pathologic diagnosis of malignant mesothelioma 2017 update of the consensus statement from the International Mesothelioma Interest Group. *Archives of Pathology & Laboratory Medicine*, 142(1), pp.89-108.

Kazan-Allen, L. (2019) Current asbestos bans. International Ban Asbestos Secretariat, viewed 17 August 2019, < http://ibasecretariat.org/alpha_ban_list.php>

Kielkowski, D., Nelson, G., Bello, B., Kgalamono, S. and Phillips, J.I. (2011) Trends in mesothelioma mortality rates in South Africa: 1995–2007. *Occupational and Environmental Medicine*, 68(7), pp.547-549.

Klebe, S., Brownlee, N.A., Mahar, A., Burchette, J.L., Sporn, T.A., Vollmer, R.T. and Roggli, V.L. (2010) Sarcomatoid mesothelioma: a clinical–pathologic correlation of 326 cases. *Modern Pathology*, 23(3), p.470.

Kraynie, A., de Ridder, G.G., Sporn, T.A., Pavlisko, E.N. and Roggli, V.L. (2016) Malignant mesothelioma not related to asbestos exposure: Analytical scanning electron microscopic analysis of 83 cases and comparison with 442 asbestos-related cases. *Ultrastructural Pathology*, 40(3), pp.142-146.

Landrigan, P.J., Nicholson, W.J., Suzuki, Y. and LaDou, J. (1999). The hazards of chrysotile asbestos: a critical review. *Industrial Health*, 37(3), pp.271-280.

Lanphear, B.P., and Buncher, C.R. (1992) Latent period for malignant mesothelioma of occupational origin. *Journal of Occupational and Environmental Medicine*, 34(7), pp.718-721.

Lemen, R.A. (2006) Epidemiology of asbestos-related diseases and the knowledge that led to what is known today. *Asbestos, Risk Assessment, Epidemiology, and Health Effects*, pp.201-308.

Liddell, F.D.K. (1997) Magic, menace, myth and malice. *Annals of Occupational Hygiene*. 41(1):3–12.

Liebenberg-Weyers, D. (2010) A multidisciplinary approach for the assessment of rehabilitation at asbestos mines in South Africa (Doctoral dissertation, North-West University).

Liebenberg, D., Claassens, S. and Van Rensburg, L. (2012) A multidisciplinary approach for the assessment of rehabilitation at asbestos mines in South Africa. *Environmental Earth Sciences*, 67(4), pp.1237-1244.

Maiphetlho, L. and Ehrlich, R. (2010) Claims experience of former gold miners with silicosis–A clinic series. *Occupational Health Southern Africa*, 16(2), pp.10-16.

Marinaccio, A., Binazzi, A., Bonafede, M., Corfiati, M., Di Marzio, D., Scarselli, A., Verardo, M., Mirabelli, D., Gennaro, V., Mensi, C. and Schallemberg, G. (2015) Malignant mesothelioma due to non-occupational asbestos exposure from the Italian national surveillance system (ReNaM): epidemiology and public health issues. *Occupational and Environmental Medicine*, 72(9), pp.648-655.

Martin, L. (2001) Asbestos lung disease: a primer for patients, physicians, and lawyers. *Journal of Controversial Medical Claims*, 8(4), pp.15-27.

Martinez-Alier, J. (2001) Environmental Conflicts,Environmental Justice and Valuation. *Just sustainabilities: Development in an Unequal World.* MIT press.

McCulloch, J. (2003) Women mining asbestos in South Africa, 1893-1980. *Journal of Southern African Studies*, 29(2), pp.413-432.

Meintjes, S., Hermanus, M., Scholes, M., Reichart, M. (2008) The Future of Penge Prospects for People and the Environment-Project Report and Guidelines-For the Asbestos Relief Trust: Centre for Sustainability in Mining and Industry (CSMI).

Milne, S.J., Nelson, G., Murray, J., Davies, J.C.A. and Phillips, J.I. (2013) A South African database of samples analysed for the presence of asbestos. *Occupational Health Southern Africa*, 19(6), pp.14-21.

Mthombeni, J. (2017) Factors influencing autopsy consent for South African miners, Masters dissertation, University of the Witwatersrand, Johannesburg.

Murray, J. and Nelson, G. (2008) Health effects of amosite mining and milling in South Africa. *Regulatory Toxicology and Pharmacology*, 52(1), pp.S75-S81.

Murray, J., Ndlovu, N., Nelson, G. (2015) Mesothelioma in South African miners 1975-2013. *European Respiratory Journal.* 46: PA1164

Muteba, K.M. (2018) Mesothelioma incidence and mortality in South Africa from 2003 to 2013, Masters dissertation, University of the Witwatersrand, Johannesburg.

Mutsaers, S.E., Prele, C.M., Brody, A.R. and Idell, S. (2004) Pathogenesis of pleural fibrosis. *Respirology*, 9(4), pp.428-440.

Ndlovu, N., teWater Naude, J. and Murray, J. (2013) Compensation for environmental asbestos-related diseases in South Africa: a neglected issue. *Global Health Action*, 6(1), p.19410.

Ndlovu, N., Rees, D., Murray, J., Vorajee, N. and Richards, G. (2017) Asbestos-related diseases in mineworkers: a clinicopathological study. *ERJ Open Research,* 3(3), pp.00022-2017.

Nelson, G., teWater Naude, J. (2016) Epidemiology of Malignant Pleural Mesothelioma in Africa. *Malignant Pleural Mesothelioma: Present Status and Future Directions.* 95(1), pp. 95-113

Neragi-Miandoab, S., Richards, W.G. and Sugarbaker, D.J. (2008) Morbidity, mortality, mean survival, and the impact of histology on survival after pleurectomy in 64 patients with malignant pleural mesothelioma. *International Journal of Surgery*, 6(4), pp.293-297.

Nolan, R.P., Ross, M., Nord, G.L., Raskina, M., Phillips, J.I., Murray, J. and Gibbs, G.W. (2006). Asbestos fiber-type and mesothelioma risk in the Republic of South Africa. *Clay Science*, 12(Supplement2), pp.223-227.

O'Reilly, K.M., Mclaughlin, A.M., Beckett, W.S. and Sime, P.J. (2007) Asbestos-related lung disease. *American Family Physician*, 75(5), pp.683-688.

Orenstein, M.R. and Schenker, M.B. (2000). Environmental asbestos exposure and mesothelioma. *Current Opinion in Pulmonary Medicine*, 6(4), pp.371-377.

Pérez-Guzmán, C., Barrera-Rodríguez, R. and Portilla-Segura, J. (2016) Malignant pleural mesothelioma in a 17-year old boy: A case report and literature review. *Respiratory Medicine Case Reports*, 17, pp.57-60.

Phillips, J.I. and Murray, J. (2010) Malignant mesothelioma in a patient with anthophyllite asbestos fibres in the lungs. *Annals of Occupational Hygiene*, 54(4), pp.412-416.

Phillips, J.I., Rees, D., Murray, J. and Davies, J.C. (2012) Mineralogy and malignant mesothelioma: The South African experience. *Malignant Mesothelioma*. Croatia: InTech, pp.1-30.

Phillips, J.I., Rees, D. and Swanepoel, A.J. (2016) Asbestos remains troublesome in South Africa after the ban: issues in occupational health. *Occupational Health Southern Africa*, 22(4), pp.20-23.

Rees, D., Myers, J.E., Goodman, K., Fourie, E., Blignaut, C., Chapman, R. and Bachmann, M.O. (1999) Case‐control study of mesothelioma in South Africa. *American Journal of Industrial Medicine*, 35(3), pp.213-222.

Rees, D., Phillips, J.I., Garton, E. and Pooley, F.D. (2001) Asbestos lung fibre concentrations in South African chrysotile mine workers. *The Annals of Occupational Hygiene*, 45(6), pp.473-477.

Republic of South Africa. Department of Health. (1973) Occupational Diseases in Mines and Works Act 78. Government Gazette no. 3970.

Republic of South Africa. Department of Labour. (2001) Occupational Health and Safety Act No.85; Asbestos Regulations, 2001. Government Notice. No. R:155.

Republic of South Africa. Department of Environmental Affairs and Tourism. (2008) Regulations for the prohibition of the use, manufacturing, import and export of asbestos and asbestos containing materials. Government Notice no. R341, Gazette No. 30904.

Rice, C. and Heineman, E.F. (2003) An asbestos job exposure matrix to characterize fiber type, length, and relative exposure intensity. *Applied Occupational and Environmental Hygiene*, 18(7), pp.506-512.

Røe, O.D. and Stella, G.M. (2017) Malignant Pleural Mesothelioma: History, Controversy, and Future of a Man-Made Epidemic. *European Respiratory Society*, 24(1), pp. 115-131.

Roggli V.L. (1990) Human Disease Consequences of Fiber Exposures: A Review of Human Lung Pathology and Fiber Burden Data. *Environmental Health Perspectives,* 88, pp. 295-303.

Roggli, V.L., Sharma, A., Butnor, K.J., Sporn, T. and Vollmer, R.T. (2002) Malignant mesothelioma and occupational exposure to asbestos: a clinicopathological correlation of 1445 cases. *Ultrastructural Pathology*, 26(2), pp.55-65.

Roggli, V.L., Gibbs, A.R., Attanoos, R., Churg, A., Popper, H., Cagle, P., Corrin, B., Franks, T.J., Galateau-Salle, F., Galvin, J. and Hasleton, P.S. (2010) Pathology of asbestosis—an update of the diagnostic criteria: report of the asbestosis committee of the college of american pathologists and pulmonary pathology society. *Archives of Pathology & Laboratory Medicine*, 134(3), pp.462-480.

Roggli, V.L. (2015) The So-called Short-Fiber Controversy; Literature Review and Critical Analysis. *Archives of Pathology & Laboratory Medicine. Vol 139:* 1052-1057.

Rudd, R.M. (2010) Malignant mesothelioma. *British Medical Bulletin*, 93(1).

Stanton, M.F., Layard, M., Tegeris, A., Miller, E., May, M., Morgan, E. and Smith, A. (1981) Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. *Journal of the National Cancer Institute*, 67(5), pp.965-975.

Suzuki, Y., Yuen, S.R. and Ashley, R. (2005) Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence. *International Journal of Hygiene And Environmental Health*, 208(3), pp.201-210.

Tertemiz, K.C., Alpaydin, A.O., Gurel, D., Savas, R., Gulcu, A. and Akkoclu, A. (2014) Multiple distant metastases in a case of malignant pleural mesothelioma. *Respiratory Medicine Case Reports*, 13, pp.16-18.

teWater Naude, J.M. (2014a) The Story of the Asbestos Relief Trust–Part 1. *Occupational Health Southern Africa*, 20(1), pp. 12–14.

teWater Naude, J.M. (2014b) The Story of the Asbestos Relief Trust–Part 2. *Occupational Health Southern Africa,* 20(2):18–19.

The National Economic Development and Labour Council. The socio-economic impact of phasing out asbestos in South Africa. Appendices to the final report. (2002) Available from: http://new.nedlac.org.za/wp-content/uploads/2014/10/Asbestos_ November2002.pdf (accessed 27 Feb 2018).

Thomas, A., Chen, Y., Yu, T., Gill, A. and Prasad, V. (2015) Distinctive clinical characteristics of malignant mesothelioma in young patients. *Oncotarget*, 6(18), p.16766.

Tilkes, F. and Beck, E.G. (1980) Comparison of length-dependent cytotoxicity of inhalable asbestos and man-made mineral fibres. *IARC Scientific Publications*, (30), pp.475-483.

Travis, W.D., Brambilla, E., Burke, A., Marx, A. and Nicholson, A.G. eds. (2015) *WHO Classification Of Tumours Of The Lung, Pleura, Thymus and Heart*. International Agency for Research on Cancer.

Van Rensburg, L., Claassens, S., Bezuidenhout, J.J. and van Rensburg, P.J. (2009) Rehabilitation of asbestos mining waste: a Rehabilitation Prioritisation Index (RPI) for South Africa. *Environmental Geology*, 57(2), pp.267-273.

van Zyl, P.A. (2017) A history of asbestos mining in South Africa. Parktown, Johannesburg: Asbestos Relief Trust.

Vimercati, L., Cavone, D., Caputi, A., Delfino, M.C., De Maria, L., Ferri, G.M. and Serio, G. 2019. Malignant mesothelioma in construction workers: the Apulia regional mesothelioma register, Southern Italy. *BMC research notes*, 12(1), p.636.

Virta, R.L. (2005) Mineral commodity profiles: Asbestos. USGS Circular 1255-KK. *US Geological Survey (USGS)*: Reston, Virginia, USA, p.56.

Virta, R.L. (2006) Worldwide asbestos supply and consumption trends from 1900 through 2003, Circular 1298. *Reston (VA): US Geological Survey*.

Vorster, T., Kgokong, N. and Phillips, J.I. (2018) Exploring the South African legacy of asbestos using routinely collected data. *Occupational Health Southern Africa*, 24(5), pp.135-139.

Wagner, J.C., Sleggs, C.A. and Marchand, P. (1960) Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Occupational and Environmental Medicine*, 17(4), pp.260-271.

Webster, I. (1973). Asbestos and malignancy. *South African Medical Journal*, 47(5), pp.165-71.

Wenk, H.R., P.E. Champness., J.M. Christie., J.M. Cowley., A.H. Heuer., G. Thomas., N.J. Tighe. (1976) *Electron Microscopy in Mineralogy*, Springer-Verlag Berlin Heidelberg New York, pg 1-564

White, N., Nelson, G. and Murray, J. (2008) South African experience with asbestos-related environmental mesothelioma: Is asbestos fiber type important?. *Regulatory Toxicology and Pharmacology*, 52(1), pp.S92-S96.

Wilk, E., Krówczyńska, M., Pabjanek, P. and Mędrzycki, P. (2017) Estimation of the amount of asbestos-cement roofing in Poland. *Waste Management & Research*, 35(5), pp.491-499.

Wolff, H., Vehmas, T., Oksa, P., Rantanen, J. and Vainio, H. (2015) Asbestos, asbestosis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations. *Scandinavian Journal of Work, Environment & Health,* pp.5-15.

Gibbs, G., Pigg, B.J., Nicholson, W.J., Morgan, A., Lippmann, M., Davis, J.M.G., Mossman, B.T., McDonald, J.C., Landrigan, P.J., Schreier, H. (1998) Environmental health criteria 203: Chrysotile asbestos. *International Programme On Chemical Safety*. Geneva, Switzerland: Available from: https://www.who.int/ipcs/assessment/public_health/asbestos_recent/en/

APPENDICES

APPENDIX A – Extraction of Asbestos Fibres from Lung Tissue

A Pathologist examines the lungs of deceased individuals and sends representative samples of all lobes of the diseased lungs in formalin to the EM Unit. The sample is recorded in a register with a unique number and job cards are created which includes the SEM and LM counting sheets, the SEM and LM findings sheets, and supporting pictures for the SEM analysis.

A competent laboratory staff member (technician, technologist or scientist) prepares the sample in terms of three weights that are required to calculate the number of asbestos fibres in the whole lung by only using a smaller representative part of the lung. The three weights are digested reference weight, the wet reference tissue weight and the dry reference tissue weight (all nett weights).

To obtain the digested reference weight, a representative sample (6 – 10 grams) is placed in a foil basket and the nett weight of the sample is calculated. After this, the sample is digested with potassium hydroxide, centrifuged and the supernatant is discarded. The remaining fluid is placed in a crucible and ashed in a furnace. Hydrochloric acid is used to help remove the remains from the crucible. The fluid sample is placed in a falcon and diluted with distilled water. Thereafter, the sample fluid is pushed through a gold-coated filter (0,2 µm) and left to dry. This sample is prepared for SEM. The sample fluid is also pushed through another filter (0,45 µm) and left to dry. This sample is for LM analysis. The 0,2 µm filter is then placed on a sticky carbon planchet and coated with gold. The 0,45 µm filter is placed on a glass slide, cleared with acetone and coverslipped.

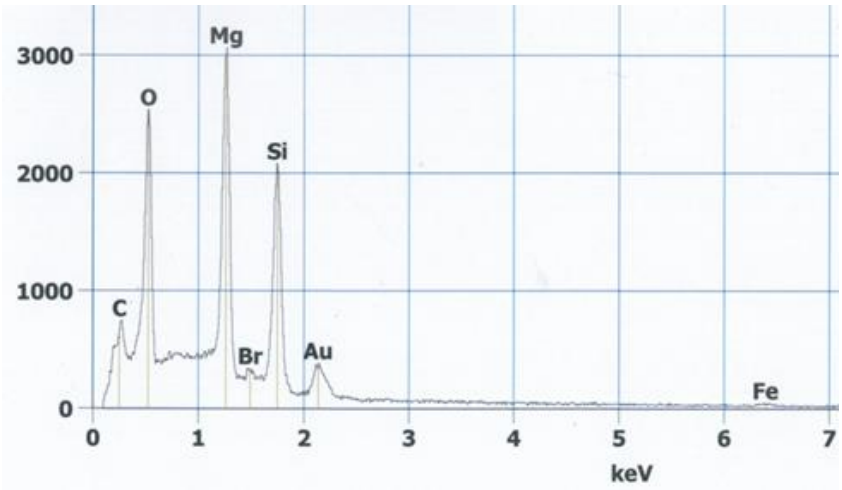
The wet reference weight is obtained by placing a representative lung sample (6 – 10 grams) in a foil basket and calculating the nett weight. This

sample foil basket is placed in an incubator for the lung to dry. The dry reference weights are then also calculated by weighing the lung once it is dry.

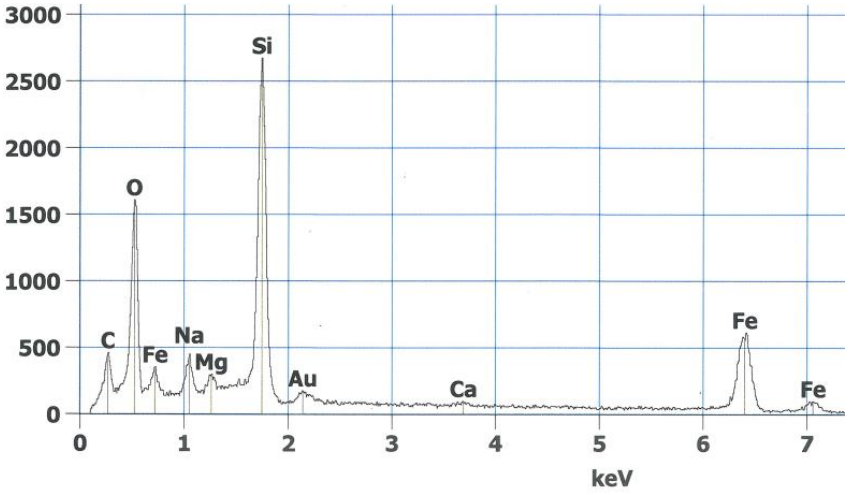
The number of fibres on the 0,2 µm filter is counted using an SEM at 2000 times magnification and counting 200 fields on each filter. This method is in accordance with the Asbestos International Association's recommended technical method (RTM-2) for counting asbestos fibres. A fibre is regarded as anything with a ratio of 3:1. All fibres are analysed using electron dispersive spectroscopy (EDS) to determine if they are asbestos fibres.

The different types of asbestos have different chemical compositions and the EDS can determine this. Chrysotile is composed of magnesium and silica and this will display as peaks on the EDS graph in an equal ratio with the magnesium peak being slightly higher than that of the silica. Crocidolite will display peaks in different ratios with silica being the highest followed by sodium, iron and magnesium. Amosite displays peaks of silica, magnesium and iron. In this way, asbestos fibre types can be determined. Examples of EDS graphs are displayed below.

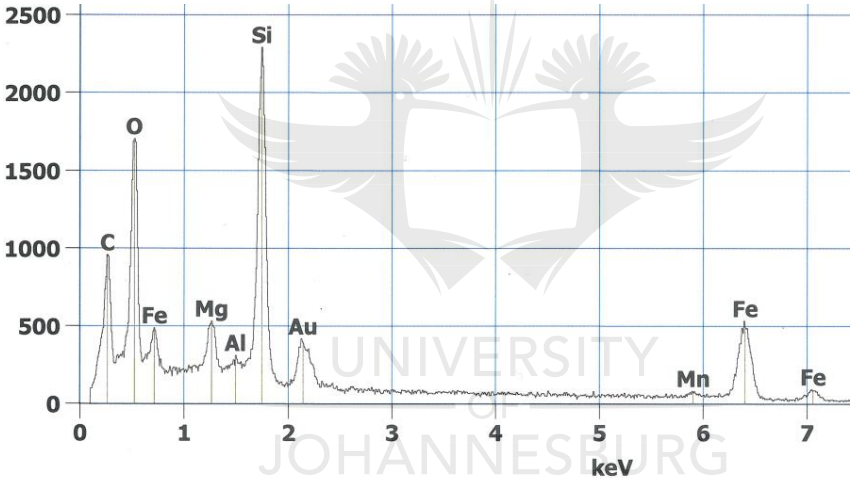
Chrysotile



Crocidolite



Amosite



The 0,45 µm filter is counted by using a phase microscope. Counting is done with a 40 x objective is in place and the 12.5 x eyepiece at a total of 500 x magnification and counting 100 fields. This method is in accordance with the Asbestos International Association's recommended technical method (RTM-1) for counting asbestos fibres. This is only done as a quality assurance check to ensure the LM and SEM counts match. If discrepancies are picked up, such as fibres were seen on under the LM microscope and not under SEM, the sample will be pushed through the two filters again and reanalysed. Fibre and asbestos body counts are reported as the number of fibres per gram of dried lung tissue.

Data obtained from the SEM and LM analysis are entered into the EM database. The database was set up to automatically calculate the wet and dry fibre and asbestos body concentrations. The calculation for the total asbestos fibres per gram of dry lung tissue using SEM is as follow:
Asbestos fibres/g tissue = (((total number of fibres counted/(0.0030672 x 200)) x 380.13) / wet tissue weight) x (wet tissue weight/dry tissue weight) x 50 ml.

Identifying asbestos is a skilled technique and requires a trained analyst. The NIOH participates in an External Quality assessment programme the Asbestos in Materials Scheme (AIMS) from the Health and Safety Laboratory (HSL) from the United Kingdom that enables them to correctly identify the different types of asbestos.

APPENDIX B – Variables used in each data set

Pathaut database variables	Pathaut variable description	ART/KRT database variables	ART/KRT variable description	EM lung database variables	EM variable description
pnum	Pathology number	idnum2	Identification number	pno	Pathology number
idnum	Identification number	status	Claim outcome	Semtotdry	Dry concentration of asbestos fibres counted
year	Year of death			Asbboddry	Dry concentration of asbestos bodies counted
Codpath 1-3	CoD by Pathology			Am5dry	Dry concentration of amosite fibres counted
pathdesc	CoD description			Am 5_10dry	Dry concentration of amosite fibres counted
popcode	Race			Am10dry	Dry concentration of amosite fibres counted
age	Age at death			Croc5dry	Dry concentration of crocidolite fibres counted
O/E	Occupational / environmental			Croc 5_10dry	Dry concentration of crocidolite fibres counted
exptype 1-4	From commodity most exposed to least			Croc10dry	Dry concentration of crocidolite fibres counted
expyear 1-4	Exposure years			Chry5dry	Dry concentration of chrysotile fibres counted
yearstar	Year mining occupation started			Chry 5_10dry	Dry concentration of chrysotile fibres counted
yearend	Year mining occupation ended			Chry10dry	Dry concentration of chrysotile fibres counted
lastmine	Last mine case worked			Oth5dry	Dry concentration of non-asbestos fibres counted
milmeso	Mesothelioma			Oth 5_10dry	Dry concentration of non-asbestos fibres counted
milmesep	Epithelioid subtype			Oth10dry	Dry concentration of non-asbestos fibres counted
milmesar	Sarcomatous subtype			Othspec	Description of non-asbestos fibres
Meso Type	Histological subtype				
gender	Sex				
province	SA province				

APPENDIX C – NIOH Consent Form for a Post-Mortem Examination

NATIONAL INSTITUTE FOR OCCUPATIONAL HEALTH

25 Hospital Street, Constitution Hill, Johannesburg
P O Box 4788, Johannesburg 2000, South Africa
Tel: 011 712 6519 or 011 712 6444
Fax: 011 712 6450

CONSENT FOR A POST-MORTEM EXAMINATION

I _____

***myself or the spouse / major child / parent / guardian / major brother / major sister (*Delete whichever is not applicable)**

Name of miner / deceased miner _____

ID number _____ **Age** _____ **Sex** _____

hereby consent to a post-mortem examination and the removal of such tissues as may be considered necessary for the purpose of the requirements of the Occupational Diseases in the Mines and Works Act (No. 78 of 1973) and for diagnostic, medical education, research and scientific purposes.

Signature _____

Witness 1 _____

Witness 2 _____

Place _____

Date _____

APPENDIX D – Research Ethics Approval

UNIVERSITY OF JOHANNESBURG

FACULTY OF HEALTH SCIENCES

RESEARCH ETHICS COMMITTEE
NHREC Registration no: REC-241112-035

3 November 2017

TO WHOM IT MAY CONCERN:

Student: VORSTER, GS
Student Number: 216057283

TITLE OF RESEARCH PROPOSAL: Malignant Pleural Mesothelioma Retrospective Analysis of the Demographic, Asbestos Lung Fibre Burden and Pathology

DEPARTMENT OR PROGRAMME: BIOMEDICAL TECHNOLOGY

SUPERVISOR: Ms J Mthombeni **CO-SUPERVISOR:** Prof JI Phillips

The Faculty Research Ethics Committee has scrutinised your research proposal and confirm that it complies with the approved ethical standards of the Faculty of Health Sciences, University of Johannesburg.

The proposal has been awarded a Code 2A – Approved with suggestions without re-submission. The attached recommendations were made by the Committee which will add value to your proposal.

Please make these amendments to the satisfaction of your supervisor/s and submit a corrected copy of the proposal to the Faculty Research Administrator after which your clearance number will be issued.

The REC would like to extend their best wishes to you with your postgraduate studies.

Yours sincerely,

Prof C Stein

Chair : Faculty of Health Sciences REC

APPENDIX E – Ethics Clearance from The Ethics Committee at Wits to Study Data Collected in The PATHAUT Database

R14/49 Prof Jill Murray

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170879

NAME: (Principal Investigator)	Prof Jill Murray
DEPARTMENT:	Public Health National Health Laboratory Services
PROJECT TITLE:	Retrospective Review of Rountinely Collected Autopsy Data for Reporting on Disease Prevalences, time trends and Associated Risk Factors
DATE CONSIDERED:	21/05/2004 (Initial Approval 21/08/2017)
DECISION:	Approved unconditionally
CONDITIONS:	Renewal for 5 Years Valid for the Period 01 September 2017 - 30 September 2022 (Previously M040421)
SUPERVISOR:	
APPROVED BY:	Professor CB. Penny Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	12/09/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review September and will therefore be due in the month of September each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

APPENDIX F – Turnitin Report

Masters Dissertation

ORIGINALITY REPORT

16%	10%	9%	12%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Hellenic Open University Student Paper	1%
2	ikee.lib.auth.gr Internet Source	1%
3	Submitted to Curtin University of Technology Student Paper	1%
4	Submitted to University of Johannsburg Student Paper	1%
5	Submitted to University of Witwatersrand Student Paper	<1%
6	malignant2mesothelioma.blogspot.com Internet Source	<1%
7	www.esp.org Internet Source	<1%
8	digitalcommons.liberty.edu Internet Source	<1%
9	wiredspace.wits.ac.za Internet Source	<1%

Submission date: 12-Dec-2019 12:18PM (UTC+0200)
Submission ID: 1233034596
File name: Trudie_Vorster_MTech_Thesis_Final.pdf (2.05M)
Word count: 16325
Character count: 91388
