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TESI DI DOTTORATO DI RICERCA:

On the use of Asbestos in Central Asian countries: findings in post-mortem lung samples, characterization of asbestos-containing materials, environmental and occupational exposure. Case studies in Kyrgyzstan.

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ABSTRACT

ENG: Asbestos is classified as a human carcinogen and banned across Europe and many parts of the world, including Italy. However, this is not the case in Central Asia. In Kyrgyzstan, asbestos-containing products are still manufactured by two companies in three locations, producing over 5 million units annually and widely used across the country and beyond. One of the aggressive consequences of asbestos-related diseases is malignant mesothelioma. The estimated age-standardized incidence of mesothelioma (both sexes, all ages) in Kyrgyzstan was 0,06 in 2020, 2nd lowest index among other Central Asian (CA) countries (Tajikistan 0,02; Uzbekistan 0,11; Turkmenistan 0,16; Kazakhstan 0,23, while in Italy it is 1.0. Several hypotheses can explain this data, including the type of asbestos used, underreporting, low environmental exposure, and “controlled use” of chrysotile in manufacturing.

This thesis aims to develop insights into asbestos production in Central Asia by analysing the chemical composition of asbestos samples taken in Kyrgyzstan; measuring the environmental exposure present in Urban areas through air sampling; and measuring the occupational exposure in one of these industries through the same method. This thesis also presents an analysis of lung samples taken from the general population of Kyrgyzstan in non-occupational settings, further aiming to examine the health risks of such exposure.

Overall, this thesis intends to fill the gap in academic knowledge concerning the use and risks of asbestos in Central Asia through the example of Kyrgyzstan and to contribute more generally to the knowledge concerning the dangers of chrysotile asbestos exposure.

ITA: L'amianto è classificato come cancerogeno per l'uomo e vietato in tutta Europa e in molte parti del mondo, compresa l'Italia. Tuttavia, questo non è il caso dell'Asia centrale. In Kirghizistan, i prodotti contenenti amianto sono ancora fabbricati da due aziende in tre località, con una produzione di oltre 5 milioni di unità all'anno, ampiamente utilizzate in tutto il Paese e oltre. Una delle conseguenze aggressive delle malattie legate all'amianto è il mesotelioma maligno. L'incidenza stimata di mesotelioma standardizzata per età (entrambi i sessi, tutte le età) in Kirghizistan è stata dello 0,06 nel 2020, il secondo indice più basso tra gli altri Paesi dell'Asia Centrale (CA) (Tagikistan 0,02; Uzbekistan 0,11; Turkmenistan 0,16; Kazakistan 0,23, mentre in Italia è dell'1,0. Diverse ipotesi possono spiegare questi dati, tra cui il tipo di amianto utilizzato, l'underreporting, la bassa esposizione ambientale e l'"uso controllato" del crisotilo nella produzione.

Questa tesi mira a sviluppare approfondimenti sulla produzione di amianto in Asia centrale analizzando la composizione chimica di campioni di amianto prelevati in Kirghizistan, misurando l'esposizione ambientale presente nelle aree urbane attraverso il campionamento dell'aria e

misurando l'esposizione professionale in una di queste industrie attraverso lo stesso metodo. Questa tesi presenta anche un'analisi di campioni polmonari prelevati dalla popolazione generale del Kirghizistan in contesti non professionali, con l'obiettivo di esaminare i rischi per la salute di tale esposizione.

Nel complesso, questa tesi intende colmare la lacuna nelle conoscenze accademiche relative all'uso e ai rischi dell'amianto in Asia centrale attraverso l'esempio del Kirghizistan e contribuire più in generale alla conoscenza dei pericoli dell'esposizione all'amianto crisotilo.

PROBLEM STATEMENT

Asbestos is banned in many countries, including Italy (from 1992), however, all Central Asian (CA) countries are still mining and manufacturing asbestos-containing products. Kyrgyzstan does not mine asbestos, but asbestos-contained products are manufactured by two enterprises and a branch of one of them and are widely used in the country. One of the factories manufacturing asbestos-containing products was founded in 1964, and another in 2013. The employees of the companies are more than 300 workers. The scale of producing and using asbestos-containing products industries is over 5 million units per year overall: slates, pipes, fibre-cement plates for facade cladding and interior decoration of industrial and office buildings.

For producing asbestos-containing commodities in Kyrgyzstan, the producers claim that only chrysotile asbestos is used, coming from a large asbestos mine operating in Kazakhstan (Zhetygarinsky chrysotile deposit, Kostanay region) and Russian Federation (Ural deposits). In these countries is also an association ("Chrysotile Association") promoting the use of chrysotile declaring that the use can be carried out without a significant health risk because of the toxic properties of this type of asbestos.

One of the reasons for the use of chrysotile asbestos in the country is its main properties of asbestos fibres that can be exploited in several industrial applications, such as thermal, electrical, and sound insulation; nonflammability; matrix reinforcement (cement, plastic, and resins); adsorption capacity (filtration, liquid sterilization); wear and friction properties (friction materials); and chemical inertia (except in acids) [1].

Asbestos is certainly the etiological factor of asbestos-related diseases (ARD) such as malignant mesothelioma, lung cancer, other cancers, asbestosis, and pleural plaques. Based on existing data, amphiboles are supposed to be the most toxic forms, associated with an increased risk of malignant mesothelioma (MM), whilst chrysotile exposure is mainly associated with lung cancer for high-dose exposures.

The estimated age-standardized incidence of mesothelioma (both sexes, all ages) in Kyrgyzstan was 0,06 in 2020, 2nd lowest index among other CA countries (Tajikistan 0,02; Uzbekistan 0,11; Turkmenistan 0,16; Kazakhstan 0,23, while in Italy it is 1.0 [2].

Several hypotheses can explain this data: 1) the type of asbestos used in CA (only chrysotile?), 2) underreporting, 3) the level of environmental exposure is low (what is the real level?), and 4) the "controlled use" of chrysotile in the manufacturing process.

Nevertheless, one hypothesis might not exclude the other; therefore, it is probably rational to use both.

RESEARCH OBJECTIVE AND CONTRIBUTION OF THE THESIS

This study aims to investigate the type of asbestos used in Kyrgyzstan, as well as the levels of non-occupational and occupational exposure to asbestos among the general population and workers in factories.

The objectives:

1. To perform systematic research of data regarding ARDs in CA;
2. To identify which type of asbestos is present in the Kyrgyzstan;
3. To estimate the levels of exposure to asbestos in the general population in one of the countries of CA;
4. To assess the level of exposure to asbestos workers in the industry producing asbestos-containing products;
5. To measure the concentration of asbestos fibres in autopsy samples of lung tissue from people of the general population of Kyrgyzstan and to determine the type of fibres present in the lungs.

Materials and methods:

1. Searched and systemized 105 articles on the topic from digital databases in Russian and English languages, analyzed with Prisma tool and manually, the data were extracted from 18 relevant articles;
2. Collected samples of raw asbestos materials and asbestos-containing products were examined by an X-ray powder diffractometer (XRPD), a scanning electron microscope combined with energy-dispersive spectrometry (SEM–EDS), a transmission electron microscope combined with energy-dispersive spectrometry (TEM–EDS) at the University of Turin, Italy;
3. Collected environmental 4 air samples in Bishkek city and 3 in Kant town. The sampling method was derived from ISO 14966:2019 and ISO 13794:2019 standards. Airborne asbestos fibres were collected on polycarbonate filters with a sampler (SKC AirChek XR 5000; flow: 2 L·min-1). The determination of the numerical concentration of airborne asbestos fibres in the atmosphere was carried out following the indications of the Italian Ministerial Decree 06/09/1994 (Annex 2B) and the ISO 13794:2019 method. The analyses of the collected samples were carried out using a scanning electron microscope – SEM-EDS with identification of chemical composition at the University of Insubria, Italy.

4. Collected 18 occupational air samples from an asbestos-producing factory in Kant town. Monitored workers (n = 18) were divided into three “Similar Exposure Groups” (SEGs; SEG-1: asbestos loading; SEG-2; asbestos-cement mixing; SEG-3: cutting of asbestos-cement sheets) according to EN 689 standard. Samples were collected through personal sampling, and subsequently examined by SEM-EDS for the compositional analysis of each fibre. The numerical concentration of airborne asbestos fibres was henceforward determined by dividing the number of fibres and the volume of sampled air. The analyses were conducted at the University of Insubria, Italy.
5. Collected 100 lung samples (formalin-fixed samples of the inferior lobe of the right lung) from the Pathology Department of Bishkek. For each deceased subject enrolled, collect medical and residential history. Lung samples collected during sanitary autopsies were ordered for whatever reason. the samples were analysed in SEM-EDS and TEM–EDS, according to the method proposed by Belluso, E. et al. [3], to determine the number of fibres, their dimensions and mineralogic type at the University of Pavia and the University of Turin.

CHAPTER 1.
Overall Introduction

Historical overview.

The term asbestos, in fact, refers to a family of six silicate minerals, containing silicone and oxygen bound in fibrous aggregates of long, thin crystals that readily separate [4]. It is divided further into two main kinds: chrysotile and amphibole. Throughout the course of the nineteenth and twentieth centuries, asbestos has transformed from one of the most ubiquitous and practicable building materials into an infamous health concern in the Western world. In many ways, asbestos is a wonder material; a fibre made of rock which is waterproof, fireproof, and stronger than steel [5], with a high tensile strength yet notable softness and flexibility [4]). It has been used in the production of everything from cloaks, tablecloths, and curtains to brake shoes, air filters, and ventilation systems [4]. When mixed with rubber, asbestos has been used to create durable steam engine components [4]. When mixed with cement, fire-resistant roofing tiles were developed [4]. The use of asbestos dates to around five thousand years ago in Cyprus, where evidence of cremation clothes, lamp wicks, and hats has been found [6]. Dioscorides, a later Greek physician describes an asbestos quarry at Mount Olympus on the island [7]. Additional archaeological studies conducted at the Lake Juojärvi Region of East Finland have demonstrated that around 4500 years ago the inhabitants knew how to strengthen earthenware pots and cooking utensils anthophyllite, an asbestos containing mineral [6]. The use of asbestos in strengthening ceramics which began in the stone age continued throughout the bronze and iron ages, spreading across Scandinavia and Russia [6]. The use of asbestos in Greece alone goes back over two thousand years and is documented by Herodotus in relation to the production of lamp wicks [6]. The mineral was well-known to the ancient Greeks, and is probably earliest referred to in the 3rd Century B.C. by Theophrastus, who writes:

> "There is also found in the mines of Scaptesylae a stone, in its external appearance somewhat resembling wood, on which, if oil be poured, it burns; but when the oil is burnt away, the burning of the stone ceases, as if it were in itself not liable to such accidents [8]."

The word itself stems from the Greek ασβεστος (asvestos): when used as a noun it refers to lime, quicklime, or unslaked lime and, as an adjective, it can mean inextinguishable or unquenchable [6]. References to asbestos are found throughout the Greek and Roman world, from the Greek geographer Strabo's refence to 'Karystian' stone obtained from quarries in the vicinity of Karystos to Plutarch's description of vestigial virgins lighting Rome's sacred fire with asbestos-wicked lamps [6]. A particularly informative account is provided in Pliny the Elder's first century manuscript *Natural History*, in which he describes its use in woven products, including easy to clean tablecloths, napkins, and funerary shrouds [7].

Over the following thousand years of human history, asbestos "continued to attract the attention of kings and chemists from Western Europe to China", and medieval alchemists even propagated the rumour that asbestos "grew as hair on fire-resistant salamanders", leading to the term *Salamandra* emerging as a new name for the stone [7]. This origin myth was later extended to lizard plumes and bird feathers as people began to forget its geological nature [7]. However, Marco Polo later corrected these wild fantasies upon returning from a Chinese asbestos mine in the late 13th century, demonstrating its formation as a rock [7]. Later, following the end of the medieval period in Europe, the newly-formed Royal Society in England, in 1660, demonstrated a renewed interest in the mineral, publishing eight reviews and letters on the subject over the proceeding forty years [7].

However, despite this rich history and the variegated considerations of asbestos across it, the general use of asbestos in international commerce dates only to the late nineteenth century [6]. Due to the need for insulation in steam technology, the reopening of asbestos deposits in Northern Italy, and the development of an international consortium of Italian and English entrepreneurs, the market for asbestos expanded rapidly during this period [6]. In the 1820s Giovanni Aldini, a prominent Italian Scientist, created the first successful modern commercial asbestos business, selling ready-to-wear fireproof apparel to urban firemen, garnering clients from Paris to Geneva [7]. Between 1860 and 1875, several new Italian companies formed and began to advance asbestos-related technology, fabricating asbestos into spun products, rope packing, and heat insulation board, and exhibits provided by these companies at the 1878 Paris Universal Exposition brought these products to international attention [6]. It was around this period that the use and development of asbestos products peaked: a New York building contractor named Henry Ward Johns developed flame-resistant tar paper for buildings, and mixtures of asbestos and cement became an established building material [7].

By the mid twentieth century, it had become a major component of a large variety of products that made use of its heat-resistant, fireproof, and other useful properties [9]. From the time of its first recorded use until 1900, asbestos production totalled only around 300,000 metric tons [6]. However, by the period 1931 to 1999, this had increased to 166 million tons [6]. Of this, by far the most common is chrysotile asbestos, which forms the bulk of global asbestos deposits. By 1939 the public perception of asbestos "could hardly have been more positive", with a display at the World's Fair the same year celebrating the mineral's service to humanity [7]. "A giant Asbestos Man greeted visitors to the company's pavilion and offered a thorough in-doctrination about the extraordinary traits of asbestos" [7]. Just prior to the outset of World War I, demand had almost exceeded the global supply [7]. The boom in construction that followed the cessation of the conflict began the final asbestos rush, as structural engineers valued its strength, durability, and fireproof

qualities, and the steel frames of high-rise buildings were sprayed with an asbestos-containing formula [7]. This final period led to some of the more bizarre uses of the mineral, including fireproof mailbags for the U.S. postal service, its use as a thread in heart surgery, and even toothpaste made with asbestos fibres [7].

Types of asbestos, their chemical composition, and physical properties.

Asbestos is classified into six fibrous silicate minerals, all of which have a crystalline structure, lengths of >5 μm, and aspect ratios of 3 or greater [10]. This crystalline structure is composed of strips or ribbons of linked polyhedral which join together in the formation of a three-dimensional crystal [11]. Many of these minerals are used to manufacture a wide variety of goods used by different populations worldwide and are conventionally divided into two distinct groups: serpentines and amphiboles [10]. Both of these groups are found in ophiolite complexes, rock formations that originate as oceanic crust and mantle and are generated by the sea-floor spreading at oceanic ridges or in marginal basins, causing an uplift that exposes the upper mantle [12].

Although the silicate tetrahedral form is the base of all asbestos fibres, asbestos has two main classes: firstly, the serpentines, of which chrysotile is by far the most common type; and secondly, the amphiboles, the main types of which are crocidolite, amosite, tremolite, anthophyllite, and actinolite [13]. Within these different types, the tetrahedral form can occur as double chains $(Si_4O_{11})^{-6}$ in amphiboles or in sheets $(Si_4O_{10})^{-4}$ in serpentine chrysotile [1]. Although they are commonly grouped, classified, and regulated together under the term ‘asbestos’, the serpentine and amphibole groups have geological forms distinct from one another and, more crucially, exhibit significant variations in crystalline structures and chemical compositions (Figure 1). Such variations translate to differences in fibre structure and dimension and result in varying biopersistence, thus causing significant differences in the overall potency of these disparate asbestos minerals in causing disease in humans [10]. The term ‘asbestos’ is therefore to be properly considered a commercial and legal term rather than a mineralogic one [10].

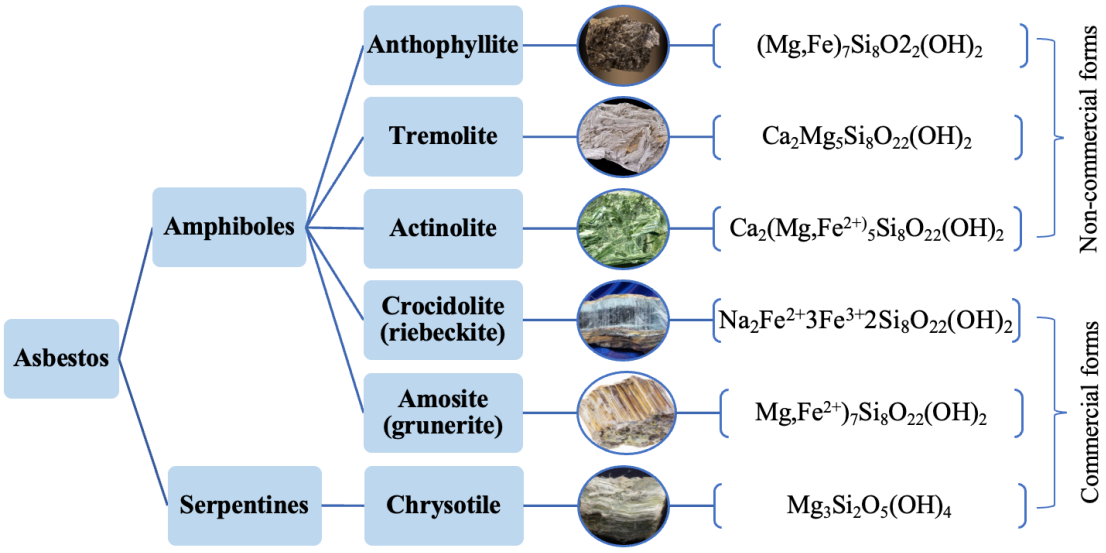

Figure 1. Classification of asbestos Forms. Source: modified from Sporn 2011 [10].

Serpentines: Chrysotile.

Chrysotile, lizardite, and antigorite are the three main mineral polymorphs within the serpentine mineral categorisation. Lizardite and antigorite are non-toxic and are often used decoratively: however, chrysotile is toxic, being the only asbestos subtype within the serpentine family. Out of all the serpentine minerals, only chrysotile is found in a fibrous form. An examination of the form of chrysotile in 1967 through circumferential lattice images observed a spiral or multi-spiral structure in the cross-section of a fibril [14]. Chrysotile is a phyllosilicate mineral, with the chemical formula $Mg_3Si_2O_5(OH)_4$ and contains around 13% water as a crystal [10]. The presence of other minerals admixed in the ore is the most likely reason for the inclusion of trace amounts of elements such as sodium, calcium, potassium, chromium, cobalt, nickel, iron, and manganese [15]. Chrysotile, also known as 'white' asbestos in its commercial use, has generally curly fibres, while the amphibole group exhibits generally straight fibres. Chrysotile fibres are very thin, with each fibre having a diameter of around 25 nanometers (0. 025 micrometres) [11]. They can be as short as a tiny fraction of a millimetre or as long as a few centimetres, but most of the fibres used are shorter than 1 cm [1]. Industrial chrysotile fibres are made up of a bunch of these tiny fibres that are usually between 0. 1 and 100 micrometres thick. Measure and shape are the foremost factors for characterizing the respirability of strands. For administrative purposes in asbestos-related working environments, fibres are generally characterised as having a perspective proportion of around 3:1, referring to the proportion of fibre length to fibre breadth [15]. Chrysotile fibres are present in low concentrations in the crustal environment, including air, water, ice caps, and soil. Both natural and human activities contribute to the aerosolization and distribution of fibres.

Anthropogenic sources of fibre exposure include dust from occupational activities such as ore recovery and processing, manufacturing, application, usage, and disposal [15].

Industrial applications of chrysotile fibres use a combination of the following properties: fibrous structure, high tensile strength, heat and corrosion resistance, low electrical conductivity, and high friction coefficient [1]. Therefore, chrysotile asbestos was predominantly used in the automobile, building and textile industries [16]. In these industries, chrysotile was considered safe when bound together with another product, such as cement. However, this has proved not to be the case.
The industrial uses of chrysotile asbestos were additionally informed by its physical properties. For example, when exposed to acids, the magnesium particles are broken down, leaving only the silica skeleton. It is thermally steady to around 550 °C, at which point it begins to dry out [15]. By 750 °C, total parchedness occurs, causing it to break down into forsterite (magnesium silicate), silica, and water [15].

Amphiboles: Anthophyllite, Amosite, Crocidolite, Tremolite, Actinolite.

In addition to chrysotile asbestos, there are five primary variants of amphibole asbestos, namely: 1) anthophyllite asbestos, which conforms to the ideal chemical formula $(Mg, Fe^{2+})_7 Si_8 O_{22} (OH)_2$, with a feasible substitution of 0 to 50 atom percent of Mg by Fe^{2+}; 2) tremolite asbestos, which ideally conforms to $Ca_2(Mg, Fe^{2+})_5Si_8O_{22}(OH)_2$, with a possible substitution of 0 to 10 atom percent of Mg by Fe^{2+}; and 3) actinolite asbestos, which has the formula $Ca_2 (Fe^{2+},Mg)_5 Si_8O_{22} (OH)_2$, with a likely substitution of 10 to 50 atom percent of Mg by Fe^{2+} [17]; amosite (brown asbestos, the commercial name of the cummingtonite/grunerite series with formula $(Mg,Fe^{2+})_7Si_8O_{22}(OH)_2$); crocidolite (blue asbestos, the commercial name of riebeckite, which has the formula is $Na_2(Fe^{2+},Mg)_3Fe^{3+}{}_2Si_8O_{22}(OH)_2$) [18].

Although not all amphibole minerals are considered asbestos and occur with morphological differences that confer a variability in flexibility and mechanical strength, they nonetheless possess the ideal geometric characteristics of asbestos, being uniformly > 5 μm in length and < 3 μm in width [18]. Typically, they have a rigid and parallel-sided structure, featuring highly diverse distributions of width-to-length ratios [18].

In general, amphibole asbestos fibres are tougher and more fragile compared to those of chrysotile. Moreover, they show better resistance to chemical attack combined with high filtration rates and outstanding hardness (4 to 6 on the Mohs scale). They can also be notably longer, measuring up to several inches [19]. However, the temperatures for the processes of dehydroxylation and recrystallization differ. Amphiboles contain less water (hydroxyl) and undergo dehydroxylation

between 400-600°C, depending on the type of amphibole, resulting in a weight loss of approximately 2% [1].

Of all amphiboles, only crocidolite and amosite had significant industrial applications [1]. Although not used commercially, tremolite has been found as a contaminant in other fibres or industrial minerals, such as chrysotile and talc [20]. Previously, chrysotile used to be blended with crocidolite or amosite to ensure a good modulus of rupture, as their fast-filtering and harsh fibres were preferred for products containing asbestos-cement [19]. Mostly, amosite asbestos could be found in ceiling tiles, fireproof products, and gaskets, whereas crocidolite asbestos was added to some cigarette filters and used in the insulation of houses.

The hazards of asbestos.

The harms of asbestos have been recognised since the late 19th century, with concerns about poor health and short life expectancies among enslaved people working in mines [21]. The term asbestosis was introduced in 1924 to identify the pneumoconiosis of subjects systematically and repeatedly exposed to the inhalation of asbestos dust. The first example of a causal association between lung cancer and asbestos is the case study by Lynch and Smith, who examined the pathological history, occupational history, and clinical course of a man from the south of the state who had worked 22 years in a cotton mill and 21 years in asbestos spinning [22]. Hueper confirmed the occupational nature of the disease in 1942, stating that asbestos workers with at least 20 years of experience have a 10-fold risk of developing lung cancer [23]. Wedler described the first correlations between asbestos exposure and the onset of mesothelioma in 1943[24]. However, Wagner et al. (1960) confirmed the hypothesis of a link between asbestos exposure and the development of mesothelioma [25].

Mesothelioma.

The incidence of mesothelioma is decreasing in the world [26]. The peak of incidence is expected for 2030–2039 [27]. Limited data from growing nations, particularly from the former Soviet Union, makes forecasting difficult [28]. However, ecological models have shown that past asbestos consumption has a high positive predictive value in estimating deaths from mesothelioma in both sexes [29]. A cross-sectional study by Zhai et al. [26]found that the global trend in mesothelioma diagnoses between 1990 and 2017 continually grows, with the most significant increase in Western Europe for patients over 70. The WHO Global Cancer Observatory reports an age-standardised incidence rate (ASIR) for mesothelioma close to zero, with only 13% of global deaths from asbestos-related diseases attributable to Asia (Figure 2). The use of asbestos in past

decades has influenced and is closely linked to today's rates of ARD, warning all countries of an imminent mesothelioma epidemic in the coming years [30].

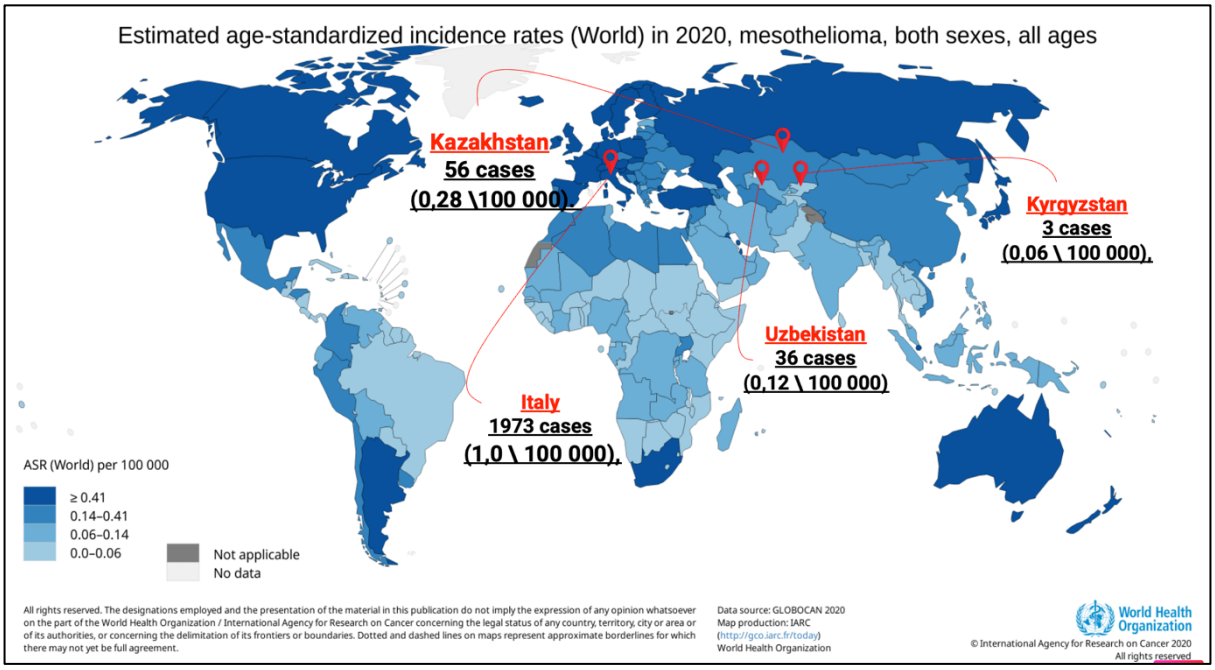

Figure 2. Estimated age-standardised incidence rates of mesothelioma in 2020, both sexes, all ages. Source: modified from Global Cancer Observatory: Cancer Today. 2020 [2]

Legislation and ban on asbestos globally.

Mesothelioma and other forms of cancer linked to asbestos exposure have been studied extensively, leading to the inclusion of asbestos as a carcinogen (Group 1) by the International Agency for Cancer Research (IARC). The U.S. Environmental Protection Agency (EPA) issued regulations prohibiting the mining, manufacturing, importing, processing, and commercial distribution of most asbestos-containing products. However, in 1991, the U.S. Court of Appeals revised these regulations, imposing a limited ban on five products and limiting the use of sprays containing more than 1% asbestos on buildings, infrastructure, pipes, and ducts [31]. Directives 83/478/EEC and 76/769/EEC further recognised asbestos health risks and limited their use in Europe. The National Mesothelioma Registry (ReNaM) was established in 2002 to estimate the epidemiological impact of mesothelioma. The International Labor Office (ILO), World Health Organization (WHO), and multilateral environmental agreements have played significant roles in promoting asbestos control, safer materials, and regulating international trade. The WHO and ILO collaborate to support countries in adopting programs and policies to stop asbestos use, suggesting strategies such as preventing asbestos use, providing information, promoting research, developing safer materials, adopting clear protection standards for asbestos disposal, and improving early diagnoses and treatments [32].

Exposure to asbestos

Asbestos fibres are found in outdoor and indoor environments, with most exposure occurring through inhalation [33]. The asbestos particles released to the environment are primarily generated during mining, crushing, grinding, screening, production, use, and disposal of waste containing asbestos. Exposure can also occur by drinking water contaminated by natural asbestos deposits or waste accumulation sites, deteriorating asbestos-cement pipes, or collecting asbestos roofing residues in rainwater [34].

Relatives of asbestos workers are at risk of experiencing increased incidences of asbestos-related pathologies, particularly pleural plaques and mesothelioma, following non-professional exposure [35]. Toxic dust deposited on hair, footwear, or work clothes can contaminate the family environment when at least one person is employed in a process that exposes them to asbestos [34]. Occupational asbestos exposure is highest among workers who come into direct contact with asbestos; mainly, they were exposed directly while mining, constructing, and industries involved in the reclamation and disposal of asbestos structures and objects [36]. In Europe, 1.2 million workers were exposed to asbestos between 1990 and 1993, with more than 96% employed in 15 industries [37]. Indirect bystander exposure of workers is observed while operating near asbestos sites or activities subjected to inadequate supervision and controls in construction companies [38].

Is chrysotile carcinogenic?

The carcinogenicity of various types of asbestos, particularly chrysotile, remains a contentious issue. While in vivo experiments have shown the high tumorigenic role of chrysotile fibres, the thesis that chrysotile has a lower carcinogenic power in humans than amphiboles has prevailed [39]. This is due to the divergent stability of fibres in the lung, the higher bio-persistence of amphibole, and the rapid clearance of chrysotile from the lung [40]. Epidemiological and toxicological studies have shown that lung cancer is closely related to fibre size, with the most significant risk stemming from long and fragile fibres [41,42]. The estimated risk of mesothelioma is 0.0012 for chrysotile, 0.099 for amosite, and 0.451 for crocidolite [43]. The association between chrysotile and lung cancer has a differential risk of lung cancer incidence for chrysotile, amosite and crocidolite [44]. However, the different latency required to generate carcinoma in humans and mice and the possibility of all fibres having the same carcinogenic value due to poor clearance in experimental animals raises questions. Further research is needed to fully understand the relationship between different types of asbestos fibres and their carcinogenic potential [45,46].

The global "pandemic" of asbestos-related diseases is affecting the general population, with over 255,000 deaths [47]. Developed economies have almost eliminated asbestos use, while low-income countries continue to use it [48]. Disease projections and trends differ between

industrialised states and those in economic transition, with measures affecting ARDs only 20–40 years after the ban [49].

Therefore, to fill the gap, particularly in countries still mining and producing asbestos and asbestos-containing commodities, studying the effects of asbestos exposure in humans can provide valuable insights into the risk factors associated with the development of ARDs, especially mesothelioma and lung cancer.

CHAPTER 2.
Asbestos in Central Asian countries: a narrative systematic review

INTRODUCTION TO CHAPTER 2.

To gain a better understanding of the overall usage of asbestos and asbestos-related diseases (ARDs) in Central Asian countries, a systematic search of published scientific findings and unpublished studies was conducted. The search was aimed at gathering comprehensive data on the prevalence and distribution of ARDs in the region, particularly in Kyrgyzstan. The search was conducted digitally using international and local databases such as Google Scholar, PubMed, Web of Science, Scopus and Elibrary.ru. These databases provided an overall picture of the use of asbestos and the findings of studies by local scientists in Central Asian countries. ARD incidence. The results of the study have been summarized in an article for submission to a peer-reviewed journal[1].

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Asbestos in Central Asian countries: a narrative systematic review

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Summary: The discovery of the detrimental effects of asbestos on human health came long after its widespread use, with scientific evidence of asbestos-related diseases emerging in the late 19th and early 20th centuries. Despite efforts to ban its use, asbestos continues to be mined and used in Central Asia (as well as in Russia, China, and other countries). To gain a deeper understanding of the situation in these countries, we have conducted an extensive and systematic review of scientific literature on the use of asbestos, exposure assessment and health consequences of asbestos exposure in Central Asia (CA). This review encompasses studies that include exposure assessments, epidemiological data, and biochemical or clinical surveys conducted in Kazakhstan, Uzbekistan, Tajikistan, Turkmenistan, and Kyrgyzstan. A total of 18 articles met the inclusion criteria, and their content is summarised. Notably, this review represents the first systematic examination of research on asbestos and its impact on the health of workers and the general population in CA countries, incorporating literature published in both Russian and English. The findings discussed here highlight the hindering of our ability to fully comprehend the extent of the impact of asbestos on health in this region.

1. INTRODUCTION

Asbestos is the name given to six silicate minerals: chrysotile (the only one belonging to the serpentines) and amphiboles (amosite, crocidolite, asbestos anthophyllite, asbestos tremolite, and asbestos

actinolite) [1]. Since the beginning of the 20th century, asbestos has been considered a valuable resource in various industrial sectors owing to its exceptional physical and chemical properties. These properties include, but are not limited to, fire resistance, electrical conductivity, thermal and acoustic insulation, and mechanical robustness. As such, asbestos has been extensively utilised in a diverse range of applications [2].

Exposure to any form of asbestos poses a increased risk of developing asbestos-related diseases (ARDs) [3]. These diseases can be divided into non-malignant conditions, such as pleural plaques and asbestosis, and malignant ones, such as lung cancer, mesothelioma [3,4]. Mesothelioma is a highly aggressive cancer arising from the mesothelial linings of pleural, pericardial, peritoneal and testicular cavities [5]. It give rise to clinical manifestations after several decades since the beginnings of exposure [6,7]. Different levels of exposure and risk exist, with certain occupations and proximity to asbestos mines or factories posing higher risks [7,8].

The World Health Organization (WHO) states that each year, over 125 million people worldwide are exposed to asbestos while working. Asbestos-related diseases cause globally around 255,000 deaths annually [9]. An increasing number of countries have banned or are banning the production of asbestos as a result of the 1986 Basel Convention [10,11] and its reconsideration in 2022 [12].

Nevertheless, Russia, China, Kazakhstan, and India continue asbestos mining. Other countries, including Central Asia (CA) countries such as Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan, are still large consumers of chrysotile. On these bases, the present paper provides an overview of the existing knowledge on asbestos use, exposure and consequences in CA (and precisely in Kyrgyzstan, Kazakhstan, Tajikistan, Uzbekistan, and Turkmenistan), aiming to understand the current situation in these countries, where asbestos-containing materials are still highly diffused.

2. METHODS

2.1. Data Sources and Search Strategy

We reviewed available publications to identify articles relating to asbestos and ARDs in CA countries, with a special focus on Kyrgyzstan. The search was performed in international repositories (Google Scholar, PubMed, Web of Science, Scopus and Elibrary.ru [13]), using the keywords and on PubMed using string (Mesh) terms reported in Table 1.

Table 1. Searching keywords and strings in international repositories.

Search string or keywords	Asbestos use and asbestos-related diseases in CA and	Database(s)
MeSH term	"Asbestos, Serpentine" AND (("Pleura" OR "Lung") OR ("Mesothelioma, Malignant" OR "Lung Neoplasms")) AND "Humans" AND "Kyrgyzstan" OR "Kazakhstan" OR "Uzbekistan" OR "Tajikistan" OR "Turkmenistan"	PubMed

Keywords	"Asbestos"; "Chrysotile"; "Asbestosis OR asbestos-related diseases OR Mesothelioma"; "Asbestos AND Kyrgyzstan"; "Asbestos AND Uzbekistan"; "Asbestos AND Tajikistan"; "Asbestos AND Turkmenistan"; "Asbestos AND Kazakhstan"; and "Asbestos AND Central Asia"	Google Scholar, Web of Science, Scopus and Elibrary.ru (in Russian)

Articles published in English and Russian from 2008 to 2022 were collected as electronic publications.

All references were imported into EndNote X20. PRISMA Flow Diagram [14,15] was used to create a systematic review flowchart. In addition, we used the Russian-language version of the Elibrary.ru (electronic library of scientific publications from Russia and CA countries. The library is integrated with the Russian Science Citation Index (RSCI)) database to search for all available journals in Russian. Also, data were collected from the Scientific Production Association 'Preventive Medicine' in Kyrgyzstan and the national statistical agencies of each CA country [16].

In addition to this, data was taken from open sources available online, such as export and import statistics, production quantities, and data concerning the consumption of asbestos in CA [17].

2.2. Selection criteria

During this search, we systematically reviewed the articles. The articles were selected first by title and abstract, then by the full text and review results. After removing duplicate articles, each study was screened based on the inclusion and exclusion criteria.

The papers were included if they contained sufficient and relevant information concerning the following topics: asbestos; occupational and environmental exposure to asbestos; production; the use of asbestos-containing products; asbestos-related diseases; mesothelioma; if they were available as a full text, written in English or Russian; if they focused on CA Countries.

The following articles were excluded: articles that could not provide sufficient data or information; articles related to laboratory studies on asbestos in chemistry and geology sciences; studies published in languages other than English and Russian; review articles; and articles unavailable as a full text.

2.3. Data Extraction

The full text of each study was categorised by its title, first author, year's publication, journal, study period, keywords, and abstract, using an Excel spreadsheet to build a database from this data.

3. RESULTS

3.1. Data Acquisition and Analysis

In total, 105 relevant research articles were found by using the following different repositories, as outlined in Figure 1, which summarises the selection process in the PRISMA [14,15] flow diagram in Figure 1.

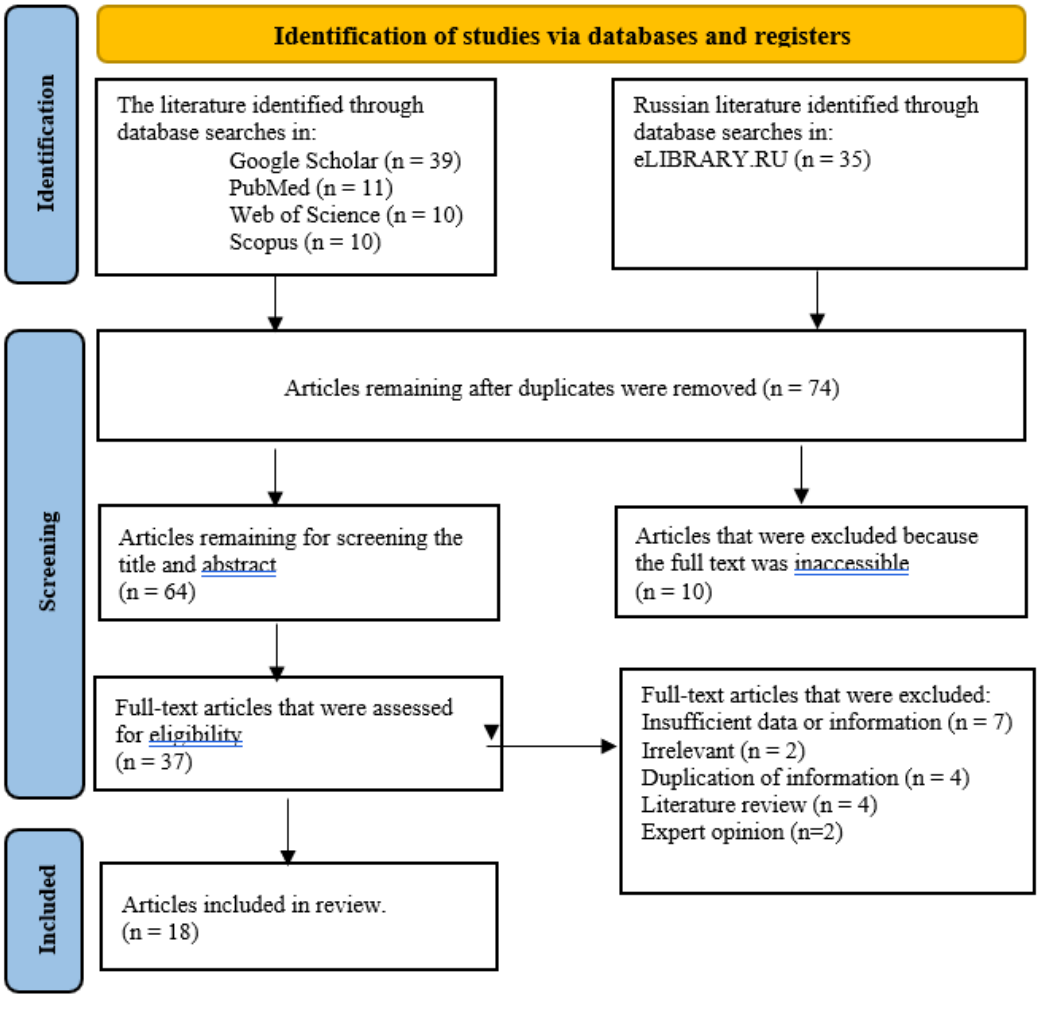

Figure 1. The inclusion and exclusion criteria for published articles on asbestos and asbestos-related diseases in CA Countries.

The main findings of the selected articles were tabulated in a data extraction Excel form. The information on the key findings of the study, year of publication, and language is summarised in Table 2.

Table 2. Summarized details of the selected articles.

#	Country	Authors	Year of publication	Language	Method	Sample	Sample s size	Dust pollution at the workplace	Human data (stat data)	Biological samples from human and clinical examination	Studies on lab animals	Non-malignant ARDs - pneumoconiosis, pleural plaques	Asbestos-related Mesothelioma (ARM)	Asbestos-related Lung cancer (ARL)
1.	Kazakhstan	Altynbekov et al. [33]	2018	Russian	analysis of the incidence of mesothelioma in the country for 2012-2016, survey	statistical data, questionnaires	257 human's data	no	yes	no	no	no	yes	no
2.	Kazakhstan	Ibraev et al. [20]	2016	Russian	The longitudinal study (every year for 7 years, the same group of workers were examined)	blood	85 humans	no	no	yes	no	no	no	no
3.	Kazakhstan	Koigeldinova et al. [21]	2015	Russian	biochemical analysis	blood	207 humans	no	no	yes	no	no	no	no
4.	Kazakhstan	Ibraev et al. [18]	2015	Russian	Calculation of the allowable length of service based on indicators of the average shift concentration of chrysotile-asbestos dust	dust	unknown	yes	no	no	no	no	no	no
5.	Kazakhstan	Amanbekova et al. [29]	2014	Russian	review of own previous studies	n/a	n/a	yes	yes	no	no	no	no	no
6.	Kazakhstan	Ibraev et al. [34]	2014	Russian	assessment of occupational disease risk	statistical data	5 years	no	yes	no	no	no	no	no
7.	Kazakhstan	Baselyuk et al. [22]	2011	Russian	cytomorphological study of cells of the nasal mucosa and buccal epithelium of the cheeks	the nasal mucosa and buccal epithelium of the cheeks	65 humans	no	no	yes	no	no	no	no
8.	Kazakhstan	Ibraev et al. [23]	2008	Russian	analysis of the function of external respiration, the study of the gas composition of arterial blood, plain radiography of the chest	human	47 humans	no	no	yes	no	yes	no	no
9.	Kazakhstan	Amanbekova et al. [24]	2012	English	retrospective cohort clinical trial and survey	nasal mucosa	106 humans	no	no	yes	no	no	no	no
10	Kazakhstan	Amanbekova et al. [25]	2012	English	retrospective cohort clinical trial	blood	85 humans	no	no	yes	no	no	no	no
11	Kazakhstan	Koigeldinova et al. [30]	2021	Russian	study of cytotoxic effect	asbestos dust	30 rats	no	no	no	yes	yes	no	no
12	Kazakhstan	Ibraev et al. [26]	2015	Russian	X-ray of chest and blood, aeration function of the lungs	human	119 humans	no	no	yes	no	yes	no	no
13	Kazakhstan	Kurkin et al. [27]	2015	Russian	Buccal epithelium cytograms	the nasal mucosa and buccal epithelium of the cheeks	108 humans	no	no	yes	no	no	no	no
14	Kazakhstan	Ainagulova et al. [31]	2022	English	Immunity monitoring	blood	40 rats	no	no	no	yes	no	no	no
15	Kazakhstan	Koigeldinova et al. [28]	2022	English	multiplex immunological assay	blood	125 humans	no	no	yes	no	no	no	no
16	Kazakhstan	Ibraev et al. [35]	2018	Russian	retrospective analysis of morbidity	statistical data	1216 human	no	yes	no	no	no	no	no
17	Kyrgyzstan	Korotenko et al. [19]	2011	Russian	various	statistical data	Not applicable	yes	yes	no	no	no	yes	no
18	Uzbekistan	Akhmadaliev et al. [32]	2021	Russian	review of the situation in the country	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	no	no	no

After applying the inclusion and exclusion criteria to all 105 articles, 18 papers were left (a summary is presented in Table 2). CA countries are former members of the Soviet Union; therefore, they predominantly publish in Russian (78% of the 18 selected articles were written in Russian). As for the country addressed, most of the studies (88.9%) concerned Kazakhstan. Only 5.6% concerned Kyrgyzstan, another 5.6% concerned Uzbekistan, and none concerned Turkmenistan and Tajikistan. The thematic content of the 18 articles can be summarised as follows:

- Only one of the reviewed articles [18] was about asbestos exposure in workplace environments.
- Only one article [19] addresses outdoor air pollution stemming from asbestos production.
- Nine studies [20–28] are based on biological samples from individuals working with asbestos and/or conducting clinical examinations, including biochemical and histological tests.
- Two studies [19,29] contained additional information concerning asbestos dust pollution at the workplace.
- The other two [30,31] described tests conducted on laboratory animals (rats).
- One [32] addressed the economic and public health-related disadvantages of asbestos use in Uzbekistan.
- Three studies [23,26,30] concerned non-malignant ARDs.
- Two articles [19,33] contained epidemiological studies on the links between mesothelioma and chrysotile asbestos.

In addition to peer-reviewed journals, we also examined the occupational disease registries of the various CA countries, non-peer-reviewed reports, conference proceedings and internal government documents, such as the report of the Scientific and Production Association "Preventive Medicine" under the Ministry of Health of the Kyrgyz Republic.

3.2. Overview of asbestos production and corresponding industries in CA countries

Three countries in the world - the Russian Federation, China, and Kazakhstan – are still producing more than 2 million metric tons of asbestos annually. Currently, 25 countries consume at least 1,000 metric tons of asbestos per year, including all the CA countries - Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan (Table 3) [36].

Table 2. Export and import of asbestos (excluding asbestos products) to CA countries from 2017-2021. Adapted from https://www.trademap.org/, accessed on 26.12.2022.

Countries	2017		2018		2019		2020		2021	
	Exported asbestos (tons)	Imported asbestos (tons)	Exported asbestos (tons)	Imported asbestos (tons)	Exported asbestos (tons)	Imported asbestos (tons)	Exported asbestos (tons)	Imported asbestos (tons)	Exported asbestos (tons)	Imported asbestos (tons)
Kazakhstan	182,304	130	184,830	44	217,839	12	209,784	407	232,366	20
Uzbekistan	0	87,403	0	129,032	0	94,168	0	116,654	0	126,115

Tajikistan	0	4,968	0	9,616	0	14,818	0	15,493	0	23,711
Turkmenistan	0	6,405	0	6,438	0	8,786	0	13,324	0	13,130
Kyrgyzstan	0	9,601	0	9,319	0	9,847	15	9,616	0	12,013

3.2.1. Asbestos mining and producing asbestos-contained commodities industries in Kazakhstan.
In terms of geographical area, Kazakhstan is the largest country among the former CA countries and has a population of 18,879,552 people [37]. The country is rich in deposits of various minerals, including chrysotile. Today, Kostanay Minerals Enterprise (KME) is the only company mining asbestos in the country [38]. The KME works the Zhitikara chrysotile deposit in the Kostanay region and employs around 2,000 people. This deposit ranks fourth in the world in terms of reserves, and the company exports to other countries of CA and beyond. Annually, the KME produces over 200,000 tons of asbestos as a raw material [38]. In addition to this company, three linked companies within the mining industry produce asbestos-cement goods, employing about 6,000 additional people [29].

3.2.2. Industries of Kyrgyzstan produce asbestos-contained commodities.
Kyrgyzstan is another of the former Soviet republics located in CA, with a population of 6.936,2 million [39]. The first enterprise in Kyrgyzstan to produce chrysotile-cement products is the Kant Pipe and Slate Enterprise (PSE) [40], which has been operating since 1967 and remains open today. The enterprise is located in Kant town, 22 km from the capital, Bishkek, and employs around 300 workers. Raw asbestos is imported from Kostanay Minerals JSC (Kazakhstan) and Ural Asbest OJSC [41] (Russia). Annually, the company sells 5 million units of asbestos-containing products. In 2020, a branch of Kant PSE was opened in the city of Kyzyl-Kyia, in the south of Kyrgyzstan, with a production capacity of 3.7 million units of 8-wave slate per year and employing around 150 workers. This production capacity is designed to meet the demand in the South of Kyrgyzstan, as well as being exported to Uzbekistan and Tajikistan [40]. The second plant to open in Kyrgyzstan producing chrysotile products is Kant Kurulush LLC [42], founded in 2013 in the city of Kant in the north of Kyrgyzstan. They produce non-pressure pipes and couplings as well as 8-wave slate. The primary raw material used is Russian chrysotile (asbestos), imported from Ural Asbest OJSC, Russia [42].
3.2.3. Industries of Uzbekistan producing asbestos-contained commodities.
Uzbekistan is the most highly populated country in the CA region, with a population of 36,024,946 people [43]. In 2020, Uzbekistan imported $37 million worth of asbestos, making it the 3rd largest asbestos importer in the world. In the same year, asbestos was the 132nd most imported commodity in Uzbekistan. Uzbekistan imports asbestos mainly from the following countries: Kazakhstan ($29.2 million); Russia ($7.63 million); China ($104 thousand); and Kyrgyzstan ($2.29 thousand) [44]. There are 44 enterprises in the country producing asbestos goods [45]. However, the total quantity of asbestos products manufactured by Uzbekistan is unknown.
3.2.4. Industries in Tajikistan producing asbestos-contained commodities.
The population of Tajikistan is around 9,700,000 [46]. In 2020, Tajikistan imported $6.19 million worth of asbestos, becoming the 9th largest asbestos importer in the world. Asbestos is ranked 138th among Tajikistan's most imported commodities. Tajikistan imports asbestos mainly from Kazakhstan ($4.54 million) and China ($1.65 million) [44]. However, information regarding the number of enterprises producing asbestos products is unavailable.
3.2.5. Industries in Turkmenistan producing asbestos-contained commodities.

The population of Turkmenistan is 6,341,855 [47]. Turkmenistan imported $4.52 million worth of asbestos in 2020, making Turkmenistan the 10th largest asbestos importer globally. In the same year, asbestos was the 147th most imported commodity in Turkmenistan. Turkmenistan imports asbestos mainly from Kazakhstan ($4.52 million) [44]. Again, the number of enterprises working with asbestos is not available data.

3.3 Detailed overview of the results

3.3.1. Physical and chemical characteristics of chrysotile asbestos

Ibraev and colleagues described the physical and chemical characteristics of chrysotile mined and extracted from Zhitikara ore [48]. The study was conducted using a scanning electron microscope (Tescan Vega\LSU) equipped with an energy-dispersive analyzer (INCA-PentaFET-x3). Notwithstanding, the authors do not present any EDS spectra, but only SEM images (compatible with pure chrysotile) and a table containing the percentage of elements contained in the analysed points. The results showed the different values of the outer diameter of the chrysotile fibres, which ranges from 94 to 167 nm; for this reason, the authors referred to them as nanofibers (no data about the lengths of the fibres were presented).

3.3.2. Asbestos concentrations in the workplace and the environment

Amanbekova and co-workers (2014) [29] found that the average daily dust concentration at workplaces in «Kostanay minerals» JSC in Kazakhstan was equal to 6 mg/m3 in 2014, which was three times higher than the maximum permissible concentration (MPC) of Kazakh regulation (the average daily MPC in Kazakhstan's legislation is 2 mg/m3 [49]). One year later, Ibraev and colleagues (2015) measured the level of dust in 2015 in the same industry; the average daily results ranged from 0.2 to 1 mg/m3, which did not exceed the MPC limits of Kazakhstan [18].

The Centre for Environmental Medicine and Human Ecology also conducted a study on air pollution in one of the industries that produce asbestos-containing commodities (Kant PSE, Kyrgyzstan) from 2019 to 2020 [16]. In total, 340 measurements were made at 162 points during the day and 18 points at night. The dust content in the air was determined through the gravimetric method. The dust dispersion and particle size characteristics were determined using a light trinocular microscope equipped with an ocular micrometre and software (BioVision, Austria). According to the results of the study, the average daily dust level in the air at the workplaces varied from 1.34 mg/m3 to 1.45 mg/m3 [16], which complies with the national regulations on acceptable MPC limits of dust containing asbestos in industries, where average daily should be no more than 2 mg/0.5 m3 [50].

However, it should be noted that the studies' findings do not comply with the Occupational Exposure Limits of the European Union, neither Kazakhstan's nor Kyrgyzstan's MPCs. Additionally, the gravimetric method is obsolete for measuring asbestos contamination since it is impossible to count the number of asbestos fibres (and thus obtain a quantitative and specific value for airborne asbestos concentrations) [3,51].

Korotenko et al., (2011) acknowledged the environmental emissions caused by Kant Pipe and Slate Enterprise (PSE) in Kyrgyzstan [19]. They highlighted that the industry released 10 various pollutants into the surrounding atmosphere, with 0.515 tonnes of asbestos-containing dust emitted in 2010. It is worth noting that this amount did not exceed the allowed annual emission of asbestos-containing dust in Kyrgyzstan, which is set at 1.47 tonnes. However, the authors did not elaborate further on their findings.

3.3.3. Research on asbestos industry workers

A study undertaken by Ibraev et al., (2008) examined 47 employees of Kostanay Minerals JSC in 2008 [23]. An X-ray examination of 20 workers with work experience of more than 20 years showed an increase in the vascular picture, a minor perivascular and peribronchial pneumofibrosis in median

zones of lungs in 60% of cases, and moderately expressed perivascular and peribronchial pneumofibrosis in media zones in 13 cases (40%). The analysis of the external respiratory function of these 47 employees revealed that 6 workers suffered from chronic bronchitis and disorders of pulmonary ventilation function, 25% of cases among them had respiratory obstruction, in some cases accompanied by hypoxemia.

Additional cytological examination of the nasal and oral epithelium of 65 workers [22] and 108 workers [27] of Kostanay Minerals JSC (2015) showed a high frequency of destructive changes of the cells of the nasal mucosa in samples of workers with occupational exposure from 5 to 20 years. The alterations in workers with more than 20 years of exposure were similar to those in the control group. The authors suggested it reflected the adaptation processes of the organism.

Amanbekova et al., (2012) studied the cell and humoral immunity of 106 workers in the Kostanay Minerals JSC, examining the «shortened» panel of monoclonal antibodies (mAbs), immunoglobulins (IgA, IgM, IgG) and secretory immunoglobulin A (SIgA) through an ELISA test [24]. They found a decreased functional activity of the T-lymphocytes in a proportion of all immune cells, accompanied by a reduced number of CD3 cells in workers who had worked more than 20 years — 58.7±0.41% ($p < 0.01$), compared to the control group (71,2±0,52 %). A similar picture was reported in CD4 cells - 40.9±0.85% (control group 45,2±0,26), CD20 cells - 6.1±0.39% (control 12,7±1,09), and IgA 1.35±0.57 g/l (control group 2,85 ± 0,27 g/l), and an increase of IgG - 19,27±0,57 g/l (control group11,27±0,14 g/l) of the employees who worked more than 20 years, respectively. Workers exposed to chrysotile asbestos over 20 years had decreased the mucous barrier of the nasal secretion in IgA - 0.16±0.03 g/l ($p<0.01$), compared with a control group (0.34±0.07 g/l).

In another study on 125 workers in the Kostanay Minerals JSC, Koigeldinova et al., (2022) found some changes in the number of CD4+ T-cells [28]. In employees occupational exposure more than 15 years, the number of CD4+ T-cells was significantly lower compared with those who had worked for less than 15 years. The levels of CD8+ T-cells were similar in these two examined groups. They concluded that most healthy workers, with a longer occupational exposure to chrysotile, have increased neutrophil phagocytic activity and a decreased total number of CD3+ T and CD4+ T cells, but an increased number of CD8+ T-cells with a lower immunoregulating index of CD4+8+. Koigeldinova et al.(year) also found that the workers of Kostanay Minerals JSC with longer occupational exposure to asbestos fibres have an increased activity of lipid peroxidation, which was more pronounced in the workers of the processing complex than the drivers and miners [21].

Ibraev et al. (2015) found significantly increased ($p<0.05$) level of alveomucin 3EG5, a marker of lung fibrosis, in the blood plasma of workers of Kostanay Minerals JSC with more than 20 years of occupational exposure compared to those of the control group [26] . They recommended the level of lipid peroxidation products and alveomucin 3EG5 in blood plasma as biomarkers of the initial stage of pneumoconiosis caused by chrysotile exposure.

In a 7-year longitudinal study of biochemical and cytochemical blood indicators in Kostanay Minerals JSC workers, Ibraev et al. (2016) found an increase in sphingomyelin (SM) and a decrease in phosphatidylcholine (PC) in workers with longer occupational exposure to asbestos-containing dust [20]. These changes in the cell membrane, involving both the plastic and energy state of cells and the level of catecholamines, occurred as a result of adaptation to asbestos exposure at the workplace. Differences in the functional state of the body were revealed in workers of the processing complex who had worked for 4 to 5 years and in employees of the mining and transport department who had worked for between 5 and 6 years. For workers of the processing complex, the authors regarded a working period of 5 years to be an occupational risk, while for employees of the mining and transport department, this risk began from 6 years.

In another cytogenetic blood study of workers in chrysotile-asbestos production, Amanbekova et al. (2012), revealed structural disorders of chromosomes represented by aberrations of chromosome and chromatid types, which could indicate chemical mutagenesis [25]. The authors observed higher rates of such induced mutagenesis in workers with more than 25 years of exposure to asbestosis. The frequency of cells with chromosomal aberrations in the peripheral blood lymphocytes of the main group significantly exceeded the control values.

Ibraev et al. (2022) studied the morphological parameters and the dust content in lung tissue taken from the autopsy material of 343 deceased individuals (including workers at Kostanay Minerals JSC and a "control" group composed of residents of Zhitikara who never worked at Kostanay Minerals JSC) [52]. They found severe sclerosis and dust particles in the form of grains (pigments) of black colour. These black dust particles were found in the lung sections of 33.3% of the workers of Kostanay Minerals, and in 44.6% of the residents of Zhitikara, who had no professional contact with asbestos. Such particles, however, are different from asbestos bodies (covered asbestos fibres observable in a light microscope), that appear as brown or dark yellow corpuscles. Moreover, the authors found more pronounced fibrotic changes, sometimes with the obliteration of alveoli, in the lung sections of Kostanay Minerals JSC workers compared to the controls, in which non-specific inflammation prevailed. The authors concluded that chrysotile occupational exposure does not increase the risk of developing pathologic changes in the lung tissue (RR-1.9 CI=0.68).

3.3.4. Epidemiological studies

Only one study of Altynbekov et al. (2018) on the prevalence of mesothelioma in Kazakhstan is available [33]. From 2012 to 2016, mesothelioma was diagnosed among 257 people across the country: 95.7 % had pleural mesothelioma, 3.9% had peritoneal mesothelioma, and 0.4% had pericardial mesothelioma. The age at diagnosis was between 40 and 70. Interestingly, occupational exposure to asbestos-containing dust was demonstrated in only 7.5 % of them, while in 92.5 % of cases, the history was negative for occupational asbestos exposure. The authors concluded that such data showed no relationship between chrysotile asbestos exposure and mesothelioma. Regarding the geographical location, 15.2% of cases were from the Almaty region (15.2 %), 12.8% from the Kostanay region, and 10.5% from the Karaganda region; notably, Almaty and Karaganda regions do not have asbestos-producing facilities.

Only one study on pleural mesothelioma was conducted in Kyrgyzstan by Golovachev in 2008 [53]. He examined 12 patients with a newly diagnosed pleural mesothelioma at the National Centre of Oncology (NCO) in 2000-2005. Among these, 7 were male (58.4%), and 5 female (41.6%); their average age was 44. The incidence rate of pleural mesothelioma in Kyrgyzstan was 0.14 per 100,000 men and 0.1 per 100,000 women in the same year. Histologically, malignant mesothelioma was confirmed in six patients (50%). In three patients (25%), the diagnosis remained histologically unverified due to their refusal to conduct diagnostic and therapeutic thoracoscopy. The rest (25%) were finally diagnosed with other types of malignant neoplasms. The patient's history showed occupational exposure to asbestos in five patients, who had worked with asbestos insulation and asbestos-cement materials. The verification of the diagnosis was based on histological methods only, an immunohistochemical assay was not performed.

Nonetheless, despite the lack of studies in CA regarding ARDs, after an extensive search, we found some data only in the database of the Bureau of National Statistics of the Republic of Kazakhstan. According to them, "pneumoconiosis caused by asbestos and other minerals" (J61, ICD-10) was registered in 1 case in 2006, 10 cases in 2015, and 1 case in 2021 [37], with an age range predominantly from 30-45. Such data reflect the extremely low registration rate of ARDs. At the same time, it is worth mentioning that the incidences of mesothelioma in CA countries, according to the WHO, are also low

compared to European rates: 0.28/100,000 in Kazakhstan, 0.06/100,000 in Kyrgyzstan, 0.12/100,000 in Uzbekistan, 0.02/100,000 in Tajikistan, 0.15/100,000 in Turkmenistan [54].

4. Discussion

In this narrative systematic review, we have presented the available data about asbestos in CA countries, including epidemiological, exposure assessment and experimental studies.

Overall, the scientific literature concerning asbestos exposure in CA does not provide sufficient data for a complete understanding of this issue. Moreover, the available data, above described in detail, have been produced using outdated and imprecise methods, lacking the accuracy of the widely shared updated recommendations. For instance, without a fibre-specific sampling method (and applicable consistent exposure limits), industrial hygiene experts cannot fully understand the complex exposure-response picture for asbestos. This means they cannot accurately and completely evaluate the risks of asbestos at the workplace. Asbestos has been recognised as different from other dusts or fibres; thus, in order to obtain data applicable to the industrial hygiene field, appropriate sampling and analysis techniques should be used. The microscopic method (e.g., ISO 14966-2019) has been used for many years to count and identify "respirable" asbestos fibres in fibre and dust samples collected on a filter.

It should be mentioned that it is claimed, and taken for granted in the examined scientific literature, that only chrysotile is used in CA. However, although chrysotile is known to be less carcinogenic for mesothelium than amphiboles, the link between chrysotile and mesothelioma is well known. Moreover, chrysotile exposure is related also to a higher risk of developing lung cancer and lung fibrosis. However, there is no data about epidemiology of lung cancer from CA countries. On the other hand, the incidence of mesothelioma, according to the examined literature, appears to be as low as 0,28/100.000 in Kazakhstan and 0,06/100.000 in Kirghizstan; low; this might be due to a significant number of undiagnosed cases. The protocol for mesothelioma diagnosis adopted in CA countries is not explained in detail in any of the consulted sources, and it does not include immunohistochemistry [55], an essential tool in the differential diagnosis of this neoplasm, which is, indeed, very complicated to diagnose unless the pathologist has a specific experience. Another explanation could reside in the younger population in CA countries (according to the United Nations website), that might imply that some individuals that would have developed mesothelioma later in life, die before manifesting the disease. This is related to the exceptionally long latency of mesothelioma.

The laboratory experiments performed on animal models in the cited studies demonstrated the development of asbestos-related pneumofibrosis, which can lead to neoplasia. However, sufficient and relevant studies on cancers related to asbestos were not found. It can be explained by not well-established methods or lack of equipment for applying the most internationally accepted approaches, in some cases due to insufficient financial support. Another issue that should be reported is that Occupational Health is not well-developed in Kyrgyzstan and other CA countries. In Kyrgyzstan, only a few specialists in occupational diseases are active across the country, and the medical examination of workers is the duty of general physicians [17], where the best option is when occupational physicians diagnose ARDs accurately. It should be stressed, however, that awareness and consideration of the problem of asbestos hazards are generally evident in CA countries through the work of scientists in the corresponding fields.

Another topic that needs to be clarified is the attribution of different hazardous effects to different types of asbestos. In the literature, there are conflicting studies on the different roles of types of asbestos in determining the impact on human health. Epidemiological studies have revealed that chrysotile has different effects compared to amphiboles, and among amphiboles, crocidolite is considered the most hazardous type of asbestos [56,57]. In addition, all of the previous studies on lung content have been conducted in Europe, US, Canada and Australia on subjects exposed to a mixture of asbestos [58–60]. On the contrary, there are no data about humans exposed to pure chrysotile.

An approach for better clarifying this aspect might be to detect and measure the asbestos fibres post-mortem in lung tissue of CA inhabitants, considering that the two key points to be addressed are the fibre content of the lung, the type of fibre, and the clearance rate of these fibres. The determination of asbestos exposure, asbestos inhalation, and persistence in lungs, as well as the link between asbestos exposure (occupational and environmental) and neoplasms (malignant pleural mesothelioma, lung cancer etc.), is an urgent public health issue in the countries of CA.

5. Conclusion

This is the first systematic review on asbestos and its impact on the health of workers and the general population of CA countries, including studies published in Russian and English. We emphasize that the arbitrary presentation of the results of reviewed studies and their notable incompleteness do not allow to understand the situation. The picture of asbestos-related issues in CA countries needs to be improved. A number of topics require attention: there are only a few studies on asbestos in the CA area, and almost no occupational and environmental exposure assessments are conducted adopting modern and internationally accepted methods. Additionally, in the cited studies, often outdated techniques are used to assess exposure. For instance, gravimetric methods are not suitable to determine the composition of the revealed dust and to distinguish between asbestos and non-asbestos fibres. There is also a need for studies linking mesothelioma and asbestos in CA, and recent studies and reliable statistical data are unavailable. Overall, a shortage of analytical foundations results in a substantial absence of inquiry and sizeable gaps in the current investigation. To fill this gap, more studies must be conducted which follow updated and validated methodologies and methods and address the problems of the existing literature. One avenue for achieving this is through the application of established methods. It approaches in collaboration with experts, improving the quality and quantity of research on what is a significant public health issue. However, this presents a chance to conduct research and fill the gap of knowledge.

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CHAPTER 3.
Asbestos in Central Asia: type of asbestos in use and assessment of environmental exposure in urban areas (a pilot study)

INTRODUCTION TO CHAPTER 3.

The results of the systematic narrative review (Chapter 2) displayed a significant lack of study on the subject and highlighted the widespread use of asbestos in Central Asian countries, despite the well-established health risks.

One argument put forward by countries in Central Asia is that they use pure chrysotile, which is not contaminated with other forms of asbestos, such as amphiboles. They claim that this is the reason why there is a low incidence or absence of asbestos-related diseases in some Central Asian countries. To investigate this hypothesis, we conducted an additional study on the composition of raw asbestos materials and asbestos-containing products. This study prompted our examination of the environmental exposure arising from chrysotile roofs present on almost every house and facade sheet throughout Bishkek. In addition, we collected and analyzed air samples from Kant town, where two asbestos-producing factories are situated. The findings of this investigation have been compiled for publishing in a peer-reviewed journal (Chapter 3).

ASBESTOS IN CENTRAL ASIA: TYPE OF ASBESTOS IN USE AND ASSESSMENT OF ENVIRONMENTAL EXPOSURE IN URBAN AREAS (A PILOT STUDY).

INTRODUCTION

In May 2007, the 60th World Health Assembly endorsed a global plan of action on workers' health 2008–2017 in which Member States requested the WHO Secretariat to include in its activities "a global campaign for the elimination of asbestos-related diseases" [50]. Before this statement, asbestos was already banned in some countries and some other countries followed the recommendation, therefore the number of countries in which asbestos is banned is now 69 [48]. This means that in several other countries, asbestos is still marketed and used. Nowadays the world leaders in asbestos production are Russia, China, Kazakhstan and India. In particular, Russia and Kazakhstan are still mining asbestos, and the neighbouring countries (especially Uzbekistan, Kyrgyzstan, and Tajikistan) are still consumers of asbestos. According to WHO estimates, more than 125 million people are exposed to asbestos while working, with many of them residing in countries that have not implemented an asbestos ban. The total number of deaths from asbestos exposure is 255,000 annually [47], with a burden of 3.97 DALYs, as estimated by a joint study performed by WHO and ILO [51]. It is important to underline that asbestos exposure is still present also in countries which banned its use, because of the presence of a high amount of asbestos-containing materials already installed in buildings and machinery [52] but it is arguable that levels of exposure and a number of exposed subjects are higher in the countries not adhering to the global ban. In these countries, one of the arguments raised to justify the decision to continue to mine and use asbestos is that they handle only chrysotile which, according to literature [53] is less pathogenic than amphiboles and produces dose-dependent effects, and a lower, but not absent, risk of mesothelioma [54]. This makes the risk posed by exposure to chrysotile would be easier to manage by applying technical risk management measures. On the other hand, some authors claim that there is a strong influence of vested asbestos-related interests affecting existing literature on chrysotile and in the end, several issues regarding the real health risk are still unresolved [55].

The pending doubts could be solved by collecting reliable information regarding the actual nature of asbestos mined in the countries which still use the mineral and regarding the levels of exposure of population and workers in these countries. Unfortunately, data from Central Asia are sparse, studies are carried out with different approaches and are not easily comparable between each other, therefore no firm conclusions can be drawn regarding asbestos exposure both of the workers and the general population [56]. However, these data are necessary because they can guide preventive actions as well as further scientific research.

The samples investigated in the present study have been collected at one of two enterprises in Kyrgyzstan producing chrysotile cement artefacts. Both plants are located in the Kant town of Chui Valley, 22 km northeast of the capital city Bishkek.

The first production of asbestos cement started in 1967 and they currently produce Chrysotile cement pipes, Chrysotile cement roofing sheets, Fibre cement flat boards, Fibre cement siding, and Cement sand roof tiles. Their asbestos-containing commodities are well spread and used all over the country and beyond, particularly in the building sector since the factories were founded.

In this frame, this study aims to define the type of asbestos used in Kyrgyzstan (specifically at Kant asbestos-cement industries) and to estimate the levels of airborne asbestos contamination in the Bishkek city and Kant town of Kyrgyzstan.

MATERIALS AND METHODS

1) RAW MATERIALS

Four powder samples and two massive samples (Figure 3), collected at Kant industry, were examined by an X-ray powder diffractometer (XRPD), a scanning electron microscope combined with energy-dispersive spectrometry (SEM–EDS), a transmission electron microscope combined with energy-dispersive spectrometry (TEM–EDS).

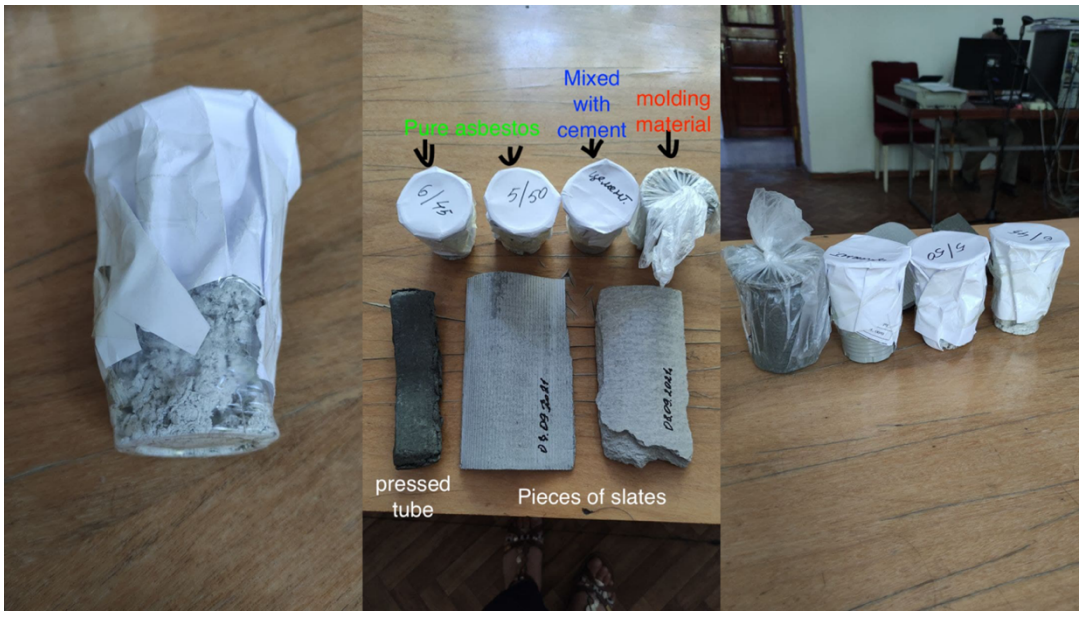


Figure 3. Bulk asbestos material and asbestos-containing products. Source: photo taken by Z.Kurzhunbaeva.

The XRPD patterns were obtained by a Bruker D8 Advance X-ray diffractometer with CuKa radiation, monochromated with a graphite sample at 40 kV and 40 mA. Scans were collected in

the range of 3 –66 (2h), with a step interval of 0.02 (2h) and a step-counting time of 3 s. EVA software (DIFFRACplus EVA) was used to identify the mineral phases and experimental peaks being compared with the 2005 PDF2 reference patterns.

SEM imaging and EDS chemical analyses were performed using a Scanning Electron Microscope JEOL JSM IT300LV SEM coupled with EDS detector Oxford INCA Energy 200, INCA X-act SDD thin window for analyses. Morphological, structural, and chemical features were examined by a TEM Philips CM12, working at 120 kV with a LaB6 filament with a double tilt holder equipped with an energy dispersive spectrometer EDAX Genesis 2000 System, TEM Quant Software PV8206/31 procession system. When fibres were detected, medium to high magnification images and selected area electron diffraction patterns were achieved to identify the nature of fibres.

For investigational purposes, any sample was previously examined at the stereomicroscope to evaluate the presence, amount, and characteristics of the fibres, when visible.

For SEM-EDS investigation, a little amount of powder or a little fragment of the massive sample was pasted on an aluminium sample holder, by using a double-sided carbon tape, and then coated with carbon to make it conductive.

For XRPD and TEM-EDS investigation, both powder and massive samples were crushed in an agate and pestle mortar by using acetone and isopropyl alcohol, respectively. For XRPD investigation a little amount of powder has been deposited on a plastic sample holder. For TEM-EDS investigation, the obtained suspension was sonicated and then two drops were deposited on a copper mesh grid coated with a 200 Å carbon film.

2) AIRBORNE ASBESTOS FIBRES SAMPLING

Airborne asbestos fibres sampling was carried out in the area of Bishkek and Kant. As mentioned above, the two enterprises in Kyrgyzstan producing chrysotile cement artefacts are hosted in Kant. For the purpose of this study, 4 air samplings were carried out in Bishkek and 3 air samplings in Kant (Table 1, Figure 4 and Figure 5). All samplings were performed in July 2023 by one of the authors (ZK), during 4 sampling days. Each sampling lasted about 8 hours (from 460 to 582 minutes). Sampling locations were mainly intended to be representative of the “Urban Background” scenario and one scenario from a Rural site (a factory was located there, and the nearest residential area was 1 km away from the factory), to assess the average urban background concentrations, possibly resulting from both (i) the transport of asbestos fibres from outside the urban area (i.e., the chrysotile cement artefacts factories hosted in Kant suburbs) and/or (ii) from

the release of asbestos fibres from materials containing asbestos possibly widespread in the urban areas.

Table 1. Location coordinates and sampling date of the airborne asbestos fibre samplings.

Area	Sample ID	Location	Coordinates (Google Map)	Sampling Date (dd/mm/yyyy)
Bishkek city	B1 (K22)	26 Kollektivnaya str. (TR)	42.92170264690117, 74.61035830716843	26/07/2023
	B2 (K19)	99 Bosogo str. (TR_B)	42.83831508434129, 74.64541705670091	26/07/2023
	B3 (K20)	124a Lev Tolstoi str. (TR)	42.864840946152405, 74.56004419344173	28/07/2023
	B4[AS2] (K_blk_1)	92 Uchitelskaya str., Novopavlovka village, Sokuluk district Chui valley (West of Bishkek) (TR_B)	42.872960773194144, 74.47396930685348	29/07/2023
Kant Town	K1 (K21)	10 Markovskogo (TR_B)	42.884917509220436, 74.84173923391842	22/07/2023
	K2 (K23)	97 Lenina (Kant Post office) (TR)	42.889606796324024, 74.84516267196803	22/07/2023
	K3 (K24)	40 Kotovskoe, Kotovskoe village, Ysyk-Ata district, Chui valley (RU)	42.91457473647124, 74.86035950555436	22/07/2023

The sampling method was derived from ISO 14966:2019 and ISO 13794:2019 standards. Airborne asbestos fibres were collected on polycarbonate filters (25 mm diameter; porosity equal to 0.8 µm) through the use of a sampling head (consisting of a cylindrical cowl and a filter holder with backing filter; the length of the cowl is 30 mm, which is more than 1.5 times the effective diameter of the filter) associated with a sampler (SKC AirChek XR 5000; flow: 2 l·min-1). All samplers were calibrated during the pre-sampling phase (±5 % of the nominal flow) and the flows checked at the end of each sampling session, verifying that the maximum variation in the pre- and post-sampling flow was not >5%. The sampling trains were arranged on tripods placed n fixed stations at the points described in Table 1, with the sampling heads 1.5 meters above ground level.

The determination of the numerical concentration of airborne asbestos fibres (expressed in number of fibres per liter of air: ff/L) in the atmosphere was carried out following the indications of the Italian Ministerial Decree 06/09/1994 (Annex 2B) and the ISO 13794:2019 method. The analyzes of the collected samples were carried out using a scanning electron microscope – SEM (XL30 ESEM – FEG, Philips; 400 microscopic fields investigated for each sample at 2000x; Working

voltage: 20 KeV; Working distance: 15 mm) equipped with an energy dispersive spectrometer (EDX, Quantax 400, Bruker Karlsruhe, Germany) for the analysis compositional of each fibre, to uniquely identify the nature of the fibres and classify the fibres according to their chemical composition. The criteria for fibre counting are those defined as per Ministerial Decree 06/09/1994 (Annex 2B) including the definition of "respirable fibres" (based on the following dimensional characteristics: length ≥ 5µm, diameter ≤ 3µm, length/diameter ratio ≥ 3). Before carrying out the analysis, each filter was placed on a 25 mm aluminium stub and coated with a thin (10 nm) layer of gold, obtained by metallizing the sample (three depositions; 20 seconds at 20 mA each - Cressington 108 auto Sputter Coater), in order to make the sample conductive. The numerical concentrations of asbestos fibres were calculated using the following relationship:

$$Concentration\ \left({}^{ff}/_{L}\right) = \frac{\frac{number\ fibres\ counted\ \ (ff)}{number\ analyzed\ fields \times \text{Microscopic field area}\ (mm^2)} \times Effectve\ filter\ surface\ (mm^2)}{Normalized\ Volume\ (L)}$$

Where:

- number of fibres counted (ff): are the fibres counted on the filter;
- number of fields counted: these are the fields observed for each count;
- Microscopic field area (mm^2): surface of the microscopic field, which corresponds to 0.00262 mm^2;
- Effective filter surface (mm^2): inspectable filter surface which corresponds to 283 mm^2;
- Normalized V (L): sampling volume corrected for the temperature detected at the time of calibration and sampling.

Lower and upper fiduciary limits were calculated, with 95% probability, assuming a Poisson random distribution of the fibres on the sampling membrane. The limit of detection (LOD) of this analytical technique corresponds to the upper confidence limit calculated based on the Poisson distribution with 95% confidence interval (CI) and depends on the number of fibres detected, the number of microscopic fields observed and the sampled volumes.

RESULTS

NATURE OF RAW MATERIALS COLLECTED IN KANT ENTERPRISE

The samples have been first examined morphologically under stereomicroscope. The results are summarized in Table 2.

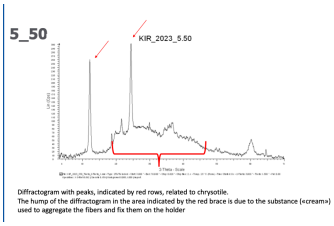
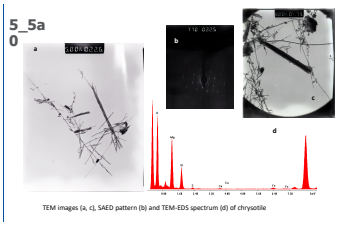
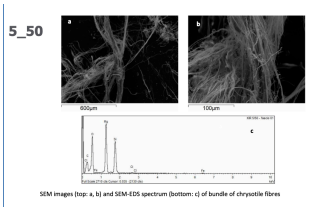
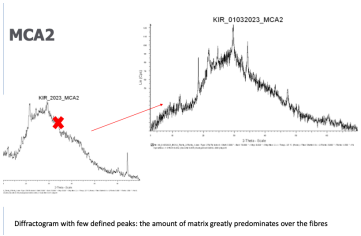
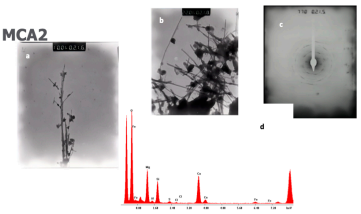
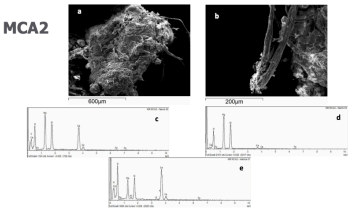
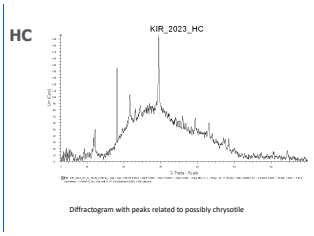
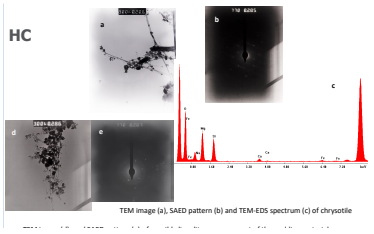
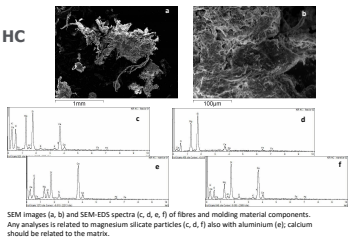
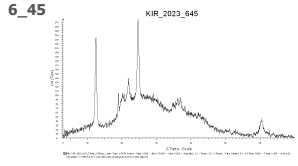
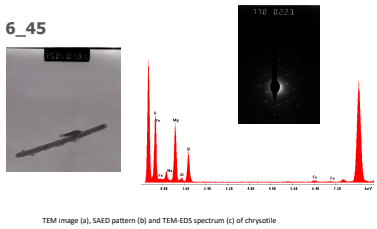
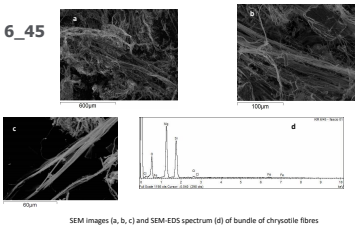
Table 2. The results of bulk materials under the stereomicroscope.

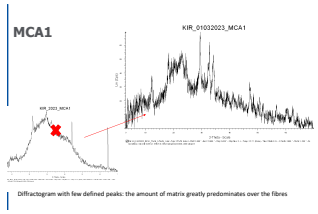
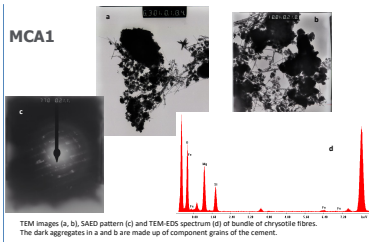
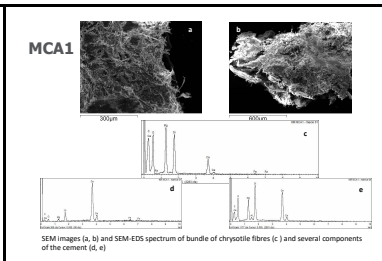
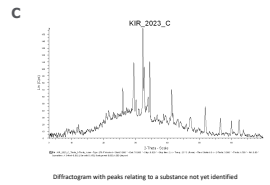
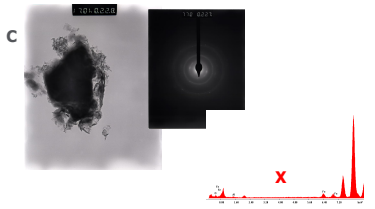
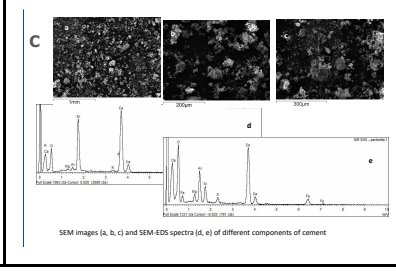
5_50	**«pure asbestos» fibre bundles, white, flexible**
6_45	**«pure asbestos» fibre bundles, white, flexible**
C	**«mixed with cement» no fibres observed**

HC	**«molding material» fibre bundles, white, flexible**
MCA1	**«pieces of slates» fibre bundles, white, flexible**
MCA2	**«pressed tube» fibre bundles, white, flexible**

Analytical techniques confirmed that Sample 5/50 was chrysotile asbestos, while Sample 6/45 was determined to be serpentine. Sample C did not contain asbestos fibres. The classification of HC as chrysotile was uncertain, and MCA1 and MCA2 were identified as chrysotile using various methods. Results are summarised in Table 3.

Table 3. Results of 6 samples in PXRD, TEM-EDS and SAED, SEM-EDS analysis.

Sample	PXRD	TEM-EDS	SEM-EDS
5_50 "pure chrysotile"			
MCA2 "pressed tube"			
HC «molding material»			
6_45 "pure chrysotile"			

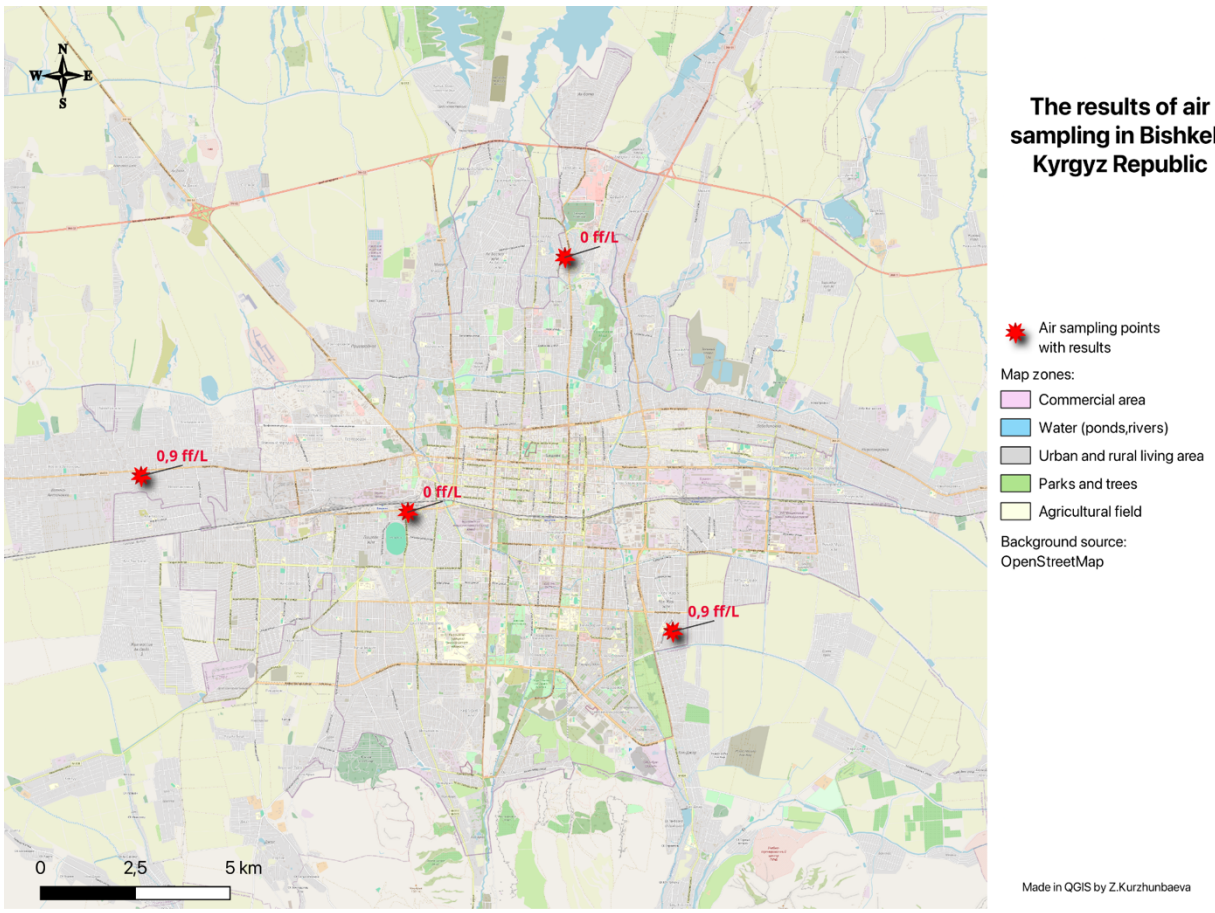
MCA1 “pieces of slate”			
C «cement»			

AIRBORNE CONTAMINATION

Poisson distribution with 95% confidence interval (CI) and depends on the number of fibres detected, the number of microscopic fields observed and the sampled volumes. Results are summarised in Table 4.

Table 4. Results of 7 environmental air samples.

Sample ID	Location	Sampling date	Analysis date	Conc (ff/L)	Theoretical uncertainty Conc (ff/L) (C.I.95%)	
					Lower limit	Upper Limit
K19	Bishkek city	26/07/23	28/08/23	**0,9**	**0,2**	**2,6**
K20	Bishkek city	28/07/23	29/08/23	**0,0**	**0,0**	**2,1**
K21	Kant town	22/07/23	28-29/08/2023	**30,2**	**24,6**	**36,6**
K22	Bishkek city	26/07/23	30/08/23	**0,0**	**0,0**	**1,0**
K23	Kant town	22/07/23	30/08/23	**9,6**	**6,6**	**13,4**
K24	Kant town	22/07/23	01/09/23	**1,2**	**0,3**	**3,1**
K_blk_1	West of Bishkek	29/07/23	01/09/23	**0,9**	**0,1**	**3,4**



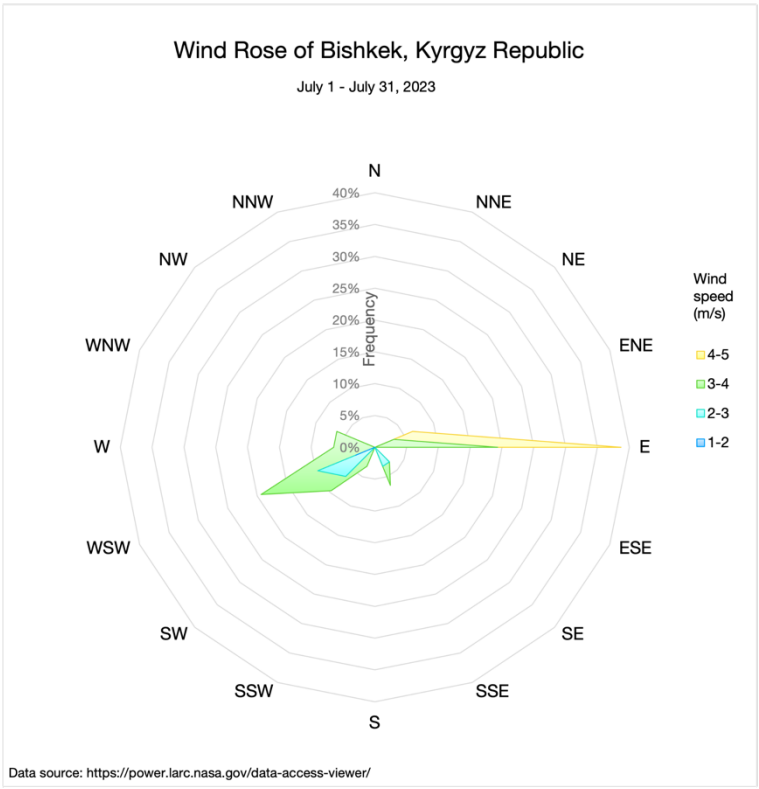
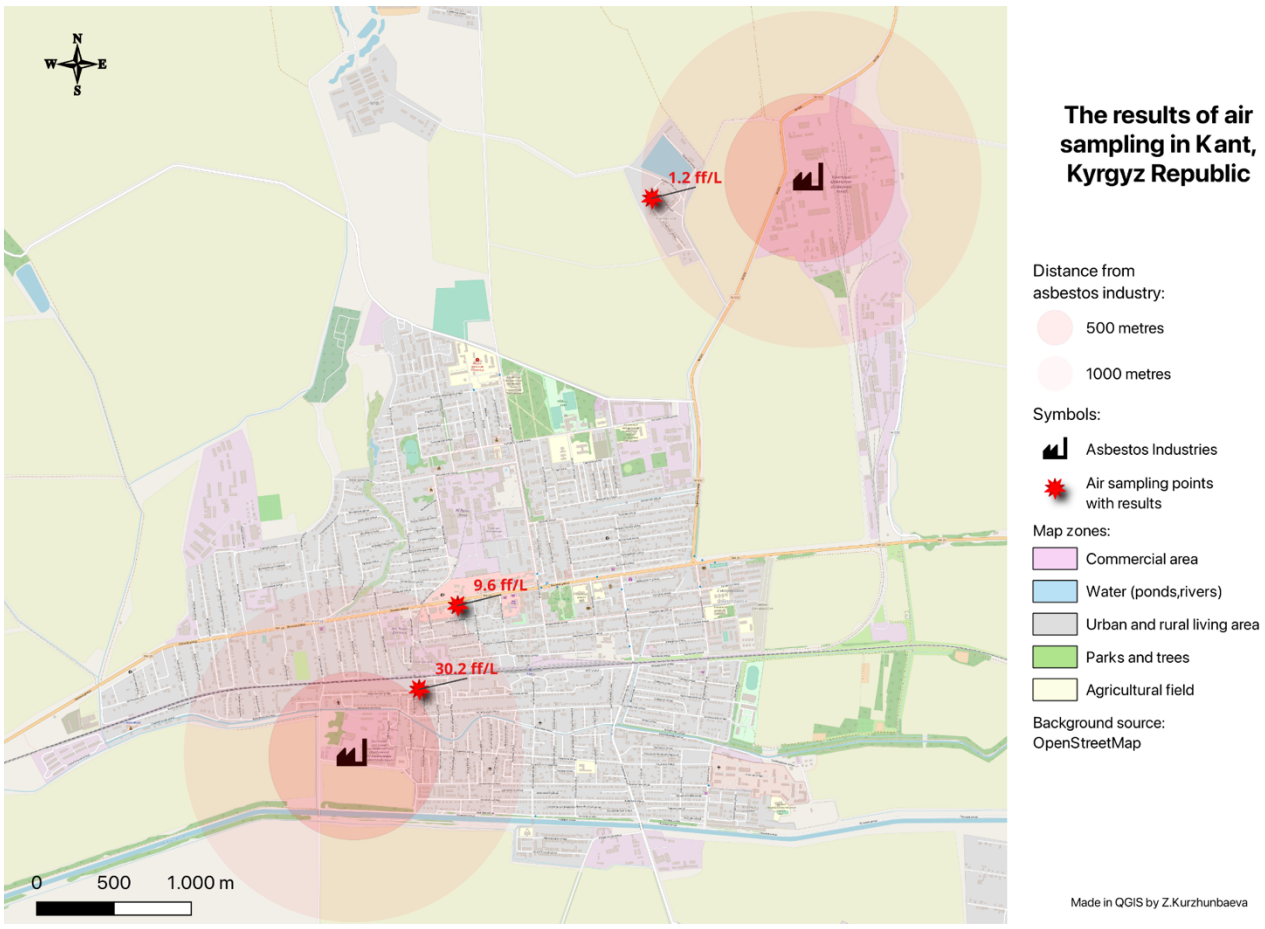


Figure 4. Location and results of air sampling were collected in Bishkek City (upper; source: made in QGIS by Z.Kurzhunbaeva) and Wind Roses of Bishkek City on July 1-31, 2023 (lower; made by Z.Kurzhunbaeva. Data source: https://power.larc.nasa.gov/data-access-viewer/)



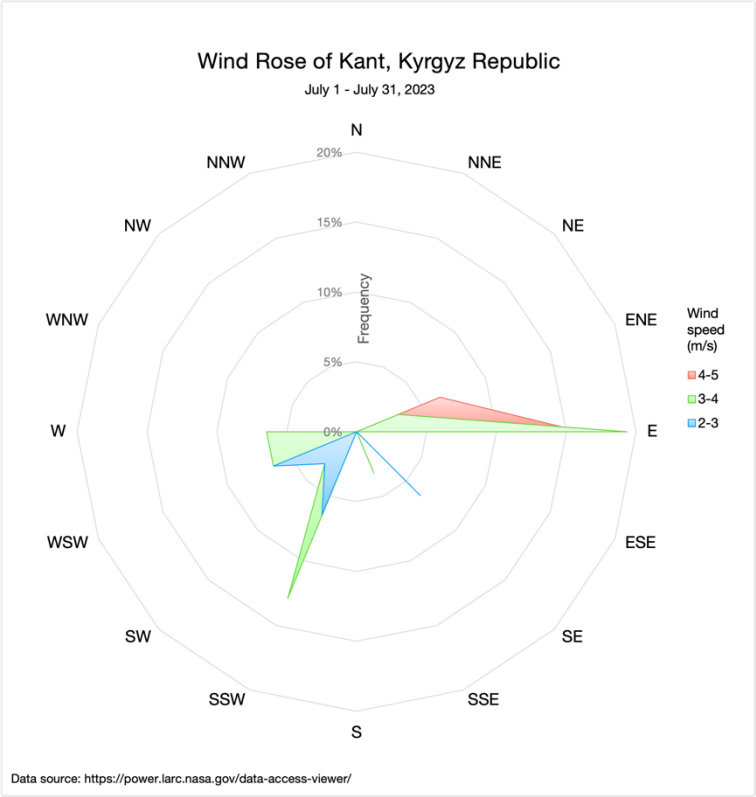
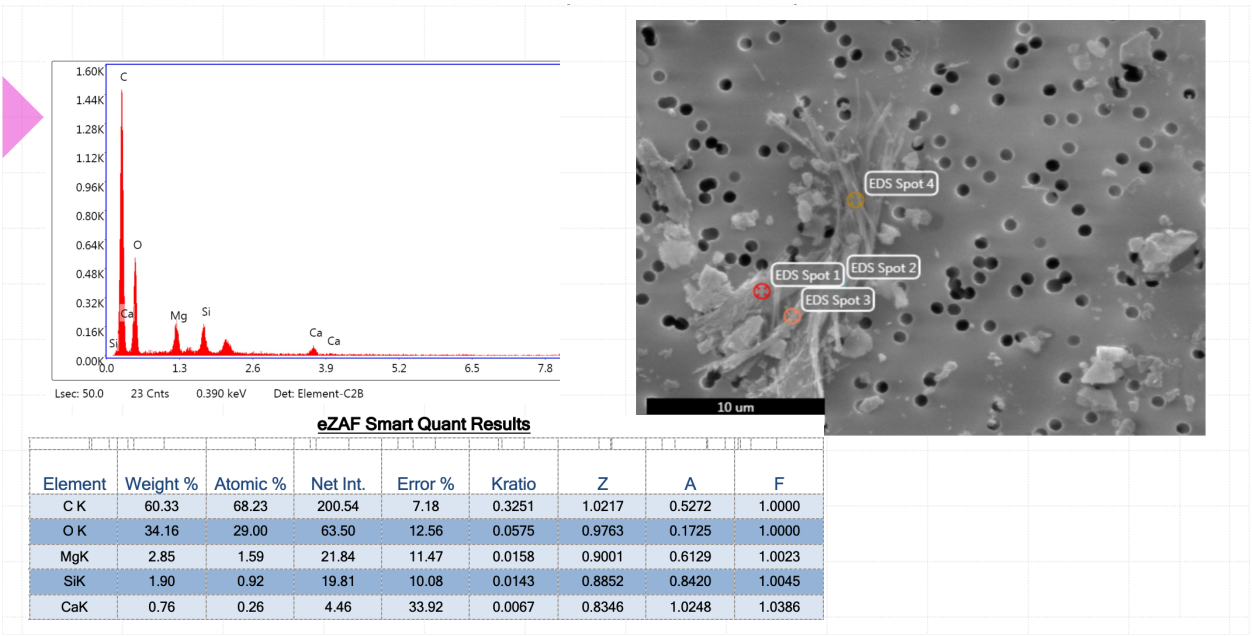


Figure 5. Location and results of air sampling were collected in Kant town (upper; source: made in QGIS by Z.Kurzhunbaeva) and Wind Roses of Kant Town on July 1-31, 2023 (lower; made by Z.Kurzhunbaeva. Data source: https://power.larc.nasa.gov/data-access-viewer/)

The results of chemical analysis in SEM-EDS and photo of captured chrysotile fibres were shown in Figure 6.



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	60.33	68.23	200.54	7.18	0.3251	1.0217	0.5272	1.0000
O K	34.16	29.00	63.50	12.56	0.0575	0.9763	0.1725	1.0000
MgK	2.85	1.59	21.84	11.47	0.0158	0.9001	0.6129	1.0023
SiK	1.90	0.92	19.81	10.08	0.0143	0.8852	0.8420	1.0045
CaK	0.76	0.26	4.46	33.92	0.0067	0.8346	1.0248	1.0386

Figure 6. Results one of the 7 air samples from Bishkek and Kant in SEM-EDS.

DISCUSSION

Numerous studies have looked into methods for locating and handling asbestos-containing items in residential settings and the resulting health impacts of asbestos domestic exposures [57]. It is well known many homes constructed before the 1980s used materials containing asbestos, which, when disturbed, can spew fibres into the air. Airborne asbestos exposure at home is a significant health risk that can result in various issues. However, particularly the countries of Central Asia still mine and produce asbestos-containing commodities, which are widely used at homes and can lead to asbestos exposure [56].

The concentrations of pollutants in urban settings have been the subject of several studies. For instance, one of the primary causes of asbestos fibre emissions into the urban air in Yazd is heavy traffic, which results in cars braking and clutching. To track the number of asbestos fibres in the surrounding air and create a GIS distribution map of the city [58]. The summer and winter mean concentrations of asbestos fibres were 11.40 ± 2.14 and 14.38 ± 2.52 ff/L, respectively, with the Baitolmoghaddas square station registering the most significant concentration.

A study conducted in South Korea in 2021 [59] examined 42 locations and various exposure sources. The results revealed the concentration of asbestos in urban areas 3.2 x 10^{-7} ff/L (0.00032 f/cc (measured by phase-contrast microscopy - PCM)) and 4.6 x 10^{-7} ff/L (0.00046 f/cc (measured by scanning electron microscopy)) and rural areas 5.6 x 10^{-7} ff/L (0.00056 f-PCM/cc) and 4.5 x 10^{-7} ff/L (0.00045 f-TEM/cc).

According to Bruno and colleagues (2023) [60], the concentration values were below 2 ff/L in 500 air samples collected from 111 Italian buildings with the possibility of asbestos fibre release into the environment from asbestos-containing materials.

It is widely accepted that there is little exposure to asbestos-containing materials in residences, however, according to WHO there is no safe level of asbestos exposure. Even after accounting for smoking patterns, research has indicated that residing in a home with asbestos-containing materials is linked to an elevated risk of lung cancer. The estimation of risk for asbestos-related lung cancer and mesothelioma was extrapolated in 2000 by WHO, where exposure to asbestos fibres in a lifetime (50 years) above the range of 1 ff/L (1000 f/m^3) would pose a risk to asbestos-related diseases (ARDs) [61].

One of the results of this pilot study of the environmental samples has shown significantly high exposure to asbestos in Kant town, home to two asbestos factories. The concentration in Kant town was from 1.2 to 30.2 ff/L depending on the vicinity of the asbestos factory (Figure 5) and these findings are concerning. However, in Bishkek, the concentration of asbestos in the air was from 0 to 0.9 ff/L (Figure 4), even though the findings are lower which can be explained by the absence of asbestos factories in the area, compared to Kant, but still, the risk of ARDs may exist from asbestos-containing materials widely used in the houses.

The analysis of samples of asbestos bulk material and asbestos-containing products has revealed that they are composed of "pure chrysotile" without any amphibole contaminants. This finding is significant as amphibole contaminants are known to be more hazardous to human health than chrysotile.

CONCLUSION

These findings highlight the need for continued monitoring of asbestos levels in urban areas and areas where asbestos factories are present. Implementing measures to reduce asbestos exposure and ensure the general public's safety is essential.

CHAPTER 4.
Occupational exposure to chrysotile in an asbestos cement factory in Kyrgyzstan

INTRODUCTION TO CHAPTER 4.

Previous research has confirmed that the asbestos currently in use is pure chrysotile (Chapter 3). However, an environmental exposure study has shown that the level of exposure to chrysotile from commodities in common use is lower compared to Kant town. Emissions from factories producing asbestos can explain these expected findings. Consequently, we investigated occupational exposure in one of the private industries to assess the actual workplace situation.

Moreover, in the narrative systematic review, we have already outlined the outdated methods used in Central Asian countries to assess the exposure at the workplace in the asbestos-producing industry, as a result, the European approach applied to this research revealed the extremely high content of fibres in samples.

The outcomes were submitted for publication in a peer-reviewed journal[2].

[2] This manuscript was submitted to Occupational Medicine for peer-reviewing on December 22, 2023. Manuscript ID: OM-23-OP-275.

Occupational exposure to chrysotile in an asbestos cement factory in Kyrgyzstan

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Running title: Occupational exposure in an asbestos cement factory in Kyrgyzstan

Teaser text

One ostensible justification for the continued usage of asbestos is the persistent and unfounded belief that chrysotile is safe when used in controlled amounts as its effects are dependent on the cumulative dose, and they are only observed at extremely high exposure levels. However, this study has revealed alarmingly elevated levels of asbestos in workplaces, pointing out that relevant situations of occupational asbestos exposure are still current in low and middle-income countries where asbestos is still mined and processed.

Abstract

Background: An increasing number of countries are banning the production and use of asbestos because of the 1986 Basel Convention and its reconsideration in 2022. Nevertheless, some countries, including Kyrgyzstan, are still miners or consumers of asbestos.

Aims: The main objective of the study is to assess the occupational exposure to asbestos of workers engaged in a production facility of asbestos-cement products in Kyrgyzstan.

Methods: Monitored workers (n = 18) were divided into three “Similar Exposure Groups” (SEGs; SEG-1: asbestos loading; SEG-2; asbestos-cement mixing; SEG-3: cutting of asbestos-cement sheets) according to EN 689 standard. Samples were collected through personal sampling, and subsequently examined by means of scanning electron microscope equipped with an energy dispersive spectrometer for the compositional analysis of each fibre. The numerical concentration of airborne asbestos fibres was henceforward determined by dividing the number of fibres and the volume of sampled air" (expressed in number of fibres per millilitre of air: ff/ml)

Results: Investigated workers resulted to be exposed to chrysotile fibres. Results outlined extremely high exposure levels for SEG-1 (2.2 ± 2.1 ff/ml) and SEG-3 (4.7 ± 1.6 ff/ml) workers and lower - but still relevant - exposure values for SEG-2 (0.91 ± 2.6 ff/ml) workers.

Conclusions: The results obtained in this case study can help to document potentially critical situations of occupational exposure to asbestos that can still occur nowadays in low and middle-income countries where asbestos is still mined and processed.

Key Words: exposure assessment; occupational exposure, chrysotile asbestos; asbestos cement; personal sampling.

Introduction

Asbestos (i.e., two mineralogical groups of silicate minerals, namely serpentine (chrysotile) and amphiboles (amosite, crocidolite, anthophyllite, tremolite and actinolite)) [1,2] have been widely used in the 20th century in many industrial sectors because of their characteristics (e.g., fire resistance, conductivity, thermal and noise insulation capacity, mechanical resistance, etc.) [3]. All types of asbestos are classified as carcinogenic (IARC, 2011, 2012; NIOSH, 2011) and exposure to asbestos increase the risk of developing non-malignant (including pleural plaques and asbestosis) and malignant (including lung, ovary, and larynx cancer and mesothelioma) asbestos-related diseases (ARDs) [1,2,5].

The highest asbestos exposure is expected in occupational environments, where manipulation processes may release asbestos fibres and expose workers [2,6–8]. Several countries have banned or severely regulated asbestos, since the 1986 Basel Convention [9,10] and its reconsideration in 2022 [11]. In these countries, scenarios of occupational exposure to asbestos fibres no longer exist, except for operators still involved in asbestos removal. Nevertheless, other countries, including the countries of Central Asia (i.e., Kyrgyzstan, Kazakhstan, Tajikistan, Turkmenistan, and Uzbekistan), are still producers and/or consumers of asbestos and asbestos-containing materials [3,12]. Nevertheless, significant gaps are still present in the existing research on exposure to asbestos and on the link between ARDs (particularly mesothelioma) and asbestos in central Asia [13]

To help fill this gap, an occupational exposure assessment study was conducted by means of environmental sampling and up-to-date analytical methods in an enterprise in Kyrgyzstan producing chrysotile cement artifacts (i.e., asbestos-cement pipes, asbestos-cement sheets (slate), fibre-cement boards, cement-sand tiles) using only chrysotile. The production process in the study company is started in late 1960's and was subjected to large-scale modernization

in 2010s. A brief description of the production process is reported in supplementary material (Text S1).

METHODS

The hypothesis of the study was that workers in the investigated company may be exposed to relevant concentrations of chrysotile fibres via inhalation while performing different tasks. The sampling campaign consisted of personal sampling. Specifically, n = 18 samples collected through personal monitoring were obtained from the same number of workers, which were divided into three "Similar Exposure Groups" (SEGs; SEG-1: asbestos loading; SEG-2; asbestos-cement mixing; SEG-3: cutting of asbestos-cement sheets; n = 6 samples per each SEG), according to EN 689 (EN 689:2019+AC:2019). These SEGs were chosen because of their expected high exposure to chrysotile fibres and since they are expected to cover all the most relevant exposure situations within the investigated company. Further Details on SEGs are reported in Table 1. Sampling method was derived from ISO 13794:2019 [15] and ISO 14966:2019 [16] standards, and from the Italian Ministerial Decree of 6 September 1994 [17]. 8-hour personal air samples were collected in the breathing zone of selected workers for the assessment of inhalation exposure. Sampling was performed with a sampling head (consisting of a 30 mm long cylindrical cowl and a filter holder with backing filter) connected to a sampler (SKC AirChek XR 5000; flow: 2 L/min) fitted with polycarbonate filters (Nuclepore™ Whatman™; 25 mm in diameter; porosity 0.8 µm). All the sampling pumps were calibrated during the pre-sampling phase (±5 % of the nominal flow) and the flows checked at the end of each sampling session, verifying that the maximum variation in the pre- and post-sampling flow was not >5%. Collected samples were subsequently analysed using a scanning electron microscope - SEM (XL30 ESEM – FEG, Philips; 400 microscopic fields investigated for each sample at 2000x; Working voltage: 20 KeV; Working distance: 15 mm) equipped with an energy dispersive spectrometer (EDX, Quantax 00, Bruker Karlsruhe, Germany) for the

compositional analysis of each fibre, to uniquely identify the nature of the fibres and classify them according to their chemical composition. The determination of the numerical concentration of airborne asbestos fibres (expressed in number of fibres per millilitre of air: ff/ml) in the atmosphere was carried out following a method derived from the Italian Ministerial Decree 06/09/1994 (Annex 2B) [17] and the ISO 13794:2019 method [15]. It is worth noting that most of the sampled filters resulted to be overloaded with dust and fibres, therefore an *ad-hoc* treatment was necessary before SEM analysis to make them readable. Specific details regarding the adopted preparation procedure and a-posteriori correction are reported in the supplementary material (Text S2). Anyhow, it is worth noting that these a-posteriori corrected results may be affected by a higher and unknown uncertainty with respect to standard methods.

RESULTS

A summary of risk management measures implemented at the study company is reported in Table 1 (but it is worth noting that compliance with risk management practices (particularly the use of PPE) by workers was not always correct and rigorous. An overall synopsis of the exposure monitoring results (after a posteriori correction) is shown in Table 2 (raw data – i.e. before correction – are presented in table S1 (supplementary material)). As expected, the investigated workers resulted to be exposed to chrysotile fibres. It should be noted that Kyrgyz government's regulations on acceptable maximum permissible concentration MPC limits of dust contained asbestos in industries, is equal to 2 mg/m^3 [18]. The gravimetric method is obsolete for measuring asbestos contamination since it is impossible to obtain a count of asbestos fibres (and thus to obtain a quantitative and specific value for airborne asbestos concentrations) and since it has not been shown to be a good indicator of risk in dose-response studies. [13,19]. The recent directive (EU) 2023/2668 [20] has established to modify the former OELV (8-hour time-weighted average (TWA)) 0.1 fibres/cm^3 established by Directive 2009/148/EC, reducing it to 0.01 fibres/cm3 (within 20 December 2029) and then progressively

to 0.002 fibres per cm^3 (from 21 December 2029). If compared to the European Union Occupational Exposure Limit, obtained results (GM ± GSD) outlined a constant non-compliance with the limit value, with extremely high exposures for SEG-1 (2.2 ± 2.1 ff/ml) and SEG-3 (4.7 ± 1.6 ff/ml) and lower - but still relevant - exposure values for SEG-2 (0.91 ± 2.6 ff/ml).

DISCUSSION

Due to the sample size and some deviations from standardized protocols, this project can be regarded as a wide exploratory rather than a confirmatory study. Nevertheless, obtained results support the hypothesis that relevant situations of occupational and environmental exposure to asbestos are still current in low and middle-income countries where asbestos is still mined and processed [3]. It should be noted that comparison with other occupational exposure assessment studies in occupational settings in the same area (Central Asia) is difficult since only a small number of recent studies are available on asbestos and its impact on the health of workers and the population in central Asia countries [13]. Further, occupational exposure assessment studies are almost completely missing, and those available (Korotenko et al., 2011; Amanbekova et al., 2014; Ibraev et al., 2015) rely on non-specific and outdated techniques [13,19]. An in-depth comparison with the results of measurements or estimates of asbestos exposure from other investigation is beyond the objectives of this study, but some are reported below, to provide a basis for comparison for the interpretation of the results. Briefly, and contextualizing it to the Italian case studies, the highest exposure in the asbestos cement sector was observed before 1974 and declined sharply after 1980. The decennium 1970-1979 was a period of transition, with high exposures at the beginning and a reduction in the last quinquennium [24]. For example, in the Eternit Plant (Casale Monferrato – Italy; active from 1907 to 1986; involved in the production of plain and corrugated sheets, chimney tubes and high-pressure pipes using both chrysotile and crocidolite) the concentration of airborne asbestos fibres was found to be

above 20 ff/ml in 11 samples out of 22 in 1971. Average concentration was 13.5 ff/ml in the production areas and 303.8 ff/ml in the area where asbestos and cement were dry mixed. In 1973, the averages of repeated measurements of asbestos fibre concentration were in the order of 13–15 ff/ml in the mixing department, 1.2–1.8 ff/ml in the production department and 0.7–1 ff/ml in the finishing department. Regular monitoring of airborne asbestos fibres started in 1978 (when working procedures were improved) and average concentrations of asbestos fibres were reported to be in the range 0.15–1.1 ff/ml in the mixing department, 0.18–1.05 ff/ml in the production department and 0.29–1.1 ff/ml in the finishing department [25]. Several comparisons can be made also with the results reported in a WHO (World Health Organization) document specifically dedicated to occupational exposure to chrysotile asbestos and dating back to 1998. It presented occupational exposure data analysed or estimated during 1970s and 1980s in selected work zones of asbestos-cement factories. Personal concentrations ranged from 0.03 to 9.5 ff/ml in bag opening and mixing units. Other activities such as cutting, drilling, grinding, and sanding caused exposures between 0.17 and 27.5 ff/ml [26]. More in general, a European Job-Exposure Matrix [27,28] exposure values estimated for asbestos cement products makers (ISCO 1968 code = 94330) in the manufacture of fibre cement (industrial sector (NAF 2000 code) = 266J - manufacture of fibre cement) were estimated to be within 1 and 10 ff/ml (estimated average level of exposure in one working day) both before 1977 and in the period 1978-1997. Exposure in this scenario was classified as “direct exposure” (i.e., the worker was expected to handle asbestos containing materials); proportion of exposed in the job for one year and frequency of exposure were estimated to be >70% of the subject and >70% of working time, respectively. Overall, the exposure values measured in this study are in the same order of magnitude as (or higher than) measurements obtained in the asbestos cement production sector in European scenarios in the late 1970s - 1980s, that is the period preceding the great decrease in exposure values resulting from the growing awareness of asbestos hazards and the final introduction of bans on asbestos [29].

The results obtained in this case study depict a scenario akin to the situation fifty years ago in countries where asbestos has been banned or strongly limited. It might be helpful to document potentially critical situations of occupational exposure to asbestos that can still occur nowadays in Kyrgyzstan, where asbestos is still processed, as well as be a starting point for any decisions in terms of risk management.

Key learning points

What is already known about this subject:

- All types of asbestos are classified as carcinogenic and exposure to asbestos increases the risk of developing non-malignant and malignant asbestos-related diseases (ARDs)
- Several countries have banned or severely regulated asbestos, nevertheless, other countries, including the countries of Central Asia, are still producers and/or consumers of asbestos and asbestos-containing materials.
- Significant gaps are still present in the existing research on exposure to asbestos and on the link between ARDs (particularly mesothelioma) and asbestos in Central Asia.

What this study adds:

- This study has revealed alarmingly elevated levels of asbestos exposure in the investigated workplace, far exceeding the permissible limits set for European asbestos factories in the 1980s.

What impact this may have on practice or policy:

- Despite this study can be considered a wide exploratory rather than a confirmatory study, obtained results make a certain contribution to the hypothesis that relevant situations of occupational asbestos exposure are still current in low and middle-income countries where asbestos is still mined and processed.
- The results obtained in this case study depict a scenario akin to the situation fifty years ago in countries where asbestos has been banned or strongly limited. It might be helpful to document potentially critical situations of occupational exposure to asbestos that can still occur nowadays in countries where asbestos is still processed, as well as be a starting point for any decisions in terms of risk management.

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Conflict of interest statement

A.S., D.M.C., C.C. served as consultants in trials concerning asbestos-related diseases. All other authors declare they have nothing to disclose.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Table 1. Description of investigated Similar Exposure Groups (SEGs) and risk management options used for different SEGs.

SEG#	SEG 1	SEG2	SEG3
SEG name	**Asbestos loading**	**Asbestos-cement mixing**	**Cutting of asbestos-cement sheets**
Description of workers' tasks	Lifting 50kg bags of asbestos to the conveyor. Opening bags with asbestos and loading asbestos on the conveyor belt for delivery to the runners or to the compartments. Stacking empty bags in bags, tying them with wire and handing them over to the warehouse.	The operator's work is automated: The mixture fed after fluffing with water is mixed with Portland cement in water to obtain a homogeneous chrysotile cement slurry in a special container with subsequent feeding into the moulding machine. Washing of the container where asbestos and cement were mixed (a cylinder 2-3 meters high and 1-2 meters in diameter) is partly manual.	Saw cutting of ready-made asbestos-cement sheets to the required dimensions.
Risk Management			
Organization, Procedures	Trainings: introduction to operation, training on compliance with safety regulations when working with equipment, training on emergency situations		
Engineering control	Open-handled process, general ventilation	Closed process, semi-automated, General ventilation	Open-handled process, vacuum suction hoses for collecting dust when cutting facade sheets
Personal Protective Equipment	Protective helmet; masks: KN95 and surgical masks, change of clothes, safety boots and gloves	Protective helmet; masks: KN95 and surgical masks, change of clothes and safety boots.	Protective helmet; masks: FFP2, KN95 and surgical masks, change of clothes, safety boots and gloves.

Table 2. Asbestos occupational exposure detected with the ISO-modified protocol through personal sampling in three Similar Exposure Groups (as described in Table 1).

SEG	Location	Sample ID	Concentration [ff/ml]	Theoretical uncertainty Concentration [ff/ml] (CI95%) (calculated assuming Poisson distribution on individual filters)	
				Lower limit	Upper limit
1	Slate Procurement Workshop	K3	1.7	1.5	1.8
		K5	1.2	0.95	1.4
		K10	1	0.94	1.2
		K11	1.6	1.5	1.8
		K15	4.5	4.3	4.8
		K17	7.5	7.2	7.8
		Summary for SEG 1	*GM = 2.2 ff/ml; GSD = 2.1 ff/ml; Shapiro-Wilk test on Log-transformed data; p = 0.210 It is assumed that the data is log-normally distributed; hypothesis of establishment of SEG is confirmed)*		
2	Sheet Procurement Workshop	K1*	3.3	3.1	3.5
		K4	0.35	0.33	0.38
		K8	1.2	1.1	1.3
		K9	0.26	0.24	0.28
		K16	0.61	0.57	0.64
		K18	2.5	2.3	2.6
		Summary of SEG 2	*GM = 0.91 ff/ml; GSD = 2.6 ff/ml; Shapiro-Wilk test on Log-transformed data; p = 0.286 It is assumed that the data is log-normally distributed; hypothesis of establishment of SEG is confirmed)*		
3	Sawmills	K2	5.0	4.6	5.3
		K6	3.1	2.9	3.2
		K7*	4.4	4.1	4.6
		K12	3.0	2.8	3.3
		K13	12.5	11.7	13.2
		K14	4.2	4	4.5
		Summary of SEG 3	*GM = 4.7 ff/ml; GSD = 1.6 ff/ml; Shapiro-Wilk test on Log-transformed data; p = 0.078 It is assumed that the data is log-normally distributed; hypothesis of establishment of SEG is confirmed)*		

Note: GM = Geometric Mean; GSD = Geometric Standard Deviation; CI95% = confidence interval 95%; ff/ml = fibres/millilitre.

** These results may be affected by uncertainty due to analytical issues.*

Occupational exposure to chrysotile in an asbestos cement factory in Kyrgyzstan

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SUPPLEMENTARY MATERIAL

Text S1

The manufacturing technology of asbestos slates consists of the following operations: asbestos mixing, stuffing, preparation of asbestos-cement mass, sheet moulding, waving, and hardening of products. The composition of the asbestos mixture is calculated in accordance with the technological needs and the availability of asbestos of the required grades in the warehouse. Asbestos of each type is dosed separately. Asbestos is unloaded from batchers and receiving hoppers onto a common belt conveyor, through which it is fed into the runners. At this stage of the technological process, asbestos-containing dust may be released and dispersed into the workplace. In runners, asbestos is pressed down, moistened. The degree of fluffing of asbestos is at least 30-35%. The final fluffing is carried out in a hydraulic fluffer, where the degree of fluffing is brought to at least 85%. The preparation of asbestos-cement mass (mixing fluffed asbestos with cement) takes place in a turbo mixer. Mixing time 3-4 minutes. The asbestos-cement mass from the turbo mixer is fed into the bucket mixer, where a certain supply of asbestos-cement mass is created, which is necessary for the continuous operation of the sheet-forming machine. From the bucket mixer, the mass is directed along the chute to the baths of the mesh cylinders of the sheet-forming machine. At this stage of the technological process, asbestos fibres emissions into the workplace atmosphere are not expected to be as significant as in the previous processes. The process of manufacturing asbestos-cement products on a sheet-forming machine consists of the following stages: first, primary layers (films) are formed on mesh cylinders, then they are transferred to technical cloth, then to a sizing drum, where the roll is formed, which is subsequently cut into formats of a given size. After that, agitation occurs on the wave-stopping unit (VSA) and the slate sheets are placed on the hardening conveyor by the stacker. After the curing conveyor, having passed through the humidifier, the sheets are stacked and placed in the finished product warehouse.

There are no emissions into the atmosphere at these stages of the technological process. A further detail is provided hereafter.

Production of sheet chrysotile cement products is carried out on a round-grid sheet-forming machine (Gatchek machine). To operate the machine and to get the finished product at the output, it is necessary to feed the initial materials into it. First, a mixture of chrysotile fibres is prepared by mixing several grades of chrysotile. After that, this mixture is thoroughly mixed with Portland cement in water until a homogeneous chrysotile cement suspension is obtained. Then, the prepared suspension is sent to the bucket agitator, from where it goes to a special chute, where it is diluted with additional water to the required concentration. And only after that, the obtained slurry is sent to the moulding machine. In metal baths, filled with chrysotile cement slurry, hollow cylinders of frame type are covered with metal mesh. Paddle stirrers stir the chrysotile cement slurry entering the baths of the mesh cylinders. The slurry is filtered through the mesh cylinders' mesh, and a wet chrysotile cement layer remains on their surface. The technical filter cloth removes the chrysotile cement layers formed on the surface of the three mesh cylinders and moves to the format drum. Passing through three squeezing stages (rollers), the layers are coiled on the surface of the format drum, forming a knurling. When the set thickness of the knurling is reached, the shearer is switched on by the signal of the thickness gauge. The knurling is cut along the formatted drum and passes to the cutting unit conveyor. After passing through the cloth cleaning devices, the technical cloth is directed to the screen cylinders, and a new working cycle starts. The chrysotile-cement roll removed from the format drum can have varied sizes, which depend on the drum's diameter and the felts' width. At the cutting machine, the roll is cut into sheets of the required size and the edges are cut. The further process depends on the type of product to be produced. The raw knurling is further corrugated mechanically in the production of corrugated sheets. After corrugation, the sheets are fed into a pre-curing conveyor and a humidifier. The corrugated sheets are stacked at the humidifier outlet and sent to the product warehouse for final curing.

In the production of flat-pressed sheets, the roll is conveyed by a conveyor belt to the stacker, which stacks the sheets. Here, special metal spacers are placed between the sheets. The stacks are then transported to the presses for additional compaction. The exact process is followed in producing flat, uncompressed sheets, except that the sheet stacks are not sent for additional compaction.

Pipe-forming machine. Production of chrysotile cement pipes is carried out on pipe-forming machines, which work on the same principle as sheet-forming machines, but instead of a forming drum, they are equipped with removable metal rollers. The diameter of these rolls corresponds to the inner diameter of the pipes to be moulded. The tubes removed from the rollers undergo heat and humidity treatment in the curing conveyor, where they are rotated around their own axis to ensure a strictly cylindrical shape. Then their ends are cut with disc knives. Some of the pipes are cut into blanks for couplings. Grooves for rubber sealing rings are bored on the inner surface of the pressure couplings. Further curing of pipes and couplings is continued in a warm warehouse until they reach the standardised strength.

Text S2

The determination of the numerical concentration of airborne asbestos fibres (expressed in number of fibres per millilitre of air: ff/ml) in the atmosphere was carried out following the indications of the and the ISO 13794:2019 method [15]. The analyses of the collected samples were carried out using a scanning electron microscope – SEM (XL30 ESEM – FEG, Philips; 400 microscopic fields investigated for each sample at 2000x; Working voltage: 20 KeV; Working distance: 15 mm) equipped with an energy dispersive spectrometer (EDX, Quantax 00, Bruker Karlsruhe, Germany) for the compositional analysis of each fibre, to uniquely identify the nature of the fibres and classify them according to their chemical composition. The criteria for the fibres' counting are those described in the ISO 14966:2019 standard [16] which defines the "respirable fibres" as those fibres having the following dimensional characteristics: length $\geq$ 5µm, diameter $\leq$ 3µm,

length/diameter ratio ≥ 3. The samples were opened and managed inside a Class II laminar flow hood to prevent any fibre dispersion into the laboratory. The overloaded samples would not have been readable and their manipulation, outside the collective protection equipment, would not have been safe. Then, they were processed before preparing the SEM analysis according to the internal procedure described below. First, excess dust and fibres were manually removed, using a spatula with extreme care taken to avoid losing mass and ruining the filter, from each overloaded filter (henceforth called "treated filter"-TF). That removed mass was weighted through an analytical balance with a precision of 0.1 mg (Eternity 100CAL, Gibertini Elettronica, Novate Milanese, Milan, Italy) and then stored in a vial. Thereafter, the TF was weighted in turn using a microbalance with a readability of 1 μg (Gibertini micro1000; Gibertini Elettronica, Novate Milanese, Milan, Italy) in accordance both with reference methods (UNI EN 14907, 2005, UNI EN 12341, 2014) and accepted standard practices. Since the individual filters had not been weighted before sampling, a consistent number (N= 15, to have good statistics) of laboratory blank filters of the same typology (i.e., polycarbonate) were weighted following the same procedures. The average mass of the blank filters was then used to calculate (with good approximation) the net dust and fibres mass that remained on each TF by differential weighing. Before SEM analysis, each filter (both non-treated and treated filters) was mounted onto a 25-mm aluminium stub and coated with a thin (10 nm) layer of gold, obtained by metallization of the sample (three depositions; 20 seconds at 20 mA each - Cressington 108 auto Sputter Coater), to make the sample conductive. The numerical concentrations of asbestos fibres were then calculated using the following relationship (Equation 1).

(equation 1)

$$Concentration\ \left({}^{ff}/_{mL}\right) = \frac{\frac{number\ fibres\ counted\ \ (ff)}{number\ analyzed\ fields \times \text{Microscopic field area}\ (mm^2)} \times Effective\ filter\ surface\ (mm^2)}{Normalized\ Volume\ (mL)}$$

Where:

- number of fibres counted (ff): are the fibres counted on the filter;
- number of fields counted: these are the fields observed for each count;
- Microscopic field area (mm^2): surface of the microscopic field, which corresponds to 0.00262 mm^2;
- Effective filter surface (mm^2): inspectable filter surface which corresponds to 283 mm^2;
- Normalized V (ml): sampling volume corrected for the temperature detected at the time of calibration and sampling.

Lower and upper fiduciary limits were calculated, with 95% probability, assuming a Poisson random distribution of the fibres on the sampling membrane. The limit of detection (LOD) of this analytical technique corresponds to the upper confidence limit calculated based on the Poisson distribution with 95% confidence interval (CI) and depends on the number of fibres detected, the number of microscopic fields observed and the sampled volumes. For the overloaded filters, the final number of fibres was estimated also considering the number of fibres present in the amount of mass removed from the filter surface. To do this, the following proportion (equation 2) was computed considering that the composition of the mass removed and the mass remaining on the filter were homogeneous with each other:

(equation 2)

$$mass\ remained\ on\ TF : mass\ removed\ from\ TF = number\ of\ fibres\ counted\ on\ TF : number\ of\ fibres\ in\ the\ mass\ removed$$

So, the final number of fibres was calculated as the sum of the number of fibres counted on TF and the number of fibres in the mass removed. Lastly, the final concentrations of the overloaded filters were computed applying the “Equation 1”, where the number of fibres counted was represented by the final number of fibres. Table 2 in the manuscript present results after this a-

posteriori correction; table S1 present raw concentrations, calculated before the application of equation 2 for the a-posteriori correction.

Table S1. Asbestos occupational exposure detected with the ISO-modified protocol through personal sampling in three Similar Exposure Groups (as described in Table 1).

SEG	Location	Sample ID	Concentration [ff/ml]	Theoretical uncertainty Concentration [ff/ml] (CI95%) (calculated assuming Poisson distribution on individual filters)		Mass on filter [mg]	Mass removed from the filter [mg]
				Lower limit	Upper limit		
1	Slate Procurement Workshop	K3	0.35	0.29	0.43	0.16	0.6
		K5	1.2	0.95	1.41		
		K10	0.27	0.22	0.33	0.386	1.1
		K11	0.31	0.25	0.38	0.52	2.2
		K15	0.47	0.39	0.56	0.242	2.1
		K17	0.32	0.27	0.39	0.408	9.0
		Summary for SEG 1	*GM = 0.42 ff/ml; GSD =1.7 ff/ml; Shapiro-Wilk test on Log-transformed data; p = 0.056 It is assumed that the data is log- normally distributed; hypothesis of establishment of SEG is confirmed)*				
2	Sheet Procurement Workshop	K1*	0.35	0.29	0.42	0.307	3.0
		K4	0.05	0.04	0.06	0.418	2.8
		K8	0.22	0.18	0.26	0.501	2.3
		K9	0.03	0.03	0.04	0.572	4.0
		K16	0.05	0.04	0.06	0.25	2.8
		K18	0.23	0.18	0.27	0.254	2.5
		Summary of SEG 2	*GM = 0.10 ff/ml; GSD = 2.8 ff/ml; Shapiro-Wilk test on Log-transformed data; p = 0.199 It is assumed that the data is log- normally distributed; hypothesis of establishment of SEG is confirmed)*				
3	Sawmills	K2	0.75	0.61	0.90	0.425	2.4
		K6	0.32	0.26	0.39	0.21	1.8
		K7*	0.39	0.31	0.47	0.307	3.2
		K12	0.51	0.42	0.62	0.345	1.7
		K13	1.8	1.5	2.1	0.367	2.2
		K14	0.53	0.44	0.63	0.298	2.1
		Summary of SEG 3	*GM = 0.59 ff/ml; GSD = 1.8 ff/ml; Shapiro-Wilk test on Log-transformed data; p = 0.414 It is assumed that the data is log- normally distributed; hypothesis of establishment of SEG is confirmed)*				

Note: GM = Geometric Mean; GSD = Geometric Standard Deviation; CI95% = confidence interval 95%; ff/ml = fibres/millilitre.
** These mass on filter may be affected by uncertainty due to analytical issues.*

CHAPTER 5.
A study on post-mortem lung samples from the general population of urban areas of Kyrgyzstan and Italy

INTRODUCTION TO CHAPTER 5.

The previous studies in Chapters 3 and 4 provided evidence of significantly high levels of occupational exposure, as well as environmental exposure in Kant town, which is deeply concerning (Chapter 4). However, one crucial part could help address the clarification and support or challenge our hypotheses. Specifically, we wanted to determine the amount of asbestos inhaled by the population living in areas with confirmed exposure to chrysotile fibres despite the low incidence of ARDs in Central Asian countries. To investigate this, we collected 100 post-mortem lung samples and analysed them in the laboratory of the University of Pavia in collaboration with the University of Turin. The following chapter presents an overview of our research and the results obtained. It is worth noting that the findings from the lung samples from the general population of Bishkek particularly confirm the investigation of environmental exposure in Bishkek. Additionally, our collaboration on the Kyrgyz samples project continues, along with similar research in Italy in which I participated, and I am co-author. The outcomes of the research helped to understand the phenomenon under study with a broader perspective and they were published in peer-reviewed journals[3]. (see Annexes 1 and 2).

[3] Journal of Thoracic Disease and Carcinogenesis

THE TYPE AND CONTENT OF ASBESTOS FIBRES IN POST-MORTEM LUNG SAMPLES FROM THE GENERAL POPULATION OF BISHKEK AND KANT, KYRGYZSTAN.

Introduction

The studies conducted in various countries, including the described new data on genetic predisposition to MPM, one of the aggressive consequences of ARD, help us better understand the disease's aetiology, pathogenesis, outcome, and burden.

The fact that there are subjects still exposed to asbestos (especially chrysotile) might help to clarify the following aspects:

What are the levels of exposure to asbestos of the subjects living in countries where the mineral is still used (general population, population living close to asbestos mining sites and asbestos materials production)?

What are the main types of fibres present, particularly if the exposed people have only chrysotile in their lungs or if other kinds of asbestos are present?

Which is the persistence of chrysotile (and, if present, other kinds of asbestos) in the lungs.

The gold standard for assessing asbestos in lungs is scanning electron microscopy with energy dispersive spectrometry (SEM-EDS) that allows counting, measuring and chemically analysing the inorganic fibres contained in the digested lung parenchyma. Asbestos bodies (AB) are asbestos fibres that have undergone a coating process in the lung microenvironment [3,62], and are counted separately. The size and chemical characteristics of fibres, and their concentration, are related to various extents to pathological processes occurring in the lungs [3,10,34,63,64].

Purpose and objectives of the study

The general purpose of the study is to study the levels of asbestos exposure of the Kyrgyz population and, if possible, to define its variability in different population subgroups (general population, population living near plant-producing asbestos manufacturers, asbestos workers).

The main objectives of the study are:

To assess the type of fibres found in the lungs of these subjects to find out if the chrysotile is pure or contaminated by other mineral fibres.

To define their characteristics in terms of size (length, width, and the ratio between these two parameters)

To measure their lung concentration (number of fibres per gram of dry weight of lung tissue- ff / gps) according to aspects such as asbestos worker, subject living close to the Kant enterprise, or general population non-exposed occupationally, but environmentally.

To compare these data with those obtained from a group of former asbestos workers currently studying in Italy.

To define and estimate the time of clearance of the fibres from the lungs of these subjects.

RESEARCH DESIGN

Study subjects

General population of Bishkek. The lung samples were collected during autopsies performed at the Republican Bureau of Pathology, Kyrgyz Republic. As biomaterials (lung samples) are routinely collected for diagnostic purposes, collecting any samples specifically for the present study was unnecessary.

2.1.2 Sample collection strategy

In the subjects under study, lung samples were collected from the inferior lobe of the right lung and fixed in formalin. Sample collection and formalin fixing was performed by the Pathology Department Republican Bureau of Pathology (Bishkek city), Kyrgyz Republic. Lung samples collected during forensic or clinical autopsies are ordered for whatever reason. Medical and residential data were collected and recorded for each deceased subject enrolled.

Research subjects

The target population is people who have died from any causes (not necessarily from ARD) in the area under study (Bishkek and surrounding areas, with particular attention to the neighbourhood of the Kant industries).

The cohort consists of 100 lung samples taken in Bishkek.

During sample collection, special attention was paid to subjects with previously demonstrated occupational exposure to asbestos or suffering from an ARD.

Study Duration and Estimates

The study has been covered the period from February 2021 to November 2023, however the samples derived from archives for the period from March 1, 2021, to December 31, 2022.

The duration of the study was 18 Months after the approval of the protocol by the Ethical Committee (Appendices 1 and 2).

Inclusion and exclusion criteria

The criteria for inclusion in the study are:

Samples from deceased persons older than18 years old

Patient deceased of any cause.

Included without regard to gender, race, or ethnicity.

The criteria of exclusion for obtaining lung autopsy samples:

Less than 18 years old

Elements for calculating sample size or cardinality.

There are few references in the literature to assess the mineralogical and dimensional characteristics of asbestos fibres depending on the type of associated asbestos pathology.

However, Dufresne et al (1996) [65] report the mean values of fibre length-to-diameter ratio found in a series of autopsy specimens found in 75 asbestos-related disease subjects with documented occupational exposure to asbestos analyzed / studied. in subgroups according to the type of pathology associated with asbestos. The authors found the following average length / diameter ratios:

- for people with asbestosis: 23,
- for persons with asbestosis and lung cancer: 25,
- for patients with mesothelioma: 28

We hypothesize that the mean value of the length / diameter ratio for subjects suffering from other asbestos-related diseases (non-mesothelioma and non-asbestosis) is comparable to the value reported by Dufresne et al. (1996) [65] for patients with asbestosis and lung cancer. Assuming greater variability in our series compared to the work of Dufresne et al. (1996) [65], considering that exposure is not only related to work, but also to the environment and family, an estimate of power was made with a standard deviation of 8.

Sample Size / Power Calculation

Taking the mean of the length-to-diameter ratio as stated above and according to the reference to the above article, the samples from 100 subjects obtained in the course of the study would allow us to compare the variable length/thickness ratio of the three disease groups (mesothelioma, asbestosis, other diseases associated with asbestos) by assessing statistically significant differences between the mean values of the groups with a degree of 89%, an alpha error of 0.05. The capacity would be estimated using the SPSS program.

Recruiting

Collection and storage of the samples have proceeded from archives of the Republican Bureau of Pathology of Kyrgyz Republic. In this case, recruiting was not applicable; moreover, it was impossible to reach a legal representative of the deceased patient; therefore, informed consent was not obtained. However, according to Decree 593 from 28 October 2014 of the Ministry of Health of the Kyrgyz Republic and Legislation 223 from April 08, 2020, biomaterials were already taken. The Ethics Committee approved the protocol for this study (see Ethical clearance).

Ethical clearance:

The ethics committee approved our protocol to study “Exposure to asbestos in Kyrgyzstan: fibre types, persistence, determinants of exposure and health risks” based on the Scientific and Production Center "Preventive Medicine" of the Ministry of Health of the Kyrgyz Republic in June 2022, extract from protocol №5 (Appendices 1 and 2). The ethics committee is internationally

certified by the US Department of Health and Human Services (HHS) Registration of Institutional Review Boards (IRB) and received IOR No. 0008909.

MATERIALS AND METHODS

The exposure to asbestos of the Kyrgyz general population was assessed using a scanning electron microscope equipped with energy dispersive spectroscopy (**SEM-EDS**). This technique is suitable to reveal:

1.The concentration and of inorganic fibres in lungs.

2.The concentration of asbestos bodies.

3.The size (length and width) of each fibre.

4.The mineralogical type of fibres.

The study was conducted on formalin fixed lung samples taken during forensic or clinical autopsies. An autopsy will be collected from the Pathology Department of Kyrgyzstan (100 samples). This part of study was done in the University of Pavia in collaboration with the University of Turin, Italy, with Prof. E. Belluso with the application of method contrubituted by her.

Sample Preparation

The sample preparation was performed in three steps: i) digestion of the biological material; ii) filtration of the suspension through a membrane; iii) filter preparation for SEM–EDS analysis. The method was tested for different kinds of biological materials, and it is the same for all types of samples.

The overall procedure is detailed in the following steps:

Digestion

A chemical digestion (instead of ashing) disregards organic materials [54]. A mass of 500 mg of tissue, previously preserved in formalin to 10% (Siac S.r.l., Italy) is digested in 30 ml of NaClO (Merck, Germany), to produce a suspension (the NaClO quantity has been optimised after different tests). The necessary time to complete the digestion strongly depends on the freshness of the sample. A mass of 5 g of the tissue is dehydrated to measure its dry weight, a quantity which is used to evaluate the concentration of fibres expressed in the number of fibres per gram of dry lung tissue.

Filtering

The obtained suspension is filtered on mixed cellulose esters membrane (Millipore, Italy) with a diameter of 25 mm (the same of the SEM pin stubs used), and with a pore size of 0.45 mm. A core surface of 19 mm in diameter of the filtering surface for a total of 284 mm2 (hereafter called ‘‘exposed area’’) is accessible to the microscopic observation.

Washing

During the filtering step, the membrane was washed thoroughly with warm distilled water to accelerate the dissolution of the micrometric crystals of NaCl grown during the chemical digestion. The NaCl precipitated on the membrane can both hide the inorganic particles and be included in the analysed volume disturbing the chemical analyses.

Dehydration of the Filter

The filter is dried at about 50 C for at least 12 hours. This temperature is enough to dry the membrane without burning it and without provoking chemical-physical alteration of inorganic particles.

Clarification

Clarification by acetone method (Merck Eurolab, France) glues the membrane to the SEM aluminium pin stub.

Sample Conductivity

Before the SEM–EDS study, the sample is made conductive by carbon sputter coating.

Observation by SEM

SEM observation is performed on a number of selected microscopic fields (MF) which is large enough to obtain a statistical sampling. Taking into account that official methods (ISO 14966, AIA-RTM 2, DM 6=9=94) to analyse airborne particles, the samples examined in 1 mm2 of the filter at 2000 M, and observed 800 MF's to cover an area (s) of 1.85 mm2 of the filter, i.e., 0.7% of the total area (S) of the filter. The observation is performed at 2000 M and the MF's are distributed along 5 parallel strips. Each strip is 16 mm long and is sampled at steps of 100 mm, thus obtaining 160 MF's per strip. The MF's along the same strip are spaced of 40 mm. The length of the steps (100 mm) and the separation of 2.5 mm between two adjacent strips are such that overlapping between different MF's cannot occur, being the dimension of each MF 40.1[66]57.8 mm.

Identification of Inorganic Fibres by SEM–EDS

The filter observation is carried out using backscattered electrons in order to detect only the inorganic particles and to disregard the organic ones. According to fibre definition, only particles with length-to-width ratio ≥3 is considered and then chemically analyzed by EDS. The fibres falling across the border of a MF contribute to 100% to the counting because the MF's are not contiguous. Chemical analyses are only qualitative because the nature of the sample does not allow the preparation required to obtain quantitative analyses.

The revealed chemical elements, the relative peak intensities and the morphology of the particles are normally sufficient features for identification.

RESULTS

This retrospective descriptive study involved the collection of lung tissue samples from 100 individuals who died from various causes in 2021. These samples were then examined through autopsies at the Institute of Pathology in Bishkek. To further analyse the samples, they were fixed in formaldehyde and archived.

A database was created using information from emergency room records and admission per diems. This database included details such as gender, date of birth, age at the time of death, month and year of death, nationality, address of residence (including town or village and district), diagnosis upon admission to the emergency room, COVID-19 nasopharyngeal swab results, cause of death, work location, and histological subtype MM.

Table 5. Gender distribution.

Gender		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	female	54	55,1	55,1	55,1
	male	44	44,9	44,9	100,0
	Total	98	100,0	100,0	

Table 5 shows the demographic characteristics of the cohort analysed. It consists of 98 subjects, of whom 54 were female and 44 males. Collecting remote pathological history, occupational history, and smoking habits was impossible.

However, some data regarding their occupational status at that moment was available and categorised into (Figure 7) workers (6), disabled (3), unemployed (9), pensioners (65), and not defined (17).

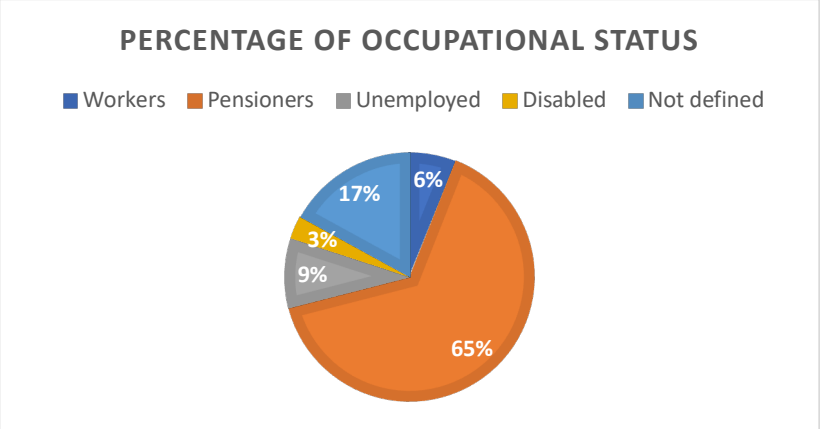

Figure 7. Percentage of occupational status.

The most frequent diagnoses on admission to the emergency department were pneumonia (86.7%) and tuberculosis (5.1%); the cause of death was then in descending order: acute circulatory failure

(50%), cardiac failure (24.5%), respiratory failure (18.4%), multi-organ failure (MOF) (3.1%), unspecified (3.1%) and cerebral oedema (1%). The mean age at death was estimated to be approximately 66 years with a minimum of 30 and a maximum of 96 years. More specifically, the average in women is 68 years, while in men, it is 64 ($P < 0.033$) and agrees with the increase in life expectancy expected in the female. As additional information it is reported that in the initial evaluation of patients an antigenic swab for COVID19 was performed, which was positive in 65.3% of cases, negative in 16.3% and not performed in 18.4% of cases.

The demographic distribution indicated that 54 resided in the Bishkek area, 17 in Alamudun, 8 in Ysyk-Ata, 4 in Issyk-Kol, 3 in Moskovskyi district, 2 in Kemin, 2 in Sokuluk, 1 in Kara Suu, 1 in Batken, 1 in Toguz, 1 in Jaiylskiy district, 1 in Jalal – Abad, 1 in Leninskyi district and 1 in Osh.

Currently, a total of 14 lung samples with the following serial numbers (ID): 13, 19, 22, 23, 25, 28, 36, 43, 41, 42, 64 and 72 have been analysed. Among these samples, numbers 19, 22, 28, and 36 were obtained from individuals residing in the Bishkek district, while numbers 13, 41, and 43 were from individuals living in the Alamudun area. Samples 23, 42, 64 and 72 were obtained from residents of Kant town.

The results of 14 post-mortem lung samples were negative for traces of chrysotile, but a single tremolite fibre was found (Table 6).

Table 6. The amount of asbestos and non-asbestos fibres found in the post-mortem samples of 14 lungs.

Program number	**Sample ID**	**Inorganic fibres (not asbestos)**	**Short fibres**	**Asbestos bodies**
1	13	0	0	0
2	19	**8**	**2**	0
3	20	0	0	0
4	22	**2**	0	0
5	23	**2**	**2**	0
6	25	0	0	0
7	28	0	0	0
8	36	**6**	**6**	0
9	43	**3**	**14**	0
10	41	**1**	**3**	0
11	47	**5**	**4**	**1**
12	42	0	0	0
13	64	0	0	0
14	72	0	0	0

Photo of single tremolite fibre found in only one lung sample in SEM-EDS is displayed in Figure 8.

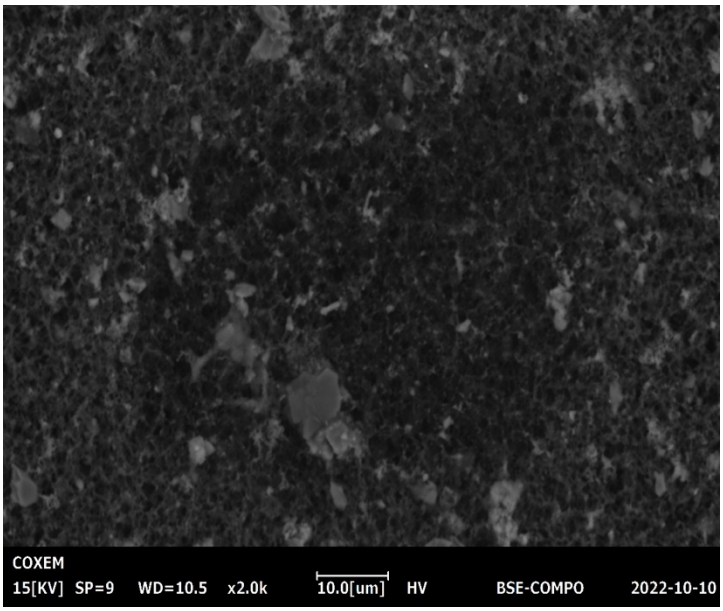

Figure 8. Photo of single tremolite fibre found in only one lung sample in SEM-EDS. Source: photo from SEM-EDS taken during the study of lung samples from Bishkek.

The diffraction spectrum obtained for the fibre in the sample and the characteristic for tremolite are shown in Figure 9.

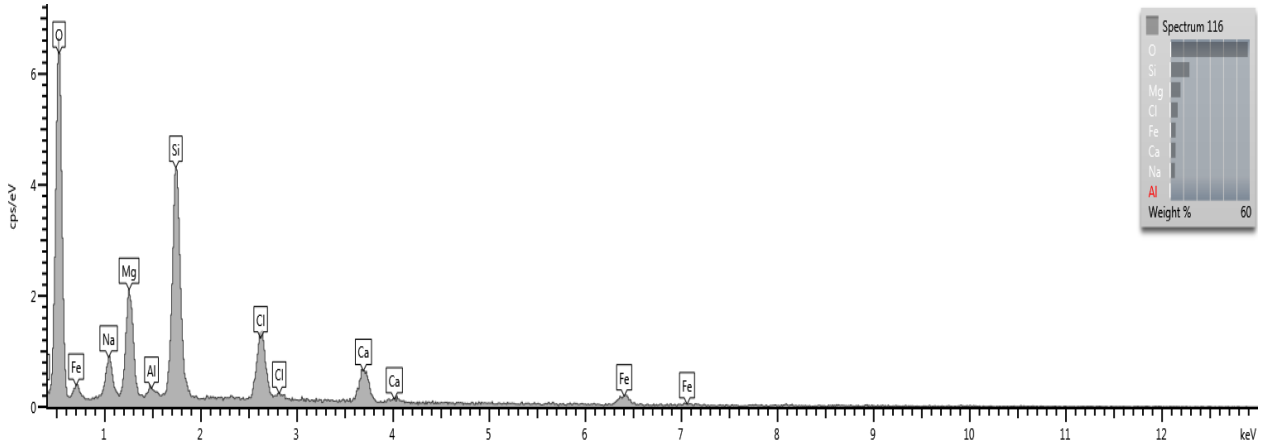

Figure 9. The diffraction spectrum obtained for the fibre in the sample and the characteristic for tremolite. Source: photo from SEM-EDS taken during the study of lung samples from Bishkek.

DISCUSSION

With this study, we evaluated the content of asbestos fibres and their type in lung parenchyma from the general population of Bishkek city, Kyrgyzstan) who are exposed environmentally (non-occupationally). However, the results revealed that 30 lung samples out of 100 do not contain asbestos apart from 1, where only a single tremolite fibre was found. These findings can be

explained by low environmental exposure, confirmed by a previous study on analysing air samples from the same urban area (see Chapter 3). Nevertheless, this study was done for the first time in the history of Kyrgyzstan, and there were no similar studies in Central Asia.

On the contrary, there are findings from another similar study from Italy where commercial and non-commercial asbestos fibres are in the lungs of the general population. Casali and colleagues (2015) [67] from Milan, where they did not have any asbestos-producing industry in the past, significantly found both fibres: amphibole and chrysotile. Nevertheless, the study revealed 58,2 % amphibole fibres and 20 % chrysotile fibres in 35 samples out of 55.

Further explanation for the absence of chrysotile fibres in the lung samples is based on the observation that chrysotile asbestos fibres are less persistent in the body [46,68,69]. In addition, it is noteworthy that there is no contamination with amphiboles in the chrysotile asbestos used in Kyrgyzstan (see Chapter 3).

Asbestos fibres are generally highly persistent in the body, excluding chrysotile. Chrysotile undergoes selective leaching in the presence of solid acid or chelating agents, leading to removing Mg^{2+} ions [34]. Further, chrysotile fibres may experience a loss of magnesium in vivo after phagocytosis by alveolar macrophages.

In contrast, amphibole asbestos fibres exhibit high biopersistence, with a difference in the persistence of chrysotile and amphiboles. Analysis of animal data indicates that chrysotile fibres tend to break into smaller segments and display some degree of bio-solubility. Human data indicates that half-lives for amphibole fibres in the lungs can extend to years or even decades, whereas for chrysotile fibres, the range is more likely measured in months [70].

The translocation of chrysotile fibres after degradation is a less likely hypothesis for the absence of fibres in samples. Asbestos fibres deposited in the lungs can translocate to the pleura, local lymph nodes, diaphragm, and distant organs. The mechanisms for translocation are not yet fully comprehended, although there is an indication of lymphatic translocation. The longer fibres are less prone to translocating from the lungs than the shorter ones [70].

CONCLUSION

This study presents intriguing yet unsurprising findings concerning the level of asbestos exposure in a Kyrgyzstan city. However, further research is needed to fully comprehend the effects of asbestos on human health in Central Asia and address the gaps in our understanding of chrysotile asbestos on a global scale. Such research would lend new insights to the ongoing debate.

Original Article

Asbestos burden in lungs of non-occupationally exposed women from Broni (Pavia, Italy): a postmortem SEM-EDS study

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Background: In Italy the incidence of malignant mesothelioma (MM) among women is remarkably high, due to the several contexts in which women had been exposed to asbestos. However, very few studies in literature focus on the inorganic lung content in women. The aim of this retrospective, observational study is to investigate the asbestos lung burden, in terms of concentration, dimensions and type of asbestos, in 42 women who died from MM and had been non-occupationally exposed to asbestos during the activity of the asbestos-cement plant located in Broni (Pavia, Northern Italy) where mainly chrysotile, crocidolite and amosite were used.

Methods: Lung samples taken during forensic autopsies have been digested using sodium hypochlorite and filtered through a cellulose-ester membrane. The filter was examined using a scanning electron microscope and the chemical composition of the fibers was analyzed using an electron dispersive spectroscopy. The number of detected inorganic fibers, asbestos fibers and asbestos bodies (ABs) were normalized to 1 gram of dry tissue.

Results: In six samples no asbestos has been detected. Overall, the most represented kind of asbestos was amosite, followed by crocidolite, tremolite/actinolite asbestos and chrysotile. The concentration of all inorganic fibers was significantly higher in women with environmental and household exposures compared with those with only environmental exposure (P=0.025), as well as the concentration of asbestos fibers (P=0.019) and ABs (P=0.049). We found a significant correlation between the concentration of asbestos fibers and the duration of exposure (rho =0.413, P=0.008), as well as with the latency of MM (rho =0.427, P=0.005). The distance of the residential address from the factory and the time spent daily in contact with asbestos did not influence the lung asbestos burden.

Conclusions: These results suggest the relevance of the lung clearance of asbestos, regarding mainly chrysotile. As a consequence, although SEM-EDS is considered the most reliable tool for assessing previous exposure to asbestos, its results should be interpreted with caution, especially in a legal context. In addition, our data confirm the relevance of environmental and household exposure in determining asbestos concentration in lungs and highlight the importance of household exposure.

Keywords: Asbestos; mesothelioma; non-occupational exposure; SEM-EDS

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Introduction

Malignant mesothelioma (MM) is a highly aggressive neoplasm, arising from serosal linings of pleura, peritoneum, pericardium, or tunica vaginalis of testis, whose causal attribution to previous asbestos exposure is well known and established. MM has a typically long latency, generally 30 to 50 years, between the beginning of exposure and the onset of the disease (1). Due to this characteristic, the MM cases currently observed are related to asbestos exposures that occurred decades ago. In Italy, asbestos extraction, use, and commercialization were banned in 1992 with the Italian Law 257/92. Oddone *et al.*, in a recent epidemiological study, predicted that the mortality of pleural MM is reaching its peak in the current years (specifically, between 2020 and 2024) and then a plateau is expected, followed by a slow decrease in the following decades (2). The peak of peritoneal MM, instead, had been reached in 2014–2016 for men and 1999–2011 for women and its mortality is currently decreasing (3). It is interesting to note that, according to Oddone's forecasts, a high number of cases of pleural MM are expected in the next 20 years (about 26,000) (2). The man-to-women ratio, which showed a continuous increase between 1970 and 2014, is expected to remain stable in the future (2). On these bases, it is clear that, even though in most European countries the use of asbestos was banned between the years 1990 and 2000, asbestos-related diseases (ARDs) and especially MM still represent a major public health problem in both sexes.

MM has been historically associated with occupational exposure to asbestos, occurring mostly in men, while in women it is more often linked with household contacts with asbestos workers or neighborhood exposure deriving from nearby industries (4). However, other domestic sources of asbestos exposure should be considered, as before the asbestos ban (in Italy, 1992) asbestos artifacts were largely present in equipment for ironing, hair dryers, kitchen supplies etc. The sex-related differences in asbestos exposure and its role in MM causation are not well understood. Previous studies on women reported a diagnosis of MM mostly due to environmental and/or household exposure to asbestos (5-7). Moreover, this exposure has always been believed to be less intense than in men, who are, instead, exposed mainly occupationally. In a recent review, Attanoos *et al.* states that, as female MM patients often show concentration of asbestos in lungs below the background level, most cases are likely to be due to alternative causes, such as exposure to other minerals or radiation, they could be idiopathic or related to germline bap-1 mutations (8). On the other hand, as demonstrated by epidemiological data collected by Italian National Mesothelioma Registry, anthropogenic environmental and household exposures (AEH) to asbestos are responsible for a remarkable proportion of MM cases in Italy, respectively of 4.9% and 4.4% of all MM cases, most of which observed in the areas adjacent to asbestos-cement industries (9). Also, Ferrante *et al.* observed a significant increase in MM incidence and mortality in women exposed to asbestos in Casale Monferrato (Alessandria, northern Italy), where an important asbestos factory was located, due to household exposure (that involves direct contact with an asbestos worker and/or the cleaning of contaminated clothes) (10). It has recently been reported that Italy presents a high incidence of

Highlight box

Key findings
- Non-occupational asbestos exposure can determine high levels of asbestos in lungs.
- Household exposure is especially remarkable in relation to asbestos lung burden.
- Chrysotile clearance influence the results of lung content analysis using SEM-EDS.

What is known and what is new?
- Malignant mesothelioma (MM) in women is associated with non-occupational asbestos exposure, a younger age at diagnosis, a better survival and a more common epithelioid histology.
- The women here analyzed, exposed to asbestos environmentally or through a family member, show high levels of asbestos in lungs.
- SEM-EDS results is influenced by asbestos clearance in lungs.

What is the implication, and what should change now?
- Environmental and familial exposures are relevant in determining asbestos burden in lungs and should be addressed in prevention programs for MM.
- SEM-EDS analysis of lung content, considered the gold standard in the evaluation of previous asbestos exposure, should be carefully interpreted.

MM among women, due to several contexts, mostly related to the former activity of asbestos industries, in which women had been exposed to asbestos. The data collected from the National Mesothelioma Registry showed that in Italy 28% of MM occur in women, with a gender ratio (female/male) of 0.4 (7). Until now, few studies systematically investigated the lung content in non-occupationally exposed individuals (11,12), and only one, to our knowledge, focused on the asbestos lung content in women with environmental and/or household exposure (5). Such studies also suggest the importance of non-occupational exposure in determining asbestos burden in lungs.

MM in women is associated with a younger age at diagnosis, a better survival and a more common epithelioid histology (13).

The present study focuses on a series of deceased women who lived in Broni, a small town located on the hills of the Pavia Province, where the Fibronit plant, involved in the production of asbestos-cement, was active. Raw products were a mixture of chrysotile, crocidolite and small amounts of amosite (14,15). The plant was active between 1932 and 1993, employing about 2,700 men and 700 women who resided prevalently in Broni or in the surrounding. Until the 1980s, no air filtration systems were present, nor other safety measures were adopted in the factory. The details of the production of the factory have been described elsewhere (6,14,16).

The aim of this study is to characterize the inorganic fiber burden, the asbestos fibers and asbestos bodies (ABs) concentration, the dimensions and type of detected asbestos, in women who died from MM, taking into account the type of asbestos exposure—anthropogenic environmental exposure (AEE) *vs.* AEH—during the activity of the above-mentioned asbestos-cement plant. Secondly, the association between the amount of time spent daily in contact with asbestos and asbestos lung content and the association between asbestos concentration and duration, latency and survival are explored. We present this article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1061/rc).

Methods

Population, study design and setting

The study population was selected among subjects who died with ARDs and had been exposed to asbestos in Broni (PV). Among the subjects for which a forensic autopsy followed by histopathological exam has been performed between 2005 and 2018, subjects with the following characteristics have been selected for the analysis:

- Female sex;
- Death due to MM;
- Anthropogenic environmental asbestos exposure (AEE) or AEH. For an explanation see the paragraph "variables".

The diagnosis of MM, already known before death, has been confirmed postmortem by immunohistochemistry according to guidelines in effect at the time (17-20). During the forensic autopsy, in each case, the whole lungs were collected, formalin-fixed, and stored for further examination. All the women investigated have been exposed to asbestos during the activity of the asbestos-cement factory which was operating from 1932 to 1993 in Broni (PV).

A retrospective cohort design was used. The study period was from 2005 to 2018.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of IRCCS Policlinico San Matteo (Pavia, Italy) (No. 20180060636) and individual consent for this retrospective analysis was waived.

Variables, data sources and measurement

The endpoints: assessment of inorganic fibers in lungs

The concentration of inhaled asbestos fibers, the dimensions and type of asbestos fibers were the endpoints. Not only asbestos, but all the inorganic fibers that fulfilled the definition of "regulated" fiber [length ≥5 µm, width <3 µm, aspect ratio greater than or equal to 3:1 (21)], but also fibers shorter than 5 µm, classified separately as short fibers, and ABs contained in 0.25 grams of wet lung (inferior lobe of right lung) were investigated by SEM-EDS in order to obtain the concentrations of total inorganic fibers, asbestos fibers, and ABs (*Figure 1*), as well as the concentrations of the various types of asbestos [chrysotile (*Figure 2*), amosite (*Figure 3*), crocidolite (*Figure 4*), tremolite-actinolite asbestos (*Figure 5*), anthophyllite asbestos] in lung samples of the selected subjects. The inorganic fibers were also measured and chemically analyzed.

The method, already described elsewhere (16,22) consists of chemical digestion (using sodium hypochlorite) of 0.25 grams of formalin-fixed lung parenchyma and filtration of the suspension through a cellulose-ester membrane (Millipore, Darmstadt, Germany) with a diameter of 25 mm

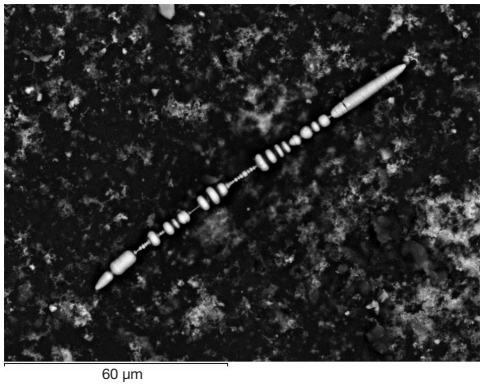

Figure 1 An example of an SEM image (backscattered electrons) of an asbestos body.

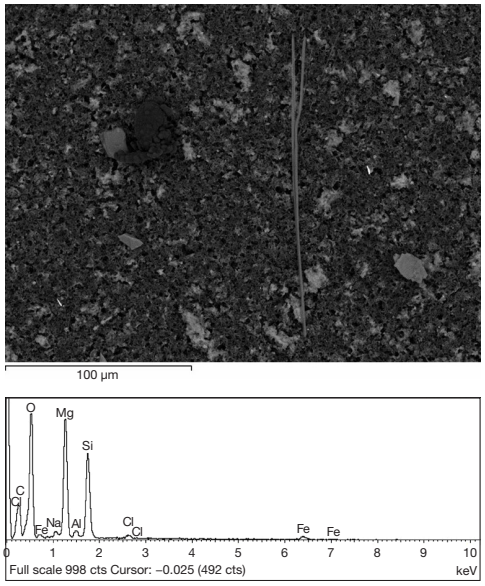

Figure 2 An example of an SEM image (backscattered electrons) of a chrysotile/asbestiform antigorite fiber, with the corresponding EDS spectra. Sodium (Na) and chlorine (Cl) peaks are related to the NaCl that is present in the background.

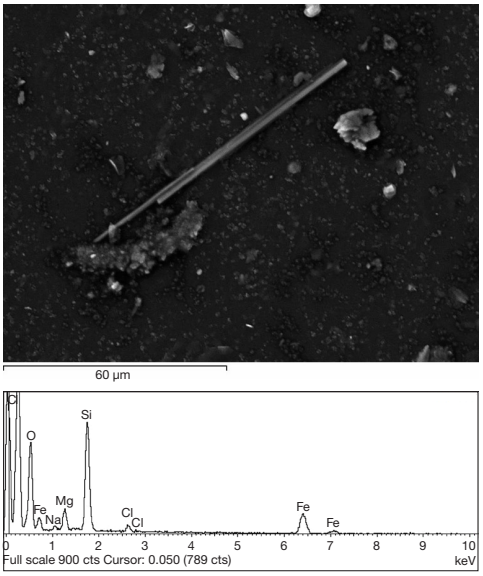

Figure 3 An example of an SEM image (backscattered electrons) of an amosite fiber, with the corresponding EDS spectra. Sodium (Na) and chlorine (Cl) peaks are related to the NaCl that is present in the background.

and a pore size of 0.45 µm.

Afterwards, the filter, dehydrated and pasted on a pin-stub using a carbon tape, was examined by SEM. The observation was performed on an area of 2 mm^2 of filter at 4,000× using backscattered electrons.

The fiber chemical composition was analyzed using an EDS, Oxford Inca Energy 200, equipped with an INCA X-act SDD detector (Oxford Instruments NanoAnalysis, Bucks, UK).

The number of detected inorganic fibers, asbestos fibers and ABs were normalized to 1 gram of dry tissue, as indicated by international guidelines (23,24), reporting concentration in terms of the burden of inorganic fibers, asbestos, and ABs per gram of dry lung tissue weight: ff/gdw.

The analytic sensitivity of the method can be identified in 3,000 ff/gdw, that corresponds to the minimum content detectable (the lung content corresponding to one fiber counted).

Moreover, the "background" concentration of asbestos in lung tissue (that is the concentration of asbestos that everyone can randomly encounter but does not increase

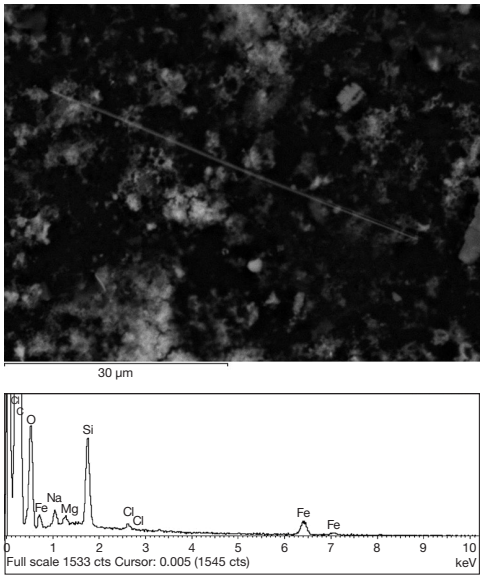

Figure 4 An example of an SEM image (backscattered electrons) of a crocidolite fiber, with the corresponding EDS spectra. Chlorine (Cl) peak is related to the NaCl that is present in the background, while sodium (Na) is related partly to the fiber composition and partly to the background.

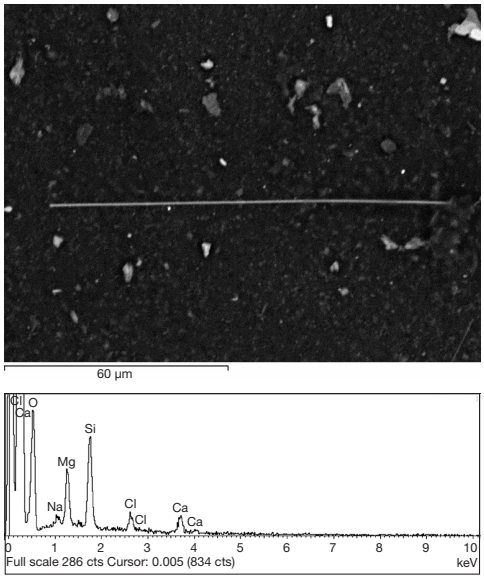

Figure 5 An example of an SEM image (backscattered electrons) of a tremolite/actinolite fiber, with the corresponding EDS spectra. Sodium (Na) and chlorine (Cl) peaks are related to the NaCl that is present in the background.

significantly the risk of MM) had been identified in our laboratory as below 100,000 ff/gdw. This threshold has been found by performing the same analysis on a series of 50 healthy subjects, who died from traumatic causes, aged more than 60 and with a negative history of asbestos exposure and lung disease.

To identify the different types of inorganic fibers, we compared the EDS spectra with a database internal to the laboratory.

Since the technique here used does not allow unequivocal identification of certain minerals with similar chemical composition and analogous morphology, it is not possible to distinguish chrysotile from asbestiform antigorite, and tremolite asbestos from actinolite asbestos. Therefore, we used, respectively, the following mineral group names: chrysotile/asbestiform antigorite and tremolite/actinolite asbestos.

Other variables

The following variables were used in the present work:

- Type of asbestos exposure (AEH, AEE). As all the women here investigated had AEE, but only a number of them had household exposure, we divided them in two groups: those with only AEE and those with both AEH. The term "AEE" is used for indicating people who lived in an area with air-dispersed asbestos from the asbestos-cement plant (25,26), in order to make clear the difference from the exposure to natural sources of asbestos (this last identified as "natural environmental exposure"). The "anthropogenic environmental and household exposure" is used for women who lived in the same area and lived together with an asbestos worker.
- Amount and daily exposure: living and working at Broni (LW-Broni) and otherwise (i.e., living at Broni and working in different place or not living in Broni and working in Broni).
- Distance in meters of the residential address and the asbestos cement plant. In case of multiple changing of address during the subject's life the mean distance

Table 1 Type of MM and exposure of enrolled women

Variables	N=42
Hystological type of MM	
Epithelial	31 (73.8)
Sarcomatoid	6 (14.3)
Biphasic	5 (11.9)
Type of exposure	
AEE	28 (66.7)
AEH	14 (33.3)
Latency (years)	49 (42.0–68.0)
Duration of exposure (years)	29 (19.0–50.0)
Survival time since diagnosis of MM (months)	14.0 (10.0–22.0)
Time since end of exposure (years)	18.0 (16.0–22.0)

N (%) or median (IQR) are shown when appropriate. MM, malignant mesothelioma; AEE, anthropogenic environmental exposure; AEH, anthropogenic environmental and household exposure; IQR, interquartile range.

was used.

- Duration of asbestos exposure.
- Histological type of MM: epithelial, sarcomatoid and biphasic.
- Time of diagnosis of MM.
- Time of death.
- Time between the end of exposure and death.

Data sources

Except the lung fibers, all the variables were extracted from the archive of Unit of Legal Medicine and Forensic Sciences of the University of Pavia from 2000 to 2018.

Statistical analysis

Quantitative variables were summarized as the mean with standard deviation if the distribution was normal, and with the median, 25th, and 75th percentiles if not. To verify normality, the Shapiro-Wilk test was used. To evaluate differences in quantitative variables across groups of exposure to asbestos (AEE *vs.* AEH) and the amount and daily exposure, was performed using a non-parametric unpaired *t*-test (Mann-Whitney test). The evaluation of differences between histological types of MM, an analogous non-parametric test of analysis of variance (Kruskal-Wallis test) was applied, followed by the appropriate post-hoc test if significant. The relationships among quantitative variables were tested using Spearman's correlation coefficient (rho). A P value less than 0.05 was considered significant. All analyses were performed using STATA 17® (StataCorp LLC., College Station, TX, USA).

Results

Among a total of 188 subjects who died from ARDs and for which a forensic autopsy followed by histopathological exam has been performed, 46 were females and died from MM. Three of these women had only occupational asbestos exposure or household exposure and one had no reported exposure. Therefore, 4 out of 46 women have been excluded from the analysis.

About 2/3 of women enrolled in the study were environmentally exposed to asbestos (AEE) and the histological type of MM was classified as epithelial in around 3/4 of cases (*Table 1*). The median time of latency was 49 years, while the survival time in at least 50% of women was 14 months. The median time elapsed between the end of exposure and death was 18 years.

Inorganic fibers in lungs by type of exposure

Among the whole series of 42 women, six cases showed no asbestos in the investigated lung sample (five of them had AEE and one had AEH), of which three subjects did not show any other kind of inorganic fibers. ABs were found in 13 cases, whereas short fibers were observed in 29 subjects.

Overall, the most represented kind of asbestos was amosite (43% of the total detected asbestos), followed by crocidolite (31%), tremolite/actinolite asbestos (24%) and chrysotile/asbestiform antigorite (2%).

The median concentration of inorganic fibers, asbestos fibers, ABs and short fibers, their dimensional characteristics in the two exposure groups are reported in *Table 2*. The concentration of inorganic fibers and of asbestos was significantly higher in women with both exposures (AEH) compared with those with only AEE, while that of ABs showed a similar pattern but the excess was borderline significant. No significant difference in fibers dimensions have been detected according to the kind of exposure.

In the AEE group, amosite was the most represented kind of asbestos (being the 42% of the total asbestos) followed by crocidolite (30%), tremolite/actinolite asbestos (25%) and chrysotile/asbestiform antigorite (3%). In the AEH group, amosite represented the 45% of the total

Table 2 Median and IQR of each variable regarding the inorganic lung content in the two groups of exposure considered for the statistical analysis (anthropogenic environmental, anthropogenic environmental and household)

Variables	Type of exposure		
	AEE (n=28)	AEH (n=14)	MW test; P value
Fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	44,387.2 (20,478.2–71,859.9)	92,700.6 (44,471.4–161,409.9)	−2.24; 0.025
Asbestos fibres per gram of dry weight lung tissue (ff/gdw), median (IQR)	20,751.1 (7,452.5–28,970.7)	42,998.1 (21,784.9–73,979.5)	−2.31; 0.019
Asbestos bodies per gram of dry weight lung tissue (ABs/gdw), median (IQR)	0.0 (0.0–0.0)	2,228.3 (0.0–13,178.5)	−2.01; 0.049
Short fibers per gram of dry weight lung tissue (sff/gdw), median (IQR)	7,804.2 (0.0–14,493.6)	5,166.4 (2,944.3–17,704.3)	−0.59; 0.563
Mean length of all fibers (μm), median (IQR)	18.3 (14.9–22.3)	16.7 (14.8–22.1)	0.42; 0.682
Mean width of all fibers (μm), median (IQR)	0.8 (0.6–0.9)	0.7 (0.4–0.9)	0.70; 0.488
Mean length of asbestos fibers (μm), median (IQR)	20.6 (11.6–26.0)	17.9 (12.3–24.2)	0.60; 0.556
Mean width of asbestos fibers (μm), median (IQR)	0.5 (0.3–0.6)	0.6 (0.4–0.9)	−1.02; 0.310
Mean length of all short fibers (μm), median (IQR)	3.9 (0.0–4.2)	4.1 (3.7–4.5)	−0.948; 0.343
Mean width of all short fibers (μm), median (IQR)	0.6 (0.0–0.7)	0.6 (0.4–0.7)	−0.70; 0.488

IQR, interquartile range; AEE, anthropogenic environmental exposure; AEH, anthropogenic environmental and household exposure; MW test, Mann-Whitney test.

Table 3 Median and IQR of the concentration of each type of asbestos in the two groups of exposure considered for the statistical analysis (anthropogenic environmental, anthropogenic environmental and household)

Variables	Type of exposure		
	AEE (n=28)	AEH (n=14)	MW test; P value
Chrysotile/asbestiform antigorite per gram of dry weight lung tissue (ff/gdw), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.445; 0.570
Crocidolite per gram of dry weight lung tissue (ff/gdw), median (IQR)	0.0 (0.0–7016.2)	11,172.6 (4,456.7–20,852.6)	−2.65; 0.007
Amosite per gram of dry weight lung tissue (ff/gdw), median (IQR)	5,843.8 (0.0–19,520.6)	15,502.6 (8,852.1–43,715.2)	−1.83; 0.070
Tremolite/actinolite per gram of dry weight lung tissue (ff/gdw), median (IQR)	3,684.3 (0.0–8,676.4)	6,970.6 (2,944.3–16,813.5)	−1.44; 0.154

IQR, interquartile range; AEE, anthropogenic environmental exposure; AEH, anthropogenic environmental and household exposure; MW test, Mann-Whitney test.

detected asbestos, followed by crocidolite (32%), tremolite/actinolite asbestos (23%) and chrysotile/asbestiform antigorite (1%).

Concerning the kind of asbestos, only the crocidolite concentration was found significantly higher in women with AEH exposure compared to only AEE, while the concentration of chrysotile/asbestiform antigorite, amosite and tremolite/actinolite asbestos did not show any significant difference (*Table 3*).

The concentration of inorganic fibers, of asbestos and of ABs was not significantly different between the women that used to spend most of their daily life exposed to asbestos (because they lived in Broni and worked in the same town or were housewives) and those who spent only part of their

Table 4 Median and IQR of each variable regarding the inorganic lung content in the two groups of daily exposure (women that used to spend most of their daily life exposed to asbestos *vs.* those who spent only part of their daily life in a condition that implied asbestos exposure)

Variables	Daily exposure		
	LW-Broni (n=29)	Otherwise (n=7)	MW test; P value
Inorganic fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	52,113.9 (30,583.6–85,749.0)	24,396.6 (5,220.9–65,354.7)	1.42; 0.165
Asbestos per gram of dry weight lung tissue (ff/gdw), median (IQR)	26,682.9 (9,702.9–50,853.6)	9,613.0 (0.0–43,968.9)	1.32; 0.193
Asbestos bodies per gram of dry weight lung tissue (ABs/gdw), median (IQR)	0.0 (0.0–4,456.7)	0.0 (0.0–0.0)	0.835; 0.488
Short fibers per gram of dry weight lung tissue (sff/gdw), median (IQR)	4,845.9 (0.0–15,140.5)	0.0 (0.0–7,994.4)	1.49; 0.140
Mean length of all fibers (µm), median (IQR)	17.3 (4.8–20.7)	17.9 (7.0–25.0)	0.02; 1.000
Mean width of all fibers (µm), median (IQR)	0.8 (0.6–1.0)	0.6 (0.3–1.6)	0.780; 0.449
Mean length of asbestos fibers (µm), median (IQR)	20.2 (13.5–24.2)	14.1 (0.0–38.8)	0.801; 0.437
Mean width of asbestos fibers (µm), median (IQR)	0.6 (0.4–0.8)	0.3 (0.0–0.5)	2.52; 0.009
Mean length of all short fibers (µm), median (IQR)	3.9 (0.0–4.3)	0.0 (0.0–4.5)	0.614; 0.556
Mean width of all short fibers (µm), median (IQR)	0.6 (0.0–0.7)	0.0 (0.0–0.9)	0.615; 0.556

LW-Broni = women who spent most of their daily life exposed to asbestos; Otherwise = spent only part of their daily life in a condition that implied asbestos exposure. LW-Broni, living and working at Broni; IQR, interquartile range; MW test, Mann-Whitney test.

Table 5 Median and IQR of the concentration of each type of asbestos in the two groups of daily exposure (women that used to spend most of their daily life exposed to asbestos *vs.* those who spent only part of their daily life in a condition that implied asbestos exposure)

Variables	Amount and daily exposure		
	LW-Broni (n=29)	Otherwise (n=7)	MW test; P value
Chrysotile/asbestiform antigorite per gram of dry weight lung tissue (ff/gdw), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	–0.709; 0.445
Crocidolite per gram of dry weight lung tissue (ff/gdw), median (IQR)	4,456.7 (0.0–13,450.8)	4,806.5 (0.0–8,714.0)	0.335; 0.761
Amosite per gram of dry weight lung tissue (ff/gdw), median (IQR)	7,124.9 (0.0–22,016.9)	4,806.5 (0.0–19,985.9)	0.507; 0.636
Tremolite/actinolite per gram of dry weight lung tissue (ff/gdw), median (IQR)	4,851.4 (2,944.3–16,813.5)	0.0 (0.0–0.0)	2.60; 0.006

LW-Broni = women spent most of their daily life exposed to asbestos; Otherwise = spent only part of their daily life in a condition that implied asbestos exposure. IQR, interquartile range; MW test, Mann-Whitney test.

daily life in a condition that implied asbestos exposure (because they lived in Broni and worked elsewhere or vice versa) (*Table 4*).

No differences in fiber length have been pointed out according to how much time a day the subjects were exposed to asbestos, whereas the asbestos fibers were significantly wider in women exposed for the whole day compared to those exposed for a lower amount of time (*Table 4*).

The concentration of tremolite/actinolite asbestos was found to be significantly higher in those who were exposed for the whole day compared to women exposed for only a part of their day (*Table 5*), while the other kind of asbestos did not show any significant difference.

Finally, a significant positive correlation between the

Table 6 Spearman's rank correlation coefficients between the variables regarding the inorganic lung content and the chronologic variables considered

Variables	Duration of exposure (years)		Latency (years)		Survival (months)		Time since end of exposure (years)	
	rho	P value	rho	P value	rho	P value	rho	P value
Asbestos fibers per gram of dry weight lung tissue	0.413	0.008	0.427	0.005	0.072	0.655	–0.186	0.249
Asbestos bodies per gram of dry weight lung tissue	0.216	0.179	0.237	0.139	0.125	0.439	0.164	0.311
Mean length of asbestos fibers	0.238	0.143	0.101	0.538	–0.241	0.137	–0.206	0.207
Chrysotile/asbestiform antigorite fibers per gram of dry weight lung tissue	–0.204	0.205	0.130	0.423	0.017	0.913	–0.050	0.757
Crocidolite fibers per gram of dry weight lung tissue	0.340	0.032	0.233	0.147	–0.068	0.676	–0.115	0.477
Amosite fibers per gram of dry weight lung tissue	0.336	0.033	0.398	0.011	0.163	0.313	–0.156	0.334
Tremolite/actinolite fibers per gram of dry weight lung tissue	0.294	0.065	0.272	0.088	–0.074	0.646	–0.120	0.460

duration of exposure and the concentration of asbestos fibers in lungs, as well as between the latency and the concentration of asbestos fibers in lungs (*Table 6*), have been observed. Moreover, the concentration of crocidolite resulted to be positively correlated with the duration of exposure and amosite concentration was positively correlated with duration of exposure and latency (*Table 6*).

Possible differences in lung content (in terms of concentration and type of inorganic fibers, asbestos fibers and their dimensions and ABs) according to the histological type of MM have been investigated, without finding any significant result (Table S1). Analyzing the histologic type according to the other variables (latency, duration of exposure and survival since diagnosis) a significantly longer survival in subjects with epithelioid MM compared to sarcomatoid and biphasic/desmoplastic MM was found.

Discussion

In this study, we investigated the characteristics of inorganic fibers contained in samples of lung tissue of a series of women non-occupationally exposed to asbestos during the activity of the asbestos-cement plant located in Broni (Pavia, Italy).

In sum, we found that, despite the well-known history of environmental and/or household exposure, not in all cases asbestos fibers and ABs have been detected at SEM-EDS investigation. Regarding the type of asbestos, amosite was found at the highest concentration, followed by crocidolite, tremolite/actinolite and chrysotile/asbestiform antigorite. Interestingly, the concentration of all inorganic fibers, of asbestos and ABs was significantly higher in women with AEH compared with those with only AEE. Moreover, the concentration of asbestos showed a positive correlation with duration of exposure (this correlation was observed also for crocidolite and amosite) and latency (this correlation was observed also for amosite). The concentration of asbestos in lungs was not different according to the daily time of exposure and the histological type.

The present study has two main limitations.

First, the subjects of the study have been retrospectively extracted from the archive of the Unit of Legal Medicine of Pavia University among those for whom a forensic autopsy was ordered by the Prosecutor in a context of a penal trial. Therefore, they do not represent the totality of women exposed to asbestos in Broni who died from MM. Notwithstanding, a selection bias is unlikely as, in the considered period of time, a forensic autopsy has been performed for most subjects who died from MM in the competence area, as the notification to the Prosecutor is mandatory in case of deaths related to occupational diseases. Furthermore, if the Prosecutor decided not to perform the forensic autopsy, the decision was based on motivations not related to the lung fiber burden (5).

The second limitation concerns the relatively low amount of lung tissue examined under SEM-EDS, as only one sample for each subject has been analyzed. Yet, on the basis of previous research (22), a lung sample taken as above explained is representative and provides the best cost-efficacy ratio. Moreover, to make sure about the suitability

of the samples, in three cases we performed the analysis on two different samples from the same subject, obtaining overlapping results.

Despite the above-described limitations, the series is sufficiently large to draw sound conclusions, as the statistical analysis confirmed; indeed, this is among the largest series of women for whom the lung content has been systematically analyzed using SEM-EDS.

Moving to the interpretation of the above-summarized results, in 14% of cases, all of which had documented exposure to asbestos (AEE or AEH) the asbestos concentration was below the detection limit. This is consistent with what was observed in our previous research, conducted on a mixed-sex population exposed to asbestos in Broni, in which we detected no asbestos in around 19% of the analyzed subjects (16,27). This is in line with previous literature, as other authors observed several cases of MM without any asbestos in lungs detected at SEM-EDS (28).

In order to explain this finding, the following hypotheses can be considered.

(I) The lungs of these subjects never contained asbestos. This is extremely unlikely, as they had documented exposure in a setting where high amounts of asbestos were air-dispersed. In addition, they died from MM and, even though MM not related to asbestos exposure has been reported (8), this does not seem to be the case, given the known history of exposure which was documented in the context of a penal trial.

(II) Asbestos was present but not detected with the used technique. Also, this explanation can be ruled out, as the hypothesis that thin chrysotile fibers can be missed by SEM-EDS is unlikely considering that in some cases of the present series chrysotile has been detected and classified with certainty.

(III) The asbestos inhaled by those subjects has been completely cleared from their lungs. This hypothesis represents the most likely explanation, as the rapid clearance of chrysotile is well known and characterized in literature (29). In studies on animal models the half-life of chrysotile has been estimated as 90 days (30). Lung clearance regards also amphiboles, which are, however, much more biopersistent compared to chrysotile: studies on animal models estimated a half-life of crocidolite and amosite in lungs of, respectively, 50 and 18 months (31). Moreover, in the present series, a long period of time elapsed between the end of exposure and death (more than 22 years).

This finding suggests that the asbestos lung content is subjected to deep changes with the passing of time after the end of exposure, especially regarding chrysotile. Therefore, SEM-EDS evaluation should be interpreted carefully, especially in a legal context, where the causal attribution of ARDs depends on this assessment, as the lung content at the moment of death, even though considered the most reliable tool for assessing previous exposure (23), does not exactly reflect the actual amount of asbestos which was inhaled by the subject during life.

Interestingly, ABs have been detected in only 30% of the investigated women. The technique here used (SEM-EDS) allows to analyze a lower amount of lung tissue compared to the optical microscopy (24), so the amount of ABs reported here might have been underestimated. However, as in several cases we found a concentration of asbestos above the threshold considered indicative of previous exposure (19) but no ABs, it is worthy to underline that ABs concentration does not always reflect the asbestos lung burden.

Therefore, we think that, in order to determine previous asbestos exposure, it is always preferable to perform SEM-EDS investigation in order to detect and count asbestos fibers rather than optical microscopy to visualize ABs.

The next consideration regards the type of asbestos detected in the present series. We know that at the asbestos cement plant located in Broni the most used asbestos types were chrysotile and crocidolite. Also, amosite was used, but in small amounts, as an additive (14).

Interestingly, the results of the lung content analysis did not reflect these data about the asbestos containing materials production at the factory. Chrysotile/asbestiform antigorite was detected in only 2% of cases. This is not surprising, as it is well known that, compared to amphibole asbestos, chrysotile is much less biopersistent (29). This characteristic is due to the different crystalline structure of chrysotile, which is subjected to dissociation of magnesium from the fiber's surface in the acid lung microenvironment (29). For this reason, chrysotile is more easily fragmented and phagocytized by the lung macrophages and removed from the alveoli through the lymphatic stream (29). In our previous work conducted on a series of subjects exposed in the same setting (mainly males) we detected no chrysotile at all (27). The scarce presence of chrysotile is in line with a previous study about lung content in female cases of MM (4).

The current scientific evidence suggests that the carcinogenic potency of chrysotile asbestos is lower compared to amphibole asbestos in humans (25,26,29,32),

due to its rapid clearance from lungs. However, the conclusion that the epidemiological evidence for lung cancer strongly supports a difference in carcinogenic potency between chrysotile and amphiboles has been questioned by a meta-analysis by Lenters *et al.* (33).

Surprisingly, in one of the four cases in which chrysotile was detected, the time since the end of exposure was 49 years, the highest among the entire series. Even though this is a single case, it may suggest that the capability to clear chrysotile (and, generally, asbestos) from lungs might be subjected to individual variability, and this could be a possible clue in the research about the MM individual susceptibility. Anyway, we must take into account that asbestos removed from lungs is gradually translocated towards the pleura, where asbestos exerts its carcinogenic potential (34). Therefore, studies about pleural asbestos content are necessary to clarify this concept.

The lack of detection of chrysotile (in spite of documented exposure), in many cases, likely reflects a less important role in causing MM, as indicated by the greater potency for amphiboles in MM causation as compared to chrysotile, well-known and confirmed by recent studies (35,36).

The most detected kind of asbestos in the present study was amosite, followed by crocidolite, tremolite/actinolite asbestos and finally chrysotile. This finding is in agreement with Barbieri *et al.*, who found amosite as the main kind of asbestos in 15 women, 8 of which were exposed in Broni (5). According to the literature data about the production of the plant in Broni (14), amosite was used in small quantities, whereas chrysotile and crocidolite were the most utilized kind of asbestos. However, it is extremely difficult to determine the exact amounts of the different asbestos used at a given time. This finding suggests that probably, at the plant located in Broni, more amosite was used with respect to what was declared and reconstructed according to the production data.

Furthermore, we found non-commercial asbestos (tremolite/actinolite asbestos): the most likely explanation is that the women here investigated have been exposed to talc-containing products (known to be contaminated with tremolite/actinolite asbestos), considering the large industrial use of talcum at the time (37). This was observed also in our previous study conducted on a mixed-sex series (27). However, non-commercial amphiboles are also contaminants of some chrysotile ores (38), so this source is possible, even if less likely considering the geographic setting of the study.

In women with both exposures (AEH) the amount of asbestos was significantly higher than in those with AEE alone, suggesting that household exposure represents a major source of asbestos exposure, that can significantly increase the asbestos amount in lungs, even in a context where AEE was heavy, since the emissions of the asbestos-cement plant in the surroundings area, never measured in 1960s and 1970s, are supposed to have been extremely intense (6,39). This finding is consistent with what was stated by Marsh *et al.* using epidemiological data (40) and with what was observed by other authors (5,38), who pointed out that familial exposure can lead to very high lung fiber burdens, comparable to occupational exposure. Therefore, this result confirms that non-occupational exposure is not synonymous to low-level exposure (41).

Unexpectedly, no significant differences in lung concentration of asbestos were detected according to how much time a day the woman spent exposed to asbestos. Even though the time spent daily in contact with asbestos is an important parameter in the retrospective assessment of asbestos exposure (42), in this series it did not seem to influence the asbestos burden in lungs. However, it must be considered that the exposure context of the present series is specific: the environmental exposure in Broni was so heavy that the daily time of exposure necessary to reach a high asbestos burden in lungs may be much lower compared to other settings of AEE.

Interestingly, no correlation between the distance of the house or workplace from the plant and the amount of asbestos in lungs has been observed. This means that the dispersion of large amounts of asbestos from the plant involved a wide area, as confirmed by the case of a woman resident in Albaredo Arnaboldi, 10 km far from Broni. She had a concentration of asbestos of 8,490 ff/gdw, very similar to what was observed in women who lived only a few hundred meters far from the plant. This result, apparently unexpected, is consistent with epidemiological data about the population around Broni, that showed an increased relative risk of MM not only in the town of Broni but also in the surrounding municipalities, especially for women (6,39). Yet, it must be considered that before 1993 asbestos was largely used in products of daily use and asbestos containing materials were widely installed. Moreover, the Fibronit plant was not the only firm that manufactured asbestos. Therefore, this subject might be exposed to other sources of asbestos, unknown (or forgotten).

In the present series, the prevalent MM histological subtype was epithelioid, consistently with other case series (4,43). As already known (4), this histological type

was significantly associated with longer survival. We investigated possible relationships between MM subtype and exposure characteristics (kind of exposure, latency, duration, asbestos burden and asbestos types observed in lungs), without finding any significant correlation, similarly to what was previously reported for mixed-sex series (27,43). On the contrary, Leigh *et al.*, investigating a series of 226 cases of MM in Australia, found a statistically significant relationship between lung fiber content and histological type of MM, observing lower asbestos concentration in epithelial MM compared to mixed and sarcomatous, which showed the highest asbestos burden (44).

As expected, we found a positive correlation between the amount of asbestos in lungs and the duration of exposure. Even though this correlation was quite weak, it is in line with what is logically predictable and with what was previously observed by other authors (45,46). This result is in contrast with our previous research (16), where we found the absence of this correlation, possibly explained by the clearance, that may compensate for the accumulation of new fibers. Probably in women, this ratio between accumulation and clearance presents some differences compared to males. The same can be hypothesized regarding the observed correlation between asbestos concentration on lungs and latency. In fact, the latency period includes duration of exposure and time since the end of exposure. Therefore, this correlation reflects the equilibrium between the accumulation and the clearance of asbestos, which seems to present sex-based differences. Those correlations regarded, in particular, amosite and crocidolite, corroborating the above explained hypothesis of a high biopersistence of these kinds of asbestos compared to chrysotile and to the other types of amphibole asbestos. Our data, instead, do not confirm that higher asbestos burdens in lungs can cause an earlier onset of MM, as suggested by Dragani *et al.* (47).

Conclusions

In the present study, we systematically analyzed the inorganic lung fiber burden (counting, measuring, and classifying regulated asbestos and inorganic fibers as well as those shorter than 5 µm) in 42 women who have been non-occupationally exposed to asbestos during the activity of the Fibronit factory, located in Broni (Pavia, Italy).

The obtained data confirm the relevance of non-occupational asbestos exposure in determining asbestos concentration in lungs, and highlight the importance of household exposure, that, if added to anthropogenic environmental one, significantly increases the asbestos lung concentration compared to environmental exposure alone.

Moreover, our results imply that the postmortem assessment does not exactly reflect the actual amount of asbestos which was inhaled by the subject during life (due to asbestos clearance). Therefore, although SEM-EDS is considered the most reliable tool for assessing previous exposure to asbestos, the results provided by this tool should be interpreted with caution, especially in a legal context.

In addition, it must be underlined that Broni was a specific scenario of asbestos exposure, as around the plant, which was located very close to the city center, the air dispersion of asbestos was very intense (6,39). Yet, this setting was similar to what is still the reality in some countries (e.g., Russia, Central Asia countries). Besides this, in Italy, people are still suffering from the consequences of exposure (not only occupational) that occurred decades ago, and the MM epidemic is expected to continue in the next years, as demonstrated by the forecasts about the future incidence and mortality due to MM (2).

Taken together, the results presented offer a novel perspective on the characteristics of inorganic lung content in non-occupationally exposed women. Indeed, very few papers in literature focus on the sex-related differences in asbestos lung concentration. If compared with our previous work about lung content in Broni inhabitants and Fibronit workers (16,27), this focus on women offers new insights about possible differences between sexes in the efficiency of lung clearance of asbestos, regarding both chrysotile and amphiboles asbestos, and offers clues for further research.

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1061/coif). C.C. and E.B. acted as experts in trials and litigations concerning asbestos (not related to the subjects of this study). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics committee of IRCCS Policlinico San Matteo (Pavia, Italy) (No. 20180060636) and individual consent for this retrospective analysis was waived.

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Supplementary

Table S1 Median and IQR of each variable regarding the inorganic lung content in the three groups of histological type of MM considered for the statistical analysis

Variables	Epithelial (n=31)	Sarcomatoid (n=6)	Biphasic/desmoplastic (n=5)	KW test; P value
Inorganic fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	52,113.9 (24,396.6–98,703.9)	43,351.9 (20,214.3–53,112.8)	77,534.2 (44,471.4–79,071.0)	0.544; 0.761
Asbestos fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	25,167.4 (6,819.3–61,929.8)	13,703.6 (8,085.7–27,367.0)	29,075.3 (26,682.9–35,142.7)	0.893; 0.541
Asbestos bodies per gram of dry weight lung tissue (ABs/gdw), median (IQR)	0.0 (0.0–4,245.0)	0.0 (0.0–8,852.1)	0.0 (0.0–13,178.5)	0.718; 0.698
Short fibers per gram of dry weight lung tissue (sff/gdw), median (IQR)	7,994.4 (0.0–15,140.5)	15,829.3 (0.0–17,704.3)	0.0 (0.0–4,623.1)	3.914; 0.141
Mean length of all fibers (µm), median (IQR)	18.7 (14.8–21.4)	16.1 (14.6–26.4)	17.4 (16.7–22.1)	0.446; 0.800
Mean width of all fibers (µm), median (IQR)	0.7 (0.6–1.1)	0.8 (0.4–0.8)	0.8 (0.6–0.9)	0.938; 0.625
Mean length of asbestos fibers (µm), median (IQR)	19.9 (11.7–24.9)	23.4 (15.5–30.0)	19.0 (16.7–24.2)	0.512; 0.774
Mean width of asbestos fibers (µm), median (IQR)	0.6 (0.4–0.7)	0.5 (0.2–0.6)	0.6 (0.5–0.8)	0.457; 0.795
Chrysotile/asbestiform antigorite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	1.527; 0.466
Crocidolite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	3,562.5 (0.0–13,450.8)	5,376.4 (0.0–6,841.8)	4,845.9 (4,806.5–8,894.3)	0.246; 0.884
Amosite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	13,070.9 (0.0–22,597.8)	5,442.3 (0.0–8,852.1)	8,894.3 (4,845.9–13,178.5)	0.817; 0.664
Tremolite/actinolite fibers per gram of dry weight lung tissue (ff/gdw), median (IQR)	3,607.5 (0.0–10,194.5)	4,447.1 (2,950.7–13,683.5)	8,894.3 (4,392.8–9,246.1)	1.151; 0.562

IQR, interquartile range; MM, malignant mesothelioma; KW test, Kruskal-Wallis test.

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Original Article

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Original Article

Asbestos burden in lungs of mesothelioma patients with pleural plaques, lung fibrosis and/or ferruginous bodies at histology: a postmortem SEM-EDS study

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Abstract

The causal attribution of asbestos-related diseases to past asbestos exposures is of crucial importance in clinical and legal contexts. Often this evaluation is made based on the history of exposure, but this method presents important limitations. To assess past asbestos exposure, pleural plaques (PP), lung fibrosis and histological evidence of ferruginous bodies (FB) can be used in combination with anamnestic data. However, such markers have never been associated with a threshold value of inhaled asbestos. With this study we attempted to shed light on the dose–response relationship of PP, lung fibrosis and FBs, investigating if their prevalence in exposed individuals who died from malignant mesothelioma (MM) is related to the concentration of asbestos in lungs assessed using scanning electron microscopy equipped with energy dispersive spectroscopy. Moreover, we estimated the values of asbestos concentration in lungs associated with PP, lung fibrosis and FB. Lung fibrosis showed a significant positive relationship with asbestos lung content, whereas PP and FB did not. We identified, for the first time, critical lung concentrations of asbestos related to the presence of PP, lung fibrosis and FB at histology (respectively, 19 800, 26 400 and 27 400 fibers per gram of dry weight), that were all well-below the background levels of asbestos identified in our laboratory. Such data suggest that PP, lung fibrosis and FB at histology should be used with caution in the causal attribution of MM to past asbestos exposures, while evaluation of amphibole lung content using analytical electron microscopy should be preferred.

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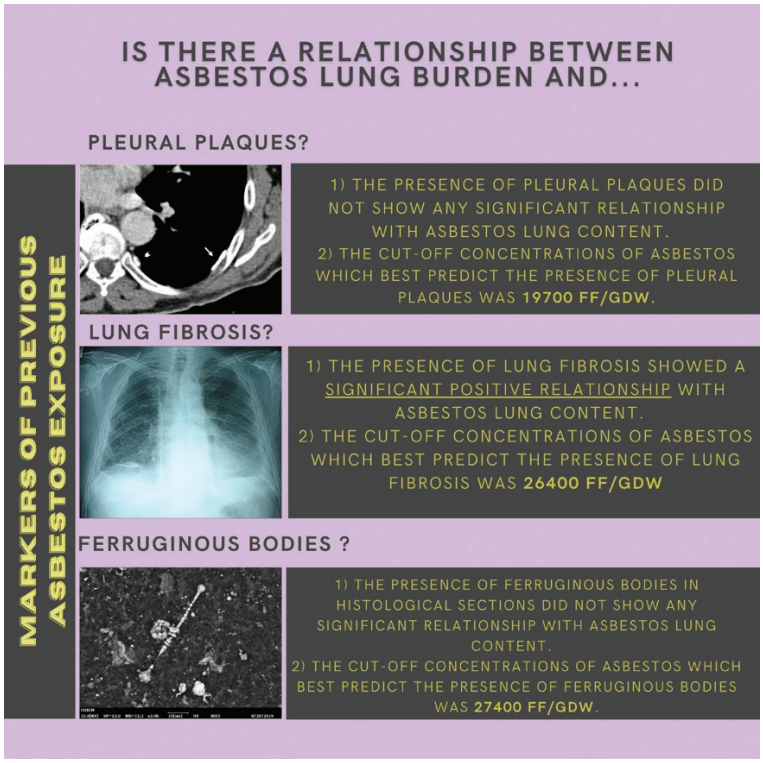
Graphical Abstract



Abbreviations: FB, ferruginous bodies; MM, malignant mesothelioma; PP, pleural plaques, ROC, Receiver operating characteristic.

Introduction

Malignant mesothelioma (MM) is a rare neoplasm, arising from the linings of serosal cavities, well known to be associated with asbestos exposures that occurred 30–40 years before the onset of the disease (1,2). The causal attribution of asbestos-related diseases (especially, MM, lung cancer and asbestosis) to past asbestos exposure is of crucial importance in clinical and medico-legal contexts. Often this evaluation is made on the basis of the exposure history assessed through specific questionnaires, but this method presents important limitations when applied to single individuals and not cohorts, because relevant past asbestos exposures may be unknown or forgotten and, on the other hand, people may report to have been exposed to 'dust' without knowing the nature of such dust; moreover, the awareness that compensation is possible when reporting past asbestos exposure may introduce a bias (3).

In order to assess past asbestos exposure, clinical and radiological markers can be used in combination with anamnestic data. These include pleural plaques (PP), lung fibrosis (which can be evaluated clinically and radiologically, as well as postmortem), and asbestos bodies (ABs) on histological sections or on digested lung tissue. PP are the most frequent benign pleural manifestations related to asbestos exposure, and are described macroscopically as discrete, raised, irregularly shaped, smooth or finely nodular areas of grayish-white to ivory white color located on the parietal pleura (4,5). They are a well-known marker of previous asbestos exposures, and the risk of developing them is positively associated with the time elapsed since the beginning of exposure (6) whilst a dose–response relationship has never been established for these manifestations, and it is still not clear which level of asbestos exposure is necessary to elicit them (7). Asbestosis is defined as a diffuse interstitial lung fibrosis occurring as a consequence of inhalation of 'excessive amounts of asbestos' (8). Even though it is widely accepted that there is a dose–response relationship with asbestosis inhalation (9), there is no agreement on the minimum amount of asbestos necessary to develop asbestosis. In addition, the differential diagnosis between idiopathic lung fibrosis and asbestosis is difficult and based on a history of exposure and the presence of asbestos in tissues (10). Asbestos bodies are inhaled asbestos fibers coated with iron and organic matter mainly composed of ferritin. They have a wide range of shapes and dimensions, and the distribution of the coating is not homogeneous (11). Their formation depends on the ability of macrophages to phagocytose inhaled asbestos fibers. If a fiber is longer than about 20 μm, a single cell is not able to ingest it entirely, and this triggers an inflammatory cascade that promotes the accumulation of iron in the cells. Iron micelles appear in the

macrophage's cytoplasm in proximity of the fiber, and their accumulation, together with homogeneous matrix material, produces a coating around the fiber (12). Asbestos fibers have the intrinsic capacity to form a complex with iron from the surrounding environment (13,14). This initiates a vicious cycle: the more iron the fiber attracts from the tissue, the more inflammation is triggered and, consequently, more iron is accumulated in close proximity of the fiber. Because the observation of AB at microscopy does not allow any chemical characterization, it is more correct to call them ferruginous bodies (FB) unless the core fiber composition is analyzed (12,15). For this reason, in this paper we prefer to use the term FBs rather than ABs, even though the vast majority of FBs are likely ABs. The presence of FB in histological sections can be a sentinel of past asbestos exposures (possibly unknown or forgotten) and, on the other hand, is often regarded as the key element in the differential diagnosis of asbestosis (8,10). However, the presence of FBs has been demonstrated in the general population without asbestos-related diseases and no history of past exposure (16,17). Since the only known cause of FBs is asbestos exposure (if we exclude the rare FB with a core different from asbestos), this finding demonstrates that very low dose exposures unknown to the affected subject might elicit them. Moreover, the amount of FBs not always correlates with the amount of uncoated asbestos fibers in lungs, assessed using electron microscopy, as there is a great individual variability in the process of coating fibers (18,19).

The most reliable tool in the evaluation of past asbestos exposure is the assessment of asbestos lung burden using analytical electron microscopy (20). Yet, the data deriving from this tool must be interpreted taking into account that the time elapsed between the end of the exposure and the subject's death modifies the asbestos lung content, due to the clearance mechanisms that take place in the pulmonary microenvironment (21). This phenomenon mainly concerns chrysotile, which, unlike amphiboles, has a more fragile crystalline structure, which can be fragmented and phagocytosed by alveolar macrophages (22). In fact, previous studies have shown that the inorganic lung content, measured by SEM-EDS, matches well with the retrospective evaluations made by experts if the concentration of amphiboles is taken into consideration (23,24).

In sum, there is a lack of knowledge on the level of asbestos exposure associated with PP, lung fibrosis and FB at histology. On these bases, the aim of the present study are: (i) to understand if the presence of PP, lung fibrosis and histological evidence of FB in MM patients is associated with asbestos lung content (and specifically by asbestos concentration, dimensions and concentration of each type of asbestos) and (ii) find cut-off values of asbestos in lungs that best correlate with each condition (PP, lung fibrosis and FB at histology).

Participants

This is a retrospective observational study conducted on subjects deceased from MM selected from the archive of the Unit of Legal Medicine and Forensic Science of Pavia University—among those who died from asbestos-related diseases between 2005 and 2019. A forensic autopsy, followed by a complete histopathological examination, was performed for each subject. The diagnosis of MM, already known in life, was confirmed postmortem according to the guidelines in effect at the time (10,25–27). During the necropsy, the whole lungs were collected, formalin fixed and stored. Most of the subjects of this study were exposed to asbestos during the activity of Fibronit factory, a large asbestos-cement plant located in Broni (a small town in Pavia Province, northern Italy), which was active between 1932 and 1993. The factory used to manufacture asbestos-cement artifacts using a mixture of chrysotile, crocidolite and smaller amounts of amosite (28).

Sample preparation for SEM-EDS

The technique used here has been described elsewhere (21,29). In summary, for each subject a sample of 0.25 g of formalin-fixed lung, taken from the inferior lobe of the right lung, was chemically digested using 13% sodium hypochlorite, and then filtered through a cellulose-ester membrane (Millipore, Darmstadt, Germany) with a diameter of 25 mm and a pore size of 0.45 µm. The membrane was then coated with graphite, and observed using a scanning electron microscope. Namely, an area of 2 mm^2 was observed at a magnification of 4000 using both secondary and backscattered electrons. The fiber chemical composition was analyzed using an EDS, Oxford Inca Energy 200, equipped with an INCA X-act SDD detector (Oxford Instruments NanoAnalysis, Bucks, UK). The amount of asbestos fibers and FBs observed in an area of 2 mm^2 was normalized to 1 g of dry tissue, reporting concentration in terms of asbestos fibers and FBs per gram of dry weight of lung tissue (ff/gdw), as indicated by international guidelines (28,29). The results have been rounded to three significant digits, considering the accuracy of the methodology. To identify the different types of asbestos fibers, we compared the EDS spectra with a reference database available in the laboratory that performed the tests. SEM-EDS cannot distinguish unequivocally chrysotile from asbestiform antigorite, and tremolite asbestos from actinolite asbestos, since they have similar chemical composition and analogous morphology, therefore we used, respectively, the term chrysotile/asbestiform antigorite and tremolite/actinolite asbestos for these minerals.

While the preparation of all samples was carried out in the same laboratory, the SEM-EDS investigation was carried out in two laboratories, and the samples were divided equally between the two labs. In order to avoid the variability deriving from different instruments and microscopists, we defined a detailed, standardized protocol for data collection. A periodic inter-laboratory control was conducted by comparing the images and spectra obtained by each laboratory. In addition, five samples were analyzed in both laboratories, and the ANOVA test for repeated measurements was used to compare the results (Supplementary Table 1). The 'background' concentration of asbestos in lung tissue (that is the concentration of asbestos that can be found in everyone randomly but does not increase significantly the risk of MM) had been identified in our laboratory as below 100 000 ff/gdw. This is threshold currently used by our laboratory to define the asbestos causation according to SEM-EDS analysis.

Variables

The following variables were extracted from the archive of the Unit of Legal Medicine and Forensic Sciences: the exposure

history, defined as occupational, household or anthropogenic environmental. Each subject was classified according to the exposure history, considering occupational exposure as prevalent over the other two, and household exposure as prevalent on anthropogenic environmental one.

The presence of PP at radiological examination performed during life and/or observed during the necropsy, defined dichotomically as present or not.

The presence of lung fibrosis at radiological examination performed during life and/or observed at the postmortem histology, defined dichotomically as present or not. All grades of lung fibrosis, from mild to severe, are considered as positive if clearly evident at radiological imaging and/or postmortem histology.

The presence of FB at histology was re-evaluated on five histologic lung sections for each subject (at least one for each lobe) stained with H&E and Perls (for trivalent iron). Also this variable was defined as dichotomic (yes or no). In this paper we did not attempt a quantification of FBs and chose to use 'yes or no' to describe this variable because all the patients with FBs in lung sections had large clusters of FBs in each section (well above the 2 FB/cm^2 requested by the asbestosis criteria (8).

The following endpoints were assessed through SEM-EDS: the concentration of asbestos fibers, expressed as number of fibers per gram of dry weight (ff/gdw); the mean length and width of detected asbestos fibers (in µm); the concentration of each type of asbestos (ff/gdw), classified as chrysotile/asbestiform antigorite, crocidolite, amosite, tremolite/actinolite asbestos, and anthophyllite asbestos; the concentration of FBs, expressed as FBs/gdw.

In the present work only asbestos fibers longer than 5 µm, thinner than 3 µm, and with an aspect ratio greater than or equal to 3:1, according to the WHO definition of 'biologically critical fiber' (30) were counted, measured and classified according to the EDS spectrum. This criterium also fits the concept of 'regulated' asbestos fiber according to the Italian law.

Statistical analysis

Samples with presence and absence of PP, presence and absence of lung fibrosis, and presence and absence of FB were compared with regards to concentrations and characteristics of asbestos fibers. Unadjusted *P* values were obtained using *t*-tests, and *P* values adjusted for age and sex were obtained from logistic regression models. Cut-point analyses were performed to obtain optimal values of asbestos concentration for determining presence of PP, lung fibrosis, and FB. Logistic models were created to assess the effect of asbestos concentration on presence of PP, lung fibrosis, and FB. Receiver operating characteristic (ROC) curves were created to assess the significance and accuracy of the predictive model. From the ROC curves, the Youden Index was calculated to determine optimal cut-point of asbestos concentration at which both specificity and sensitivity are maximized. All statistical analyses were performed using SAS, version 9.4. Statistical significance was defined as $P < 0.05$.

Ethics approval and consent

Since this is a retrospective study on autopsy samples, it is not possible to obtain informed consent, which is therefore not necessary according to the Provision containing the prescriptions regarding to the treatment of special categories of data (art. 21, paragraph 1 of Legislative Decree 10 August 2018, n. 101). The study has been approved by the Ethical Committee of Policlinic San Matteo of Pavia.

Results

The study includes 95 subjects who died from MM (93 pleural, 2 peritoneal) between 2005 and 2018; 52% of them were males and 48% were females. The mean age at death was 70 years (SD 11). The past asbestos exposure (according to the medical history and the forensic records) was occupational in 40% of cases, household in 18%, anthropogenic environmental in 41%. One subject had no known history of exposure. Concerning the histological type of MM, 74% had epithelial MM, 10% had sarcomatoid MM, 13% had biphasic MM and 3% had desmoplastic MM. The mean duration of exposure was 25 years (SD = 15.6), the mean latency between the beginning of exposure and the MM diagnosis was 50 years (SD = 12.98), while the mean time elapsed between the cessation of exposure and death was 26 years (SD = 10.64). The mean survival since MM diagnosis was 17 months (SD 15).

Asbestos concentration ranged between 0 and 7 570 000 ff/gdw (mean = 158 000 ff/gdw, SD = 240 000), whereas FBs concentration ranged between 0 and 3 000 000 FBs/gdw (mean = 75 000 FBs/gdw, SD = 333 000) where 0 means below the detection limit. The concentration of asbestos fibers was below the detection limit in 26.3% of subjects, 1–9999 ff/gdw in 15.8%, 10 000–99 999 ff/gd in 43.2%, 100 000–999 999 ff/gdw in 13.7% and above 1 million ff/gdw in 1%. The mean length of asbestos fibers ranged between 6 and 55 µm (mean = 23.7 µm, SD = 12.42), while the width ranged between 0.21 and 1.9 µm (mean = 0.68 µm, SD = 0.28). Considering asbestos as a whole, 0.54% of fibers were classified as chrysotile/asbestiform antigorite, 40.58 % as crocidolite, 48.33% as amosite, 0.85% as anthophyllite asbestos and 9.70% as tremolite/actinolite asbestos.

Among the 95 MM cases investigated, PP was identified in 53.68% of them; the information was missing in 5 (5.26%) cases (*n* for PP = 90). No statistically significant difference was observed in the asbestos lung content in subjects with and without PP (adjusting for age and sex), (Table 1). Namely, The asbestos' concentration, the concentration of each type of asbestos and the dimensional characteristics of asbestos (mean fibers length and width) were not different between subjects with and without PP. The cut-off concentration of asbestos which best predicts the presence of PP (with a sensitivity of 0.6471 and a specificity of 0.6667) was 19 800 ff/gdw (Figure 1a, table 4).

As shown in Table 2, 28.42% of the analyzed individuals presented with lung fibrosis. The concentration of asbestos fibers, as well as the concentration of crocidolite, amosite and tremolite/actinolite (adjusted for age and sex), were higher in subjects with lung fibrosis compared to those without it ($P = 0.0043$, 0.0124, 0.0184, respectively). Instead, the concentration of FBs and the dimensional characteristics of fibers (adjusted for age and sex) showed no statistical difference according to the presence of lung fibrosis. The cut-off asbestos concentration which best predicts the presence of lung fibrosis was 26 400 (with a sensitivity of 0.70 and a specificity o 0.72) (Figure 1b, Table 4).

Table 1. Asbestos burden in MM patients according to the presence of pleural plaques (PP); *n* = 90

	PP *n* = 51 (56.67%) Mean (SD)	No PP *n* = 39 (43.33%) Mean (SD)	*P*-value	*P*-value*
Asbestos concentration (ff/gdw)	11400 (311 000)	27600 (51 300)	0.0539	0.0764
Concentration of asbestos bodies (FB/gdw)	124100 (449 000)	12900 (36 400)	0.0843	0.3901
Concentration of chrysotile asbestiform antigorite fibers (ff/gdw)	406 (1780)	525 (2150)	0.7741	0.7673
Concentration of crocidolite fibers (ff/gdw)	47900 (130 000)	8600 (24 300)	0.0398	0.1216
Concentration of amosite fibers (ff/gdw)	57800 (189 000)	113000 (23 800)	0.0880	0.0676
Concentration of anthophyllite asbestos fibers (ff/gdw)	918 (4080)	446 (2790)	0.5160	0.7269
Concentration of tremolite actinolite asbestos fibers (ff/gdw)	7950 (121 000)	6790 (11 600)	0.6481	0.8303
Asbestos fibers length, (μm)	23.97 (13.17)	23.37 (11.53)	0.8484	0.7134
Asbestos fibers width, (μm)	0.69 (0.17)	0.69 (0.40)	0.9633	0.1820

ff/gdw, fibers × gram of dry weight.
*Analysis adjusted for age and sex.

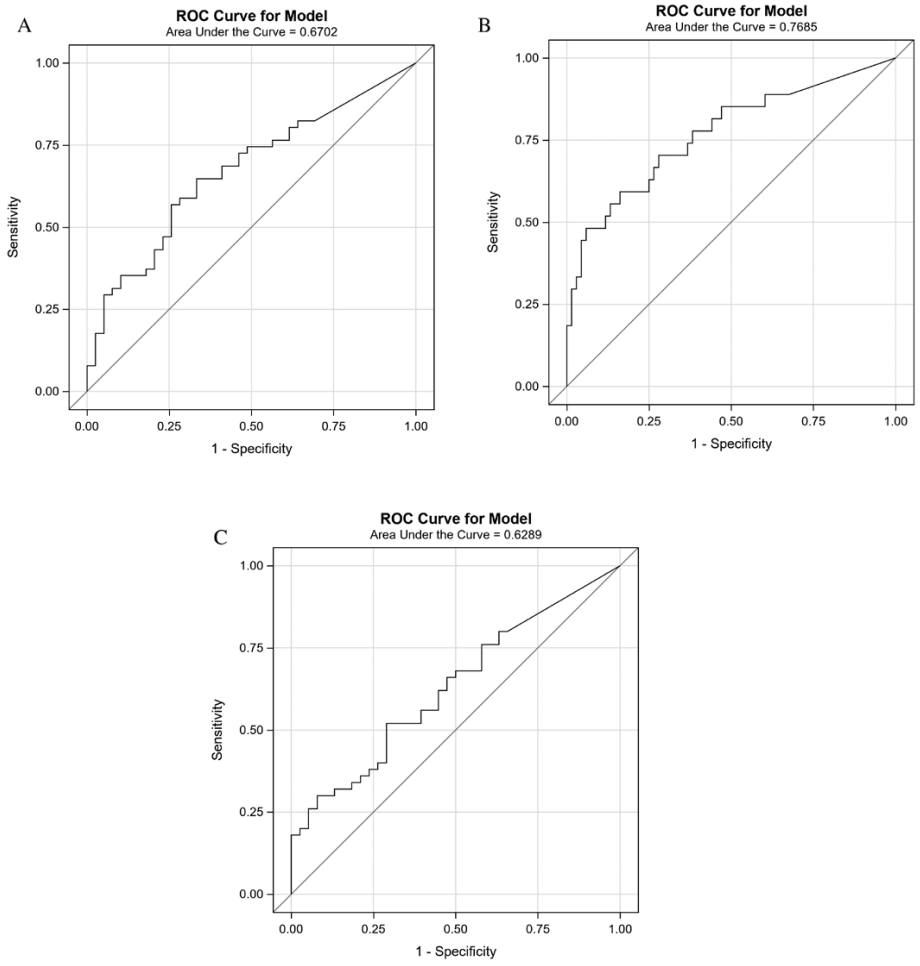


Figure 1. The ROC curves and 'critical' concentrations of asbestos for developing PP, lung fibrosis and FB at histology (a–c).

Table 2. Asbestos burden in MM patients according to the presence of lung fibrosis (n = 95)

	Lung fibrosis n = 27 (28.42%) Mean (SD)	No lung fibrosis n = 68 (71.58%) Mean (SD)	*P*-value	*P*-value*
Asbestos concentration (ff/gdw)	212 000 (421 000)	27 100 (47 000)	0.0313	**0.0043**
Concentration of asbestos bodies (FB/gdw)	206 000 (595 000)	22 400 (97 000)	0.1210	0.3643
Concentration of Chrysotile asbestiform antigorite fibers (ff/gdw)	432 (1750)	434 (1950)	0.9968	0.2013
Concentration of crocidolite fibers (ff/gdw)	92 000 (177 000)	8600 (21 000)	0.0217	**0.0124**
Concentration of amosite fibers (ff/gdw)	105 000 (257 000)	12 000 (21 600)	0.0712	**0.0086**
Concentration of anthophyllite asbestos fibers (ff/gdw)	1730 (5520)	255 (2110)	0.1863	0.1852
Concentration of tremolite actinolite asbestos fibers (ff/gdw)	12 700 (15 500)	5760 (11 400)	0.0417	**0.0184**
Asbestos fibers length (µm)	24.86 (14.23)	23.33 (11.47)	0.6294	0.4885
Asbestos fibers width (µm)	0.73 (0.18)	0.66 (0.32)	0.2864	0.4719

ff/gdw, fibers × gram of dry weight.
*Analysis adjusted for age and sex. In bold are significant p values.

Table 3. Asbestos burden in MM patients according to the presence of ferruginous bodies (FB); n = 88

	FB n = 50 (56.82%) Mean (SD)	No FB n = 38 (43.18%) Mean (SD)	*P*-value	*P*-value*
Asbestos concentration (ff/gdw)	127 000 (323 000)	29 000 (43 800)	0.0393	0.0890
Concentration of asbestos bodies (FB/gdw)	137 000 (452 000)	6390 (15 100)	0.0466	0.1040
Concentration of Chrysotile asbestiform antigorite fibers (ff/gdw)	265 (1380)	735 (2520)	0.3049	0.5008
Concentration of crocidolite fibers (ff/gdw)	53 500 (136 300)	10 000 (23 800)	0.0313	0.1415
Concentration of amosite fibers (ff/gdw)	63 300 (194 000)	11 500 (16 400)	0.0655	0.0749
Concentration of anthophyllite asbestos fibers (ff/gdw)	937 (4110)	458 (2820)	0.5193	0.5515
Concentration of tremolite actinolite asbestos fibers (ff/gdw)	8950 (13 200)	6480 (13 500)	0.3918	0.7451
Asbestos fibers length (µm)	23.99 (13.05)	22.60 (11.37)	0.6658	0.7774
Asbestos fibers width (µm)	0.68 (0.26)	0.72 (0.32)	0.6032	0.1499

ff/gdw, fibers × gram of dry weight.
*Analysis adjusted for age and sex.

Table 4. The asbestos cutoffs with sensitivity, specificity and Youden index for each variable

	Asbestos cut-off (ff/gdw)	Sensitivity	Specificity	Youden index
Pleural plaques	19 800	0.6471	0.6667	0.3137
Lung fibrosis	26 400	0.7037	0.7206	0.4243
Ferruginous bodies	27 400	0.5200	0.7105	0.2305

Furthermore, FB were present at histology in 52.63% of cases, whereas in seven cases (7.37%) the sections could not be retrieved from the archive and the information was not reported clearly in the autopsy report (n for FB = 88). The asbestos lung content in subjects with FB at histology (adjusted for age and sex) did not show any statistically significant difference compared to those without FB (Table 3). The cut-off asbestos concentration which best predicts the presence of FB at histology was 27 400 (with a sensitivity of 0.52 and a specificity of 0.71) (Figure 1c, Table 4).

Discussion

In this study we found no association between the presence of PP or of FB at histology and the amount, the dimensions and the types of asbestos in lungs. Instead, the onset of lung fibrosis seems to be related to higher amounts of asbestos in lungs, and specifically of crocidolite, amosite and tremolite/actinolite. We also observed that the critical amount of asbestos necessary to develop, respectively, PP, lung fibrosis and FB observable at histology is 19 800, 26 400 and 27 400 ff/gdw,

The main limitation of this study is that we did not analyze FBs in digested lung tissue at light microscopy, which is the most suitable technique to count FBs in lung tissue (31). Instead, we identified and counted them at SEM-EDS in the same sample we used to detect uncoated asbestos fibers. Therefore, the concentration of FBs might be underestimated, as we counted them in a smaller lung sample than those usually examined at light microscopy. A second possible limitation regards the choice to analyze only fibers longer than 5 µm, thinner than 3 µm, and with an aspect ratio greater than or equal to 3:1 (30), according to the WHO definition of 'biologically critical fiber', even though in literature a relationship

between fibers with a low aspect ratio and MM has been reported (18,32). Notwithstanding, the current, shared opinion is that the pathogenicity of asbestos is mostly determine by fibers longer than 10 μm (33).

In our series, PP have been observed in 53.68% of MM patients, a lower incidence compared to other studies on PP prevalence in asbestos exposed individuals. For example, Kato *et al.* (34) found PP in 89.4% among 2132 subjects previously exposed to asbestos and Barbieri *et al.* (35) found PP in 89.51%. Furthermore, we found that asbestos concentration did not correlate with the presence of PP. In contrast, Paris *et al.* (36) pointed out a relationship between PP and both cumulative dose of exposure and time since the first exposure. However, in the study by Paris *et al.* the cumulative dose of asbestos exposure was evaluated through questionnaires and not demonstrated using analytical electron microscopy. Recently, a strong positive relationship between the presence of PP and asbestos lung burden has been described (35): for example, Sichletidis *et al.* (37) found PP in dentists as a consequence of very low exposures (measured in air using contrast phase microscopy) repeated daily. The hypothesis that very low doses of inhaled asbestos can provoke the onset of PP seem to be in line with our data, as eight of our cases with PP had no asbestos at SEM-EDS investigation. However, as chrysotile is subjected to clearance in the lung, we cannot draw conclusions about the actual amount of inhaled asbestos and its relationship with PP in those cases where the analysis of lung content was negative for amphiboles (22,38). Hourihane and Mc Caughey (39) suggested that the dose of asbestos necessary to develop PP is intermediate between the dose necessary to develop MM and asbestosis, while Whitwell found that all people with PP had more than 20 000 ff/gdw (40), identifying for the first time a 'critical dose' of asbestos related to the development of PP. This result is in line with our estimation of the cut-off value of asbestos concentration in lungs that present the best relationship with the presence of PP (19 800 ff/gdw), that is considered a low level of exposure compared to the typical asbestos burdens in lungs of asbestos workers (17,18), and even lower than background exposure observed in some studies on general population (16). In our laboratory, the observed 'baseline' level of amphiboles in lungs of individuals without a known exposure to asbestos is below 100 000 ff/gdw. This means that in our experience, most individuals never exposed to asbestos (occupationally, familiarly or environmentally) have a lung burden below 100 000 ff/gdw. However, we observed many cases of MM with well-documented exposure with a lung content below that cut-off. Therefore, in a legal context, each case must be evaluated in the light of all the available data and a lung burden below 100 000 amphibole ff/gdw should not exclude compensation if a compelling history of exposure or the presence of the other markers strongly suggest previous asbestos exposure.

Warnock *et al.* (41) identified significantly higher median concentrations of amosite and crocidolite in subjects with PP, while we did not find any relationship between the concentration of any specific type of asbestos and the risk of developing PP. Kraynie *et al.* (42) found that the occurrence of PP in patients with mesothelioma indicated a probability of having an elevated tissue asbestos content of around 99%, even though asbestos is not the only cause of PP. In addition, Roggli *et al.* (43) found that around 50–55% of mesothelioma patients had PP, similar to what reported in the present study. These findings indicate that the presence of PP in mesothelioma patients strongly point at an asbestos etiology, but the absence of plaques does not rule out that possibility.

We found lung fibrosis in 28.42% of our MM patients, a prevalence that is in line with a previous study on asbestos exposed individuals (34). Instead, Dodson (18) found lung fibrosis in 29 out of 55 cases of MM, in a cohort of cases mainly exposed occupationally. Moreover, lung fibrosis appeared to be related to significantly higher lung concentrations of asbestos, but not with higher FBs concentrations, confirming what was previously stated by Roggli and Shelburne (44). Dodson *et al.* (18) found a higher concentration of both asbestos fibers and ABs in MM patients with asbestosis, even though they pointed out that the dose–response relationship for asbestosis is not constant, given the wide range of asbestos and FBs concentrations reported in asbestosis patients. Bellis *et al.* (45) found low asbestos fibers in some asbestosis patients. Our results are in line with these data, as our estimation of the critical concentration of asbestos in lung that show the best relationship with lung fibrosis (26 400 ff/gdw) is, indeed, lower that the value typically observed in patients with asbestosis (several million fibers per gdw) (46). Furthermore, in the present study, 15 cases with lung fibrosis showed less than 100 000 ff/gdw, and in two cases no asbestos fibers at all; on the other hand, two cases with more than 100 000 ff/gdw had no lung fibrosis. Such data confirm the known dose–response relationship for asbestosis, but also the impossibility to predict the onset of this disease based on asbestos lung content, as previously stated by Dodson *et al.* (18). In addition, the present data suggest that the presence of lung fibrosis is associated with higher concentration of amosite, crocidolite and tremolite/actinolite. This correlation is likely to be due to the longer persistence of amphiboles in lungs compared to chrysotile, rather than to an intrinsic more intense fibrogenicity of amphiboles, and is in line with previous studies (46,47).

Finally, despite the finding of FBs at histology sections is generally regarded as a pillar in the diagnosis of asbestos-related diseases, especially asbestosis (8,48) we did not find any correlation between the asbestos concentration in lungs and the presence of FBs at histological sections. This may be due to the fact that MM and asbestosis correlates better with uncoated asbestos fibers than FBs (49). Indeed, the tendency to cover asbestos fibers is related to each individual response, and some individuals are 'poor coaters' (18), as demonstrated by the wide range of ratios between uncoated fibers and FBs (21,49). A different coating efficiency has been described even in different areas of the same individual's lung (50). Interestingly, the 'critical' concentration of asbestos in lungs necessary to develop FB at histology was estimated as 27 400 ff/gdw, a value well-below the threshold considered indicative of past exposures to asbestos and currently used for causal attribution of asbestos-related diseases (100 000 ff/gdw) (10). Therefore, even though the presence of FBs is suggestive of asbestos exposure, the present data suggest that FB in histological sections, as well as in digested tissue, should not be regarded as a compulsory criterion for causal attribution of a disease to past asbestos exposure, and they cannot provide any reliable quantitative information about asbestos lung content. On the other hand, the low value of asbestos concentration we estimated as 'cut-off' for FB suggests that FB can be identified in people with background exposure (sometimes higher than 4000 ff/gdw) in the general population (16).

In conclusion, especially considering the cut-off values of asbestos concentration in lungs we estimated for each condition examined, these data corroborate the recommendation of the quantification of uncoated asbestos fibers at electron microscopy for the causal attribution of MM to past asbestos exposure.

Conclusions

This study investigated the relationship between the asbestos lung content, assessed using SEM-EDS on digested lung tissue, and the presence of PP, asbestosis and FB at histological section. Only the development of asbestosis resulted to be associated with asbestos lung burden, while PP and FB did not show any significant correlation to the amount of asbestos in lungs. Moreover, we were able to identify, for the first time, critical values of asbestos concentration in lungs that showed the best relationship with the onset of each condition (PP, lung fibrosis and FB at histology), that were all well-below the expected values. Such data suggest that PP, lung fibrosis and FB at histology should be used with caution (and combined with history of exposure) in the causal attribution of MM to past asbestos exposures, while evaluation of amphibole lung content using analytical electron microscopy should be preferred, especially in the legal context. However, lung content analysis should be carefully interpreted, and each case should be evaluated thoroughly, considering all the available data.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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OVERALL DISCUSSION AND CONCLUSIONS

The aim of the present study was wide-ranging research on the use of asbestos and asbestos-containing products in Central Asia, in particular in Kyrgyzstan. Literature data on conducted studies of the impact of asbestos on human health in Central Asia, analysis of asbestos raw materials and asbestos-containing materials which are widely used among the population for private and industrial facilities, as well as air samples from workplaces of one of the enterprises producing asbestos-containing materials, moreover the air samples from the environment of Bishkek and Kant (pilot study), in addition lung tissue samples from those who died from any cause from the general population of Bishkek.

Nowadays there is global awareness of asbestos hazards, especially the long-term prognosis for ARDs like malignant mesothelioma is discouraging [47]. The literature confirms that even brief exposure to low concentrations of asbestos can result in mesothelioma, supported by detectable asbestos fibres in the lung tissue of non-occupationally exposed individuals as well as occupationally exposed [71].

In order to gain a comprehensive understanding of the situation at hand, our initial step involved conducting a systematic narrative review. This review focused on examining studies pertaining to the correlation between asbestos and asbestos-related diseases (ARDs) in both Russian and English publications. Our sources included peer-reviewed journals as well as other credible references [56]. The primary conclusion drawn from this review underscores a significant knowledge gap in the scientific literature concerning asbestos exposure, especially in the context of Central Asian (CA) countries' occupational settings [56]. The available data relies on outdated and imprecise methods [72–74] and does not accurately evaluate asbestos-related risks in the workplace and does not correspond to appropriate sampling and analysis techniques, such as the microscopic method (ISO 14966-2019).

Secondly, it is important to underscore that the chrysotile used in Central Asia is not mixed with other asbestos types; it is referred to as "pure chrysotile". In comparison, the most commonly mined form of asbestos in Italy was chrysotile, however, Italian plants used various asbestos fibre types, including blue long-fibre (crocidolite) and yellow mid-fibre (amosite) from South Africa, white mid-fibre from Canada, and white short-fibre (chrysotile) produced locally [75]. We were plagued by unanswered questions, with the issue of whether it was truly pure chrysotile looming over us. The results obtained from the analysis of materials and artefacts from one of the asbestos-producing enterprises demonstrated the absence of

contaminants and confirmed that the industry's products are composed solely of pure chrysotile (see Chapter 3), as noted in a prior review [76].

In this field of research, our investigation progressed to the subsequent stage, where we examined the extent of asbestos exposure among the inhabitants of the urban area where chrysotile is extensively utilized. The investigation involving scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) of environmental air samples from Bishkek has revealed that the level of asbestos exposure is comparably lower than in Kant town, moreover, it is significantly higher than in Italian results of a study the ambient air [77]. It could be explained by the presence of two industries producing asbestos-containing commodities.

Additionally, the investigation into occupational exposure reveals the actual "controlled use" during the production of artefacts. This extensive exploratory study, albeit with limited sample size, yielded remarkably high results when compared to the occupational exposure limit which is 0,01 fibres/ cm3 (f/ ml) as an 8-hour time-weighted (Directive (EU) 2023/2668 from 22 November 2023). It is important to note that this study provides objective data on the potential hazards of occupational exposure in the industry of Kant.

It is worth noting that comparing this study with other occupational exposure assessments in the same Central Asian region is challenging due to the scarcity of recent studies on asbestos and its impact on the health of workers and the general population in these countries [56]. Moreover, occupational exposure assessment studies are nearly nonexistent, and the few available [78–80] rely on outdated and non-specific techniques. Therefore, conducting a detailed comparison with the results of asbestos exposure measurements or estimates from other studies goes beyond the scope of this research.

It is an interesting fact, that chrysotile is reported to have different effects compared to amphiboles, with crocidolite being deemed the most hazardous asbestos type [81,82]. Furthermore, studies on lung content have been conducted on individuals exposed to a combination of asbestos fibres [67,83–85]. However, this study shows 0 chrysotile fibres in the autoptic samples from the general population of Bishkek, which is explained by the results of analysis of air samples from ambient air in the same city where extremely low fibres found or were not found at all (see Chapter 3).

Nevertheless, they do not consider other ARDs as dangerous mesothelioma and omit the fact that chrysotile has been linked to lung cancer [86]. When it comes to searching some data

about lung cancer, there is a lack of accessible data on this subject in CA countries as well. Moreover, the incidence of mesothelioma in these regions appears to be low, which could be attributed to either underdiagnosis or distinct diagnostic protocols, although the specifics of these protocols remain unclear [56]. Diagnosing mesothelioma is a complex task that presents challenges. The pertinent issue is the not enough developed Occupational Health departments in some of the CA countries, with only a few specialists in occupational diseases serving the entire country and general physicians, who are not well trained in precise ARD diagnosis among workers of asbestos-producing enterprises [73].

The study aimed to evaluate the health risks associated with the ongoing use of asbestos in the country. Additionally, it sought to measure the asbestos fibre content in a representative sample of 100 individuals from the general population of Kyrgyzstan. The 14 samples have yielded negative results for the presence of chrysotile, with only one identification of a tremolite. This divergence is noteworthy as it vastly differs from the average asbestos fibre concentration found in the general population, which is around 1x10^6 per gram of dry tissue [87]. The peculiarity of these results is impressive; no other investigation carried out in similar circumstances and with similar objectives has yielded equivalent outcomes. In comparison to other similar studies that have been conducted all over the world asbestos fibres are found [67,84,85,88], even in the study conducted in the Russian Federation [89].

The absence of fibres in the results may be attributed to low environmental exposure in Bishkek, and poor biopersistence [90]. It is noteworthy that four lung samples were collected from Kant town residents, where environmental exposure is significantly higher than in Bishkek, and still no fibres were detected. The distance of their residence from industrial activities (over 2,5 km) and the brief latency period of chrysotile in the lungs may be the reason for this outcome.

Overall, the study results indicate that there is a range of non-occupational exposure levels in the general population of Bishkek city. However, the absence of asbestos fibres in post-mortem lung samples from the same city may support the theory of the short biopersistence of chrysotile. Nevertheless, it is a very important to highlight the exposure to asbestos in Kant poses significant health risks to both residents and workers, whether it is due to occupational or non-occupational exposure, and the industries are a major contributor to these risks. This is a public health issue in need of urgent attention. This requires extended further research in Central Asian countries.

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APPENDIX 1

ВЫПИСКА ИЗ ПРОТОКОЛА № 5

Экстренного заседания этического комитета при научно-производственном объединении «Профилактическая медицина» МЗ КР

г. Бишкек, ул. Байтик- Баатыра 34, 30 июня 2022 г.

Председатель – Байызбекова Д.А.
Секретарь - Мергенова И.О.

В обсуждении приняли участие – 5 членов ЭК (кворум состоялся).

Вопрос № 4. Этическая экспертиза пакета документов на проведение исследования «Воздействие асбеста в Кыргызстане: типы волокна, стойкость, детерминанты воздействия и риск для здоровья».

Заявитель: Ответственная за проведение исследования д-р Жылдыз Куржунбаева (докторант/аспирант в области медицинских наук).

1. Департамент медицинских наук Миланского университета, Отделение гигиены труда и Международный центр здоровья в сельской местности больницы Сан-Паоло и Сан-Карло, Милан, Италия.

2. Департамент общественного здравоохранения, экспериментальной и судебной медицины, секции судебной медицины и судебной медицины Университета Павии, Павия, Италия.

3. Научно-производственное объединение «Профилактическая медицина» (НПО ПМ), Бишкек, Кыргызстан.

4. Республиканское бюро патологии, Бишкек, Кыргызстан.

5. Патологоанатомическое бюро ТЦОВП Ысык-Атинского района, г. Кант, Кыргызстан.

Техническая экспертиза. Представленные документы в достаточном объеме для этической экспертизы.

Пакет документов на этическую экспертизу получен 08.06.2022 г.

1) Сопроводительное письмо-заявление.
2) Протокол проведения исследования с приложениями:
   - ПРИЛОЖЕНИЕ 1. Форма информированного согласия
3) ПРИЛОЖЕНИЕ 2. Шаблон файла Excel, который будет использоваться для извлечения данных на русском и английском языках
4) Резюме и копии дипломов исследовательской команды:

EXTRACT FROM PROTOCOL
NO. 5

Emergency meeting of the Ethics Committee of the Scientific Production Association "Preventive Medicine" of the Ministry of Health of the Kyrgyz Republic

г. 34 Baitik-Baatyr Street, Bishkek, 30 June 2022

Chairperson -
BaiyzbekovaD.A. Secretary -
MergenovaI.O.

Five EC members took part in the discussion (a quorum was present).

Question 4. Ethical review of the research package 'Exposure to asbestos in Kyrgyzstan: fibre types, persistence, determinants of exposure and health risks'.

Applicant: Dr Zhyldyz Kurzhunbaeva (doctoral/postgraduate student in medical sciences) was responsible for the study.

1. Department of Medical Sciences, University of Milan, Division of Occupational Health and International Centre for Rural Health, San Paolo and San Carlo Hospital, Milan, Italy.

2. Department of Public Health, Experimental and Forensic Medicine, Section of Forensic Medicine and Forensic Science, University of Pavia, Pavia, Italy

3. Scientific and Production Association "Preventive Medicine" (NPO PM), Bishkek, Kyrgyzstan.

4. Republican Bureau of Pathology, Bishkek, Kyrgyzstan.

5. Pathologist's Office of the TSPC of Ysyk-Ata District, Kant, Kyrgyzstan.

Technical expertise. The documents submitted are sufficient for ethical review.

Ethics review package received 08.06.2022

1) Accompanying letter of application.
2) Study protocol with annexes:
   - ANNEX 1: Informed Consent Form
3) ANNEX 2. Template Excel file to be used for data extraction in Russian and English
4) Summary and copies of the research team's diplomas:

APPENDIX 3

LIST OF FIGURES

LIST OF TABLES